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POSTER SESSIONS

CSI-01 – Cell Cycle & Checkpoints

SUN-001
Adaptations of the DNA replication licensing process in mouse embryonic stem cells

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Mouse Embryonic Stem Cells (mESCs) have the ability to proliferate and self-renew indefinitely in culture and, when stimulated, to differentiate towards all three germ layers. They exhibit an unusual cell cycle which consists mainly of S phase cells, a short G1 phase and a lack of major checkpoint responses. Maintaining genome stability is pivotal for embryonic stem cells, as they give rise to all mature cell types. In order for committed cells to maintain genome integrity, DNA replication is restricted to only once per cell cycle. This is accomplished through the assembly onto chromatin of multisubunit protein complexes which license DNA for a subsequent round of replication. Replication licensing takes part in G1 phase and consists of the loading of the hexameric MiniChromosome Maintenance 2-7 (MCM2-7) complex onto chromatin, a step which is dependent on the licensing factor Cdt1. We previously showed that the loading of MCM2-7 onto chromatin takes place in two waves in live mammalian cells: upon mitotic exit and at the G1/S phase transition.

We are interested to understand the molecular mechanisms which ensure genome stability in mESCs, and govern DNA replication licensing, and how these are compared to differentiated and cancer cells. We show that mESCs have a very short G1 phase and move to S-phase with high synchrony following mitotic arrest. During S-phase, replication factories are visualized by immunofluorescence against the replication protein PCNA. They show characteristic early, middle and late S-phase localization, reminiscent of replication factory dynamics in differentiated and cancer cells. Cdt1 is specifically detected during the G1 phase of the mESC cell cycle and degraded following entry to S-phase. MCM2 and MCM7 can be immunodetected onto chromatin following mitotic exit and in S phase, and exhibit differential localization along the cell cycle. Following DNA damage by UV-irradiation in G1, Cdt1 is rapidly proteolysed, while changes in chromatin-loaded MCM proteins are evident. When mESCs are irradiated during mitosis, Cdt1 degradation is delayed until entry into G1, suggesting the Cdt1 is protected from proteolysis in mitosis. mESCs irradiated during mitosis or in G1 fail to progress to S-phase. We will be using functional live-cell imaging to assess licensing in mESCs by Fluorescence Recovery After Photobleaching (FRAP) of the GFP-tagged MCM protein subunits, through the cell cycle of uncommitted cells, or when cells are moving towards differentiation, with a concomitant lengthening of G1 phase.

Keywords: DNA replication licensing, mouse embryonic stem cells.

SUN-002
Analysis of the mechanism of DNA damage and replication arrest-induced histone mRNA decay

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Histone mRNA decay (HD) is the process which ensures that histone mRNA is rapidly degraded following completion of DNA replication at the end of S-phase. Strict coordination between histone protein production and DNA replication is essential for the correct packaging of newly replicated DNA, as imbalances can lead to deleterious effects such as genomic instability. Interestingly, histone mRNA stability is controlled by the presence of a stem-loop structure at the 3′ end of histone mRNA, and a protein, HBP/SLBP (hairpin/stem loop binding protein) which specifically binds to this structure, plays a major role in histone mRNA metabolism. Importantly, previous studies have shown HD is also one functional target of an intra S-phase checkpoint activated when DNA synthesis is inhibited, ensuring that histone mRNA is rapidly destroyed when global DNA replication is blocked. Consistent with this, replication stress-induced histone mRNA decay is blocked in the presence of inhibitors of the PIKK family of checkpoint protein kinases, implicating PIKK family members in the regulation of this process. Therefore, we aim to utilise a proteomic approach to to identify HBP/SLBP-associated proteins and post-translational status in order to elucidate the detailed mechanism of checkpoint activated HD. We have established isogenic cell lines stably expressing functional, tagged HBP/SLBP by using the Flp-InTM T-RexTM Expression system. Our results indicate that isogenic Flp-In HeLa cell lines inducibly expressing tagged forms of SLBP under the control of a doxycycline promoter are a useful model system for the molecular analysis of SLBP function during replication stress.

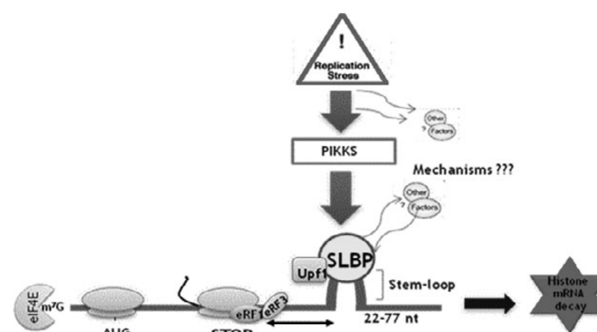

Fig. 1.

Keywords: hairpin/stem loop binding protein (HBP/SLBP), histone mRNA decay, Intra S-phase checkpoint.

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SUN-004
Antioxidant and anticancer effects of *Pterocarpus mildbraedii* (Papillionaece) extracts

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The incidence of cancer in Nigeria is about the highest in sub-saharan Africa. There are growing concerns over the harmful side-effects of synthetic drugs used in cancer therapy. Consequently, there is focus on plants with antioxidant properties as potential therapeutic agents against cancer and other diseases.This study investigates the antioxidant activity of *Pterocarpus mildbraedii* extracts (PME) in vitro. Our study also reports the cytotoxicity of PME in five human tissue cancer cell lines namely, promyelocytic leukemia (HL-60), monocytic leukemia (THP-1), prostrate adenocarcinoma (PC-3), colon adecarcinoma (COLO-205) and lung carcinoma (A549) by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Changes in mitochondrial membrane potential (MMP) and morphological alterations associated with apoptosis were examined by flow cytometry and phase contrast microscopy, respectively.Total phenolic and flavonoid contents were calculated using the Folin-Ciocalteu and the aluminum chloride colorimetric methods and found to be 0.215 ± 0.005 and 0.082 ± 0.003 mg GAE/g respectively. The PME showed a maximum 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity of 55%. Furthermore, the extract exhibited a promising activity against HL-60 cells with IC_{50} value of 8.5 μg/ml. The mechanism of antiproliferative activity revealed that after 24 h, at a concentration of 30 μg/ml, the extract induced apoptosis in HL-60 cells by loss of mitochondrial membrane potential. Microscopic examination of cells revealed the presence of nuclear fragments- containing apoptotic bodies. In conclusion, extract of *P. mildbraedii* showed evidence of antioxidant activity in vitro as well as anticancer effect against HL-60 cell lines and shows great potential for the treatment of leukemia, which still has a high mortality rate, in Nigeria.

Keywords: None.

SUN-005
BubR1 deacetylation is a crucial signal for spindle assembly checkpoint silencing

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BubR1 acetylation is essential in mitosis. We have shown previously that the mice heterozygous for BubR1 acetylation deficiency (*K243R*/+) succumb to high incidence of tumorigenesis due to chromosome mis-segregation, manifested by aneuploidy and broken chromosomes. Detailed investigation of BubR1 acetylation revealed that it is required both for stable chromosome-spindle attachment and in maintaining SAC. For chromosome congression, BubR1 acetylation was required to recruit PP2A-b56alpha to kinetochores to counteract excessive Aurora B activity and stabilize kinetochore-microtubule (KT-MT) attachments. Thus, *K243R*/+ cells have very high chances of chromosome mis-attachments but chromosomes segregate with these errors due to weakened SAC. Biochemical analysis of the mitotic checkpoint complex (MCC) showed that acetylation-deficient BubR1 was readily degraded by APC/C-dependent ubiquitination, resulting in failure to sustain MCC. These data strongly suggested that BubR1 acetylation at K250 is required for SAC maintenance and deacetylation may be a cue to SAC silencing, the mechanism less understood compared to SAC activation. Here we report that BubR1 is deacetylated for MCC disassembly and SAC silencing. We found that HDAC2 binds to BubR1 in metaphase when SAC is satisfied. Cells expressing acetylation-deficient form of BubR1 (K250R) fail to maintain MCC in the presence of microtubule poison, the situation when SAC should be active. On the contrary, introduction of acetylation-mimetic form (K250Q) resulted in arrest in mitosis even though the chromosomes were congressed. Furthermore, we show that there is an BubR1 acetylation-phosphorylation code in SAC activation and silencing in cell division. Collectively, we suggest that BubR1 acetylation/deacetylation is a critical decision point for cells to be in mitosis or exit from mitosis.

Keywords: BubR1, deacetylation, mitosis.

SUN-006
CCAAT/Enhancer-binding protein beta is a critical regulator of Wee1 in G2/M cell cycle progression

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CCAAT/enhancer-binding proteinβ (C/EBPβ) is a transcription factor implicated in the regulation of cellular proliferation, differentiation and development. Mitosis is regulated by cyclinB/CDK1. The Wee1 kinases phosphorylate cyclinB/CDK1 at tyrosine-15(Tyr15) and/ or threonine-14(Thr14) and dephosphorylation of Tyr15 and Thr14 by the Cdc25 is required for the activation of CDK1 and further entry into mitosis. In this study we investigated the roles of C/EBPβ in non-small cell lung cancer cells, as a regulating factor of molecules associated with cell cycle. We found that knockdown of C/EBPβ by RNA interference arrested A549 cells at the G2/M phase of cell cycle. Also, C/EBPβ-deficient cells displayed increased Tyr15 phosphorylation of CDK1 and, at the same time, increased expression of Wee1 at the mRNA and protein levels and decreased protein levels of CDC25B. To demonstrate that C/EBPβ regulates Wee1, a key regulator of G2/M progression, we have conducted cell cycle analysis using flow cytometry with C/EBPβ knockdown cells treated with a Wee1 inhibitor. We observed that in C/EBPβ knockdown cells treated with MK-1775, Tyr15 phosphorylation of CDK1 was reduced to the similar levels to that in wild-type cells treated with control and that C/EBP β knockdown cells were recovered to from G2/M arrest. Taken together, these results indicate that C/EBPβ is a potential regulator of mitosis inhibitor protein, Wee1, ultimately regulating G2/M progression.

Keywords: C/EBPbeta, cell cycle, G2/M transition.

SUN-007
Cdc25 inhibitors in melanoma cells

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Cell division cycle 25 (Cdc25) proteins are dual specificity phosphatases involved in the progression of the cell cycle. In particular, mammalian cells express three isoforms of Cdc25, called Cdc25A, -B and -C: Cdc25A mainly controls G1/S progression, whereas Cdc25B and Cdc25C predominantly activate G2/M transition. Moreover, over-expression of Cdc25A and B activities has been frequently observed in a wide variety of human tumors with poor prognosis. Thus, the design and study of small molecules endowed with an inhibitory activity towards Cdc25 proteins represent a promising strategy for the development of new anti-cancer therapies. A previous work from this group described the

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properties of a set of small molecules, which were able to inhibit Cdc25 functions (Lavecchia et al. *J Med Chem* **55**, 2012, 4142–4158). Among them, compound **11**, possessing a quinonic structure, acted as an irreversible inhibitor of Cdc25B and showed a strong antiproliferative action on some cancer cell lines.

To expand the structure-activity relationship study and to explore potential new structure analogues of **11**, we performed a multiple ligand-based chemoinformatics approach against full ZINC database (~35 million purchasable compounds) and NCI Open database (~260 000 compounds). 25 structures identified from these searches (hereafter called **AL1–AL25**) were analyzed, and selected compounds were tested in vitro to assess the Cdc25B inhibition activity. In particular, compounds **AL11**, **-12**, **-13**, **-14**, **-15**, **-16**, **-23**, **-24** and **-25** maintained a percentage of Cdc25B inhibition comparable to that exerted by compound **11**, and some of them were even more powerful. The effect of these active analogues was also evaluated on melanoma cell lines (A2058 and SAN), because of the limited information on the effect of Cdc25 inhibitors in this cellular system. In both melanoma cells, after 48-h treatment with 100 mM of each inhibitor, a significant reduction of cell growth was observed only with **AL11**, whereas the other active analogues, as well as compound **11**, were ineffective. Furthermore, cytofluorimetric analysis and measurements of the activity of caspase-9 and -3 indicated that **AL11** exerted a pro-apoptotic effect on melanoma cells. In addition, the cellular treatment with **AL11** produced an alteration of the cellular redox state and a reduction of the mitochondrial membrane potential. Studies are in progress to clarify the mechanisms involved in the cytotoxic potential of **AL11** and other related molecules in melanoma cell lines, with the aim at the refinement of the inhibitory action of the studied compounds.

Keywords: Cdc25 inhibitors, Cdc25 phosphatase, Melanoma cells.

SUN-008
Cell-cycle arrest at the G1-S phase boundary through mimosine-induced ATM activation

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The pre-replication complex (pre-RC) is assembled on the replication origin during G1 phase. In S phase, activation of the pre-RC triggers unwinding of DNA, and a bidirectional replication fork is formed. Excess thymidine is widely used for cell synchronization in G1/S phase. Mimosine is also used for cell synchronization in late-G1 phase by preventing the formation of replication forks. However, the mechanisms of G1 arrest remain unclear.

Using highly synchronous cell populations, we show here that mimosine arrests cells at the G1-S phase boundary through ATM activation [1]. HeLa S3 cells are exposed to thymidine for 15 h, released for 9 h by washing out the thymidine, and subsequently treated with 1 mM mimosine for further 15 h (Thymidine→Mimosine). In contrast to thymidine-induced S-phase arrest, mimosine treatment synchronizes >90% of cells having G1-phase DNA content. Cdc45, one of the pre-RC-activating factors, is not loaded onto chromatin in mimosine-synchronized cells, suggesting that mimosine treatment arrests cells at the G1-S phase boundary by blocking the activation of the pre-RC.

Since mimosine treatment strongly activates ATM-mediated checkpoint, we treated mimosine-synchronized cells with the ATM-specific inhibitor KU-55933 and shRNA for ATM. Inhibition of ATM activity is found to induce mimosine-arrested cells to enter S phase. Intriguingly, ATM activation by mimosine treatment is induced without DNA damage. The levels of DNA damage in mimosine-synchronized cells are much lower than those in thymidine-synchronized cells.

The method presented in this study enables us to highly enrich cells at the G1-S phase boundary. After release from mimosine treatment, cells can progress through the cell cycle in a highly synchronous manner. Thus, mimosine synchronization enables us to examine the initial events in DNA replication, which include the activation of the pre-RC and the loading of the replication fork proteins onto chromatin. In thymidine-synchronized cells, these initial events take place prior to release from thymidine treatment. Further studies using mimosine synchronization will help us to gain new insights into the precise mechanisms that control the onset of DNA replication.

Reference

1. Kubota et al., Activation of the prereplication complex is blocked by mimosine through reactive oxygen species-activated Ataxia telangiectasia mutated (ATM) protein without DNA damage. *J. Biol. Chem.*, 289: 5730–5746, 2014.

Keywords: cell cycle, checkpoint control, DNA replication.

SUN-009
Depletion of biliverdin reductase sensitizes glioma cells to chemotherapy

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Enhanced cellular cytoprotective activities are reported to play a major role in the development of intrinsic drug resistance in malignant glioma. Here, we show that human biliverdin reductase (hBVR), recently identified as a major redox regulator, play a key role in the developmenet of multidrug resistance in glioma. Using stepwise-selected drug-resistant human glioma cell lines, we show that hBVR activity increased in the drug-resistant sublines and knock down of hBVR led to a significant increase in chemosensitivity in response to chemotherapeutic drugs and drug-induced cell death. We further found that this sensitizing effect was associated with increased ROS generation and dissipation of mitochondrial transmembrane potential. These results show that hBVR is an important regulator of glioma resistance to chemotherapy.

Keywords: biliverdin reductase, chemoresistance, Multi drug resistance.

SUN-010
Depletion of SUMO ligase hMMS21 inhibits HCT116 colorectal cancer cell growth

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We demonstrate that depletion of SUMO ligase hMMS21 leads to a slower growth of HCT116 cells by reducing S phase progression, which involves a decrease in the amount of thymidylate synthase (TYMS) and inhibition of CDK2 by an increased amount of p27. The decrease in TYMS is caused by a faster degradation, rather than an effect on gene expression. In addition, we showed that although the decrease in TYMS amount caused by depletion of hMMS21 provoked a low degree of DNA damage, preventing DNA damage by supplementing thymidine did not rescue the growth rates of HCT116 cells. Moreover, ectopic expression of TYMS did not rescue the slow growth of HCT116 cells depleted of hMMS21, consistent with the notion that other factor, e.g., p27, may also be involved. Thus this study may provide a new perspective for treating colorectal carcinomas.

Keywords: hMMS21, HCT116 colorectal cancer cell, thymidylate synthase.

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SUN-011
Differential proliferative responses of fetal and adult human skin fibroblasts to TGF-β: implications for wound healing

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Wound healing in adults is characterized by intense inflammation, collagenous scar formation and contraction, while fetal repair – in the first two trimesters of gestation – is marked by minimal inflammatory reactions and the absence of scar formation. Transforming growth factor-β (TGF-β) is a cytokine with a broad range of activities in tissue repair and a pleiotropic regulator of cell proliferation. In this context we have shown previously that TGF-β regulates the proliferation of normal human skin fibroblasts according to their developmental origin, i.e. it inhibits fetal fibroblasts via the activation of Protein Kinase A (PKA) and the induction of the cyclin-dependent kinase inhibitors (CKIs) $p21^{CIP1/WAF1}$ and $p15^{INK4B}$, while it stimulates the proliferation of adult donor cells by the release of basic Fibroblast Growth Factor (bFGF) and – through the FGF receptor-1 (FGFR-1) – the subsequent activation of the MEK-ERK pathway. Recently, we have shown that both signalling pathways are SMAD-dependent.

During the early wound healing phases fibroblasts grow and migrate on a provisional extracellular matrix (ECM) containing mainly fibronectin, while at the late stages they are surrounded by a matrix containing mostly fibrils of polymerized collagen. Accordingly, we investigated the interaction of these ECM components with TGF-β and found that the responses of fetal and adult cells remain unaltered when cultured either on plastic surfaces or on surfaces coated with fibronectin or collagen type-I, as well as, on top or within three-dimensional matrices of polymerized collagen. Moreover, the differential effect of TGF-β persists in both stressed and relaxed collagen lattices.

Chronic wounds in adults have been linked to the existence of senescent cells in the wound area. On the other hand, it has been recently reported that senescence may control the quality of tissue repair by inhibiting fibrosis. In this vein, we have found that a long-term exposure to TGF-β provokes premature senescence in both fetal and adult skin fibroblasts. The mechanisms underlying this phenomenon will be discussed.

In conclusion, these data indicate a dynamic interplay between skin fibroblasts, ECM components and TGF-β at different developmental stages towards tissue repair and remodeling.

Keywords: extracellular matrix, growth factors, Signaling pathway.

SUN-012
Dihydroxynaphthyl aryl ketones as tubulin polymerization and cancer cell growth inhibitors

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Dihydroxynaphthyl aryl ketones (arene = 2/3 thiophene, **1** and **2**; furane, **3**; pyrrol, **4**; 3,4,5 trimethoxybencene, **5**) have been evaluated for their ability to inhibit tubulin polymerization and colchicine binding, their ability to bind tubulin and their cytotoxic activity against the cancer cell lines DU-145 (prostate), T24 (bladder) and MCF7 (breast). These compounds are of interest because they are readily available via our synthetic method and because they are structurally related to several molecules that are known to inhibit tubulin polymerization. Compounds **4** and **5** displayed competitive inhibition against colchicine binding, and docking analysis showed that these compounds bind to the colchicine binding site pocket of tubulin. Remarkable differences in biological activity were observed between the assayed compounds, and these differences are related to the structure and position of the aryl substituent bonded to the carbonyl group. For compounds **2**, **3** and **4**, which contain a heterocyclic ring, higher affinity was observed compared to that of carbocyclic analogue **5**. The compound with the best affinity of the series was compound **4**, which exhibited an IC_{50} value of 2.1 mM for microtubule polymerization inhibition and a tubulin dissociation constant of 1.0 ± 0.2 mM, as determined by thermophoresis. Among these compounds, only ketone **5** showed selectivity for cancer cells over healthy, non-transformed cells. In T24 cancer cells treated with compound **5**, a concentration-dependent decrease in cell proliferation was observed. In addition, cells lost their ability to migrate when treated with compound **5**. While compound **4** is slightly more efficacious in disrupting tubulin polymerization in biochemical studies, the greater lipophilicity of compound **5** likely facilitated increased membrane penetration in cell-based assays for cytotoxicity, which showed compound **5** to be more potent.

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Keywords: cytotoxicity, microtubules, thermophoresis.

SUN-013
Direct interaction between CENP-C and protein phosphatase 4 regulatory subunit 3 affects Drosophila kinetochore integrity

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Reversible phosphorylation of proteins is one of the major intracellular regulatory mechanisms, which controls almost all cellular events, including progression through mitosis. Although roles of the multiple kinases responsible for protein phosphorylation are largely understood, the functions and substrate architecture of the multiple protein phosphatases (PPase) that reverse these events are for the most part mysterious. To fully understand mitotic progres-

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sion, it is necessary to comprehend mitotic PPase functions, one of the major remaining challenges in the field of mitosis. Protein Phosphatase 4 (PPP4) is an evolutionarily conserved multisubunit enzyme that has a relatively unexplored mitotic role. PPP4 belongs to the PP2A subfamily of Ser/Thr phosphatases and is composed of one catalytic (PP4c) and two regulatory (R2 and R3) subunits in *Drosophila melanogaster*. Although its role has been shown in various mitotic processes including centrosome maturation and nucleation of spindle microtubules, no mechanistic details or mitotic substrates have been revealed so far. In this study we found that PPP4 forms a complex with the key centromeric protein CENP-C, the major and essential link between the mitotic centromere and kinetochore network. We narrowed down this interaction to the very amino-terminus of the Regulatory 3 subunit of PPP4 and a 19 amino acid-long region within CENP-C. Then we solved the crystal structure of the heterodimer of these minimal regions, which helped us to better understand the molecular basis of the interaction. We unequivocally proved that the presence and catalytic activity of PPP4 at the centromere is essential for proper kinetochore integrity: depletion or deactivation of PPP4 causes partial removal of CENP-C and the entire kinetochore from the centromeres followed by accumulation on the mitotic spindle and around spindle poles during mitosis. We also revealed that CENP-C is a mitotic substrate of PPP4 and its phosphorylation status critical for kinetochore integrity. Therefore we propose CENP-C as a novel mitotic substrate of PPP4 and suggest PPP4 as a regulator of kinetochore integrity.

Keywords: CENP-C, *Drosophila melanogaster*, Protein phosphatase 4.

SUN-014
DNA repair in retinal pigment epithelial cells arrested in G0/G1 phase

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DNA repair and cell cycle arrest are main responses to DNA damage in eukaryotic cells. Little is known about the DNA repair in cells arrested in G0/G1 phase. It is known, however, that all eukaryotic DNA repair systems operating in proliferating cells also operate in cells arrested in G0/G1 phase. Recent studies indicate a potential function of cell cycle checkpoints in the DNA repair in neurons arrested in G0 phase. The aim of our research was to investigate the kinetics of DNA repair in cells arrested in G0/G1 phase in response to acute and chronic oxidative stress. The cell response to acute stress determines the cell fate – to die or live, whereas the response to chronic stress may induce an adaptive response.

We employed ARPE-19 cell line, derived from retinal pigment epithelial cells and induced their cell cycle arrest through a combination of contact growth inhibition, restriction of growth factors and the presence of retinoic acid (RA). For the generation of acute oxidative stress we used hydrogen peroxide or *tert*-butyl peroxide and for chronic oxidative stress – glucose oxidase – which continuously produce hydrogen peroxide in the presence of medium. The cytotoxicity was assessed using trypan blue exclusion assay and DNA damage by comet assay.

RA induced density-dependent cell cycle arrest associated with morphological changes in APRE-19 resembling those featured in terminally differentiated cells in vivo. We observed that there was no difference in the level of DNA damage after the generation of oxidative stress and at the late stage of DNA repair (from 30′ to 60′) between ARPE-19 cells arrested in G0/G1 phase and cycling cells. Analysis of repair kinetics in ARPE-19 cells arrested in G0/G1 phase showed a clear defect at the intermediate (from 5′ to 15′) time points following generation of acute oxidative stress. In contrast, there was no repair defects at any time point in ARPE-19 cells arrested in G0/G1 phase after exposure to chronic oxidative stress.

The ability to cross G0/G1 checkpoint may be required for regular DNA repair in non-cycling ARPE-19 cells in response to acute oxidative stress.

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Keywords: cell cycle arrest, DNA repair.

SUN-015
Downregulation of NOX4 NADPH oxidase leads to senescence of vascular smooth muscle cells

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Senescence is proposed as one of the mechanisms that drives organism aging as well as age-related diseases. This process was shown to participate in pathogenesis of cardiovascular diseases since senescent vascular smooth muscle cells (VSMCs) were found in atherosclerotic plaques. Cellular senescence is related to unrepairable DNA double strand breaks and the activation of the DNA damage response (DDR) pathway as well as increased reactive oxygen species (ROS) production. Although defective mitochondria are considered to be the main source of ROS a number of studies prove that ROS-generating enzymes like NADPH oxidases (NOX) could also play an important role in this process. Data suggest that NOX4 could be involved in the regulation of both proliferation and aging. The aim of this study was to analyze the role of NOX4 in the senescence of human VSMCs (hVSMCs). We observed increased ROS production in VSMCs undergoing stress-induced and replicative senescence. Treatment of the cells with DPI – NOX family inhibitor, decreased the level of ROS in senescent as well as in proliferating hVSMCs, however did not affect senescence induction. In contrary, it caused permanent cell cycle arrest and increased the level of other senescence markers in proliferating VSMCs. The DPI-induced senescence was not correlated with activation of the DDR pathway. To address the NOX4 exclusive role in these process we downregulated its expression with siRNA. Cells with reduced NOX4 transcript accumulated in mitosis due to abnormal mitotic spindle formation. Downregulation of NOX4 correlated with the impaired level of SAC and CPC proteins involved in the regulation of mitotic checkpoint. After a few days of culture, cells transfected with NOX4 siRNA underwent senescence. Majority of those cells were multinucleated proving that they underwent improper mitotic divisions on the road to senescence. What is interesting, disturbed nuclei morphology was also observed in replicatively senescing hVSMC what corresponds with gradual decrease of NOX4 mRNA level. These findings suggest that NOX4-derived ROS play an important role in regulation of the cell cycle in VSMCs and decreased level of this enzyme could be responsible for senescence induction.

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Keywords: mitosis, NOX4, senescence.

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SUN-016
Early contribution of C1q and Calreticulin to the recognition and elimination of apoptotic cells

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Efferocytosis is an immunologically silent process by which phagocytes remove rapidly apoptotic cells before their entrance in secondary necrotic phase. C1q, the first component of complement cascade, enhances uptake by bridging the apoptotic cell to the phagocyte. C1q contributes also to the anti-inflammatory response by modulating cytokine release by phagocytes. Autoantibodies against C1q were found to target specifically early apoptotic cells, thus suggesting a fast involvement of this protein in the efferocytosis process. Furthermore, it was shown that C1q interacts with surface-exposed Calreticulin (CRT), an early eat-me signal capable of eliciting an immunogenic response. Based on these findings, we hypothesized that C1q acts early with CRT to remove apoptotic cells.

Jurkat cells were rendered apoptotic by 312 nm UVB-irradiation (1000 mJ/cm^2). PMA stimulated THP-1 cells were used as macrophages for in vitro efferocytosis assay. Binding of human serum-purified C1q or its globular heads (C1qGR), and CRT surface exposure were studied by flow cytometry and epifluorescence microscopy. The interaction between CRT and C1q was examined by fluorescence resonance energy transfer (FRET) confocal microscopy imaging. Our results indicate that opsonisation of 'freshly' UV-irradiated Jurkat cells by C1q significantly enhances their uptake by THP-1 macrophages. Moreover, as measured by flow cytometry, CRT is rapidly retained at the cell surface after UV irradiation, even before the phosphatidylserine exposure to the outer membrane leaflet. At this 'pre-apoptotic' stage, C1qGR highly bind to the cell surface and co-localize with CRT. Interestingly, data obtained by FRET experiences show efficient early interaction between C1q or C1qGR and cell surface-exposed CRT.

Taken together, these results point to the fast contribution of C1q on efferocytosis, by acting with CRT to the recognition and elimination of early very apoptotic cells.

Keywords: efferocytosis, early apoptotic cells, C1q, Calreticulin.

SUN-017
Effect of a cathepsin L variant on colorectal cancer HCT116 cell proliferation

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We have studied a variant of cathepsin L protease homologous to SpH protease, a kind of protease initially described in sea urchin. This variant of cathepsin L is entitled SpH-Cathepsin-L, and has been also identified in cancer cells. This particular protease has a molecular weight of 60 kDa, and has nuclear localization during S phase of the cell cycle; it colocalizes with alpha-tubulin during mitosis in colorectal cancer and cervical cancer cell lines. SpH-Cathepsin L was also identified the colorectal cancer HCT116 cell line.

To analyze the effect of SpH-cathepsin L on cell proliferation of the HCT116 cell line, we silenced the SpH-cathepsin L gene by using siRNA technique. Cell proliferation was measured by means of bromodeoxyuridine (BrdU) incorporation to DNA. In addition, we analyzed cell viability by using a MTT assay.

Our results show that siRNA-mediated depletion of SpH-cathepsin L decreases HCT116 cell proliferation reflected by diminished incorporation of BrdU. Besides, we show that siRNA-mediated depletion of SpH-cathepsin L did not generate a cytotoxic effect in HCT116 cells. Based on these results, we concluded that SpH cathepsin L participates in proliferative processes of the HCT116 cell line, which is consistent with results obtained with other variants of cathepsin L.

Grant: VRID-Enlace 214.037.018-1.0.
Keywords: Cathepsin L, cell proliferation, siRNA.

SUN-018
Effects of bisphenol a (BPA) on SHSY5Y cells: evaluation of nitric oxide related pathways

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Bisphenol A (BPA) is a chemical widely used as monomer of polycarbonate plastics, nylon and PVC. It is clearly known that BPA is endocrine disrupting chemical reacting with estrogen and androgen receptors. Endocrine disruptors have been widely reported to effect nitric oxide synthase (NOS) activity but the mechanism hasn't been explained cleary yet.

In this study we aimed to enlight BPA's effect on SHSY5Y cells through cell viability and nitrite concentration of cells. The cytotoxicity of BPA on SHSY5Y cells was evaluated by MTT assay. MTT assay was performed in a 96 well plate for 24 h at increasing concentrations of BPA(1 nM–100 mM), and calculated as percentage of the viability of untreated cells. Cell viability was reduced significantly to % 50 with 10 μM BPA. Nitrite concentration of SHSY5Y cells were determined according to colorimetric Griess's reaction spectrophotometrically at 540 nm with reference to a standard curve. Preliminary results show a relation between BPA concentration and nitrite levels on SHSY5Y cells. Relation between decreased cell viability and nitrite concentration is assumed to be a result of increased nitrosative stress of cells and altered nitrosylation of proteins. Further studies will be done to investigate detailed interactions of BPA and NOS related pathways.

Keywords: Bisphenol A, Nitric Oxide, SHSY5Y Cells.

SUN-019
Elucidating the impact of tumor marker nucleoporin Nup88 on the pRb pathway, cell proliferation and nuclear organization

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Nuclear pore complexes (NPCs) are macromolecular structures that are embedded in the double membrane of the nuclear envelope (NE) and mediate bidirectional nucleocytoplasmic transport. The vertebrate NPC consist of about 30 nucleoporins (Nups). Nup88 is found over-expressed in a wide variety of cancers characterized by their high aggressiveness and metastatic potential; within the tumors, Nup88 expression levels are particularly high at the tumor periphery and their invasive front. In tumors, Nup88 is partly displaced from its localization at the NPC and is also found in the cytoplasm. Nup88 has been proposed as a tumor marker, but its physiological

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function is poorly understood and the regulation of its expression has remained elusive. We have previously identified Nup88 as a novel lamin A interacting partner. Since the lamin A-LAP2α complex is known to repress pRb and to regulate G1-S transition, we investigated whether modulating Nup88 expression affects the lamin A-LAP2α complex and cell cycle progression. In this context, we studied the impact on a selection of pRb/E2F target genes. In addition, we aimed at further characterizing the role of Nup88 in nuclear organization in particular via its effect on lamin A interactors. By ectopical expression of Nup88 and siRNA-mediated Nup88 depletion, we could show that over-expression of Nup88 leads to a destabilization of the lamin A-LAP2α complex, possibly by recruiting lamin A to the nuclear periphery, and a weakening of its repressor function at pRb/E2F gene loci, which in turn leads to accelerated G1/S progression. On the other hand, depletion of Nup88 slows down S-phase, leaving the lamin A-LAP2α complex intact. Consistently, cyclin E1, TK1 and PCNA mRNA levels were increased in Nup88 knock-down cells, as shown by RT-qPCR. In addition, over-expression of Nup88 was shown to mislocalize emerin, a protein of the inner nuclear membrane, which specifically needs lamin A for proper localization at the NE. Here, we propose Nup88 as a regulator of the pRb/E2F pathway via its ability to regulate the repressor complex lamin A-LAP2α activity and as a key player in the organization of nuclear lamina.

Keywords: Cell cycle, nuclear lamina, Nucleoporins.

SUN-020
Essential functions of Orb2 in spermatogenesis of *Drosophila melanogaster*

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During this work, we investigate functions of CPEB family Orb2 protein in spermatogenesis of *Drosophila melanogaster*. CPEB family proteins are known to participate in cytoplasmic polyadenylation of mRNA tails and in masking of mRNAs. However, it is not shown for Orb 2 to date. Using whole-mount immunostaining and confocal microscopy we analyze patterns of Orb2 distribution in the wild-type testes and characterize a failure of spermatogenesis in flies with different Orb2 mutations (1793, 6090 and 36). We reveal that Orb2 in spermatocytes localizes in germinal granules, in which processing of short RNAs (part of piRNA-pathway) also occurs. Nevertheless, the absence of Orb2 doesn't lead to disruption of germinal granules and disturbances in the piRNA-pathway with further derepression of harmful transcripts stellate. We also determine a meiotic arrest on two stages of spermatogenesis in the absence of Orb2 (line 36): about 60% of mature cysts arrested in G2-phase and about 40% in prometaphase. Arrest in prometaphase is assosiated with failures of meiotic spindle formation. Using PAT-analysis (analysis of the length of poly-A tails in mRNA) we find some potential targets of Orb2 in the testes. There are cyclines A and B, and another CPEB – Orb. We showed shotering of poly-A tails in mRNAs of the both cyclines in mutants without Orb2. According to previous data, Orb2 in brain forms functional amyloid aggregates. We display for the first time that in the testes Orb2 also forms amyloid aggregates.

Keywords: CPEB, Orb2, Spermatogenesis.

SUN-021
Glutathione is essential to preserve nuclear function and cell survival under oxidative stress

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Organisms growing in an aerobic environment must cope with Reactive Oxygen Species (ROS), such as superoxide, hydrogen peroxide and hydroxyl radical. Although ROS damage all the cellular macromolecules, they play a central role in a range of biological processes, including signaling. This later process requires a tight control of their abundance. Thus, redox homeostasis is achieved by antioxidant systems involving a large collection of enzymes that scavenge or degrade the ROS produced endogenously during cell growth. In addition to this enzymatic protection against ROS, cells also contain small antioxidant molecules, such as glutathione (GSH). With an intracellular concentration between 1 and 10 mM, GSH is the most abundant non-protein thiol in the cell and is considered as the major redox buffer of the cell. To better characterize its essential function during oxidative stress conditions, we studied the physiological response of H_2O_2-treated yeast cells containing different amounts of GSH.

We showed that the transcriptional response of GSH-depleted cells is severely impaired, despite an efficient nuclear accumulation of the transcription factor Yap1. Moreover, oxidative stress generates high genome instability in GSH-depleted cells, but does not activate the checkpoint kinase Rad53. Surprisingly, scarce amounts of intracellular GSH are sufficient to preserve cell viability under H_2O_2 treatment. In these cells, oxidative stress still causes the accumulation of oxidized proteins and the inactivation of the translational activity, but nuclear DNA and nuclear functions are protected against oxidative injury, as exemplified by low mutation frequency, moderate histone carbonylation, activation of the checkpoint kinase Rad53 and of the H_2O_2 transcriptional response.

We conclude that the essential role of GSH is to preserve nuclear function, allowing cell survival and growth resumption after oxidative stress release. We propose that cytosolic proteins are part of a protective machinery that shields the nucleus by scavenging reactive oxygen species before they can cross the nuclear membrane.

Keywords: Glutathione, Oxidative stress, Rad53.

SUN-022
GNL3L is nucleo-cytoplasmic shuttling protein: role in cell division cycle regulation

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GNL3L (Guanine Nucleotide Binding Protein-Like-3-Like) is an evolutionarily conserved high molecular weight nucleolar GTP binding protein belonging to HSR1-MMR1 subfamily of GTPases. Given the increasing importance of nucleolar proteins in the regulation of cell growth and proliferation, we have attempted to address the relationship between nucleo-cytoplasmic transport of GNL3L and its functional significance during cell proliferation. Employing a combination of immunofluorescence, mutagenesis and protein-protein interaction assays, current investigation demonstrates that GNL3L shuttles between nucleus and cytoplasm in a CRM1-dependent manner. Deletion mutagenesis analysis reveals that the C-terminal domain (amino acids 501–582) is necessary and sufficient for the export of GNL3L from the nucleus

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and the exchange of hydrophobic residues (M567, L570 and L572) within the C-terminal domain impairs this process. In addition, our data indicates that the C-terminal domain encodes a non-canonical nuclear localization signal (NLS), and is able to import cargoes into the nucleus in an importin-alpha and importin-beta independent manner. Cell cycle analysis indicated that less number of cells accumulated in S-phase upon overexpression of nuclear export defective GNL3L, which is localized in the nucleus. Interestingly, BrdU labeling assay showed a significantly higher amount of DNA synthesis despite the fact that less number of cells accumulated during 'S' phase of cell cycle. Furthermore, the expression of E2F1, cyclin A2 and cyclin E1 were up-regulated along with phosphorylation of Rb protein upon ectopic expression of $GNL3L^{\Delta NES}$, resulting in faster 'S' phase progression. Collectively, the present study provides evidence that GNL3L is exported from the nucleus by a CRM1 dependent mechanism and the nuclear localization of GNL3L is critical to promote 'S' phase progression during cell division cycle.

Keywords: Cell cycle, nucleo-cytoplasmic shuttling, nucleolar GTPase.

SUN-023

Green tea, coffee and cocoa polyphenols exhibit different effects on HeLa cell viability and proliferation

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One of the leading causes of cancer death within female population is cervical cancer, especially in developing countries. Therefore, lot of effort has been invested in understanding of molecular mechanism of cancerogenesis and development of mechanistically targeted therapies. Common beverages such as green tea, coffee and cocoa are rich in antioxidants, especially phenolic acids and derivatives of catechins, known for their health promoting properties. Epidemiological and experimental studies indicate that green tea extract acts as effective chemo preventive agent targeting different organ specific cancers. In accordance with the claims set forth above, the aim of this investigation is to identify and confirm if there is any significant biological effects of cocoa, coffee and green tea extracts on cervical cancer cell (HeLa cell line) survival.

Total phenolics of extracts were determined by Folin-Ciocalteu's method. Cytotoxic activity of extracts in HeLa cell line was assayed by viability testing using MTT. Estimation of cell proliferation was assayed using flow cytometry and CFSE dye diluting metod. For detecting cells in early stages of apoptosis and for distinguishing them from dead cells, Annexin V-FITC was employed. All tested extracts exhibited cytotoxic activity in HeLa cell line, with green tea extract (GT) having the highest cytotoxic activity followed by coffee (CF) and cocoa extract (CC). Statistically significant decrease in proliferation was observed only with GT, while increase in proliferation index occurred in the case of CF and CC after 72 h of treatment. Quantification of apoptotic effect *via* Annexin-FITC labeling, showed that all three tested extracts behaved as apoptotic agents for HeLa cell line. As expected, GT induced the highest degree of apoptosis, while CF and CC induced similar degrees of apoptosis after 72 h of treatment.

As a conclusion, GT provides the most potent apoptotic signals to the tumor cells, coffee extract shows modest effects on cell viability and apoptosis induction, while the weakest effects shows cocoa extract.

Keywords: apoptosis, Cancer, Polyphenols.

SUN-024

HCF-1 cleavage and glycosylaton are distinct activities of OGT

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O-GlcNAc Transferase (OGT) catalyzes a dynamic and a reversible glycosylation of cellular proteins that regulate intracellular signaling, transcriptional regulation and stress responses. Structural features of OGT include a N-terminal tetra-tricopeptide repeat (TPR) domain and a C-terminal catalytic domain. While the TPR domain of OGT is involved in substrate recognition and binding, the catalytic domain is required for substrate glycosylation. Recently, a novel proteolytic activity of OGT was described for a transcriptional co-factor called Host Cell Factor-1 (HCF-1) (Capotosti et al; *Cell 2011*). HCF-1 also undergoes OGT-mediated glycosylation during this process. X-ray crystallography analysis shows a unique interaction between OGT and HCF-1 proteolytic site (Lazarus et al; *Science 2013*). Using molecular dynamics simulations, we observed a strong interaction between OGT TPR domain and the HCF-1 proteolytic site. However, the mechanism of OGT's-dual activities against HCF-1 (proteolysis and glycosylation) remains unexplained. We performed extensive mutational analysis of OGT and assayed the mutant OGTs for HCF-1 cleavage and glycosylation activities. Certain mutants of OGT retained HCF-1 cleavage activity despite losing HCF-1 glycosylation activities or vice-versa, suggesting that glycosylation and cleavage of HCF-1 are two distinguishable properties of OGT. Interestingly, mutational analysis of the TPR domain suggests a critical role of the TPR domain for the proper presentation of the HCF-1 proteolytic site within OGT catalytic core.

Keywords: Epigenetics, Glycosylation, O-GlcNAc transferase.

SUN-025

Hormonal signalling and nuclear receptor: new players in the regulation of intestinal crypt cells division

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The intestinal epithelium is characterised by a continuous renewal dependent of the multipotentes stem cells residing at the bottom of intestinal crypts. They give rise to proliferative progenitors that differentiate while migrating along the crypts. The intestinal homeostaαsis is achieved by balanced proliferation and differentiation processes, which are tightly controlled by signalling pathways such as Wnt, BMP and Notch. Our group identified the thyroid hormones (TH) and their nuclear receptor TRalpha1 as a regulator of intestinal homeostasis, acting in particular via cross talks with the Wnt pathway. Mice overexpressing TRα1 in intestinal epithelium (vil-TRα1 mice) display increased cell proliferation and develop adenomas; in a Wnt-activated background, TRα1 overexpression accelerates tumorigenesis (vil-TRα1/Apc+/1638N mice). Intriguingly, the epithelium of the vil-TRα1 mice displays an aberrant architecture suggesting an alteration of crypt cells division axis.

In order to characterize the effects of TRα1 on cell division, we used an approach of *in toto* crypt immunolabelling and analysed in vivo the division axis of stem and progenitors cells in the colon of wild type or vil-TRα1 mice, by measuring the angle between the spindle poles (the centrosomes) and the apical pole. We confirmed that at anaphase the mitotic spindle aligns with the apical pole in both types of cells in a control situation. In contrast, we observed that upon overexpression of TRα1 this planar orientation is lost. Interestingly, we already described that Aurora kinase A (AURKA) and TACC3, regulators of the cen-

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trosomes, are positively-regulated TH target genes. By immunolabelling, we showed increased centrosomal levels of both in vil-TRα1 compared with WT crypts.

Taken together, our current hypothesis is that overexpression of AURKA and TACC3 in vil-TRα1 mice disturbs the mechanism of centrosomes-driven spindle orientation thus leading to an aberrant division. The misplaced daughter cells within the crypts might escape the control of their physiological microenvironment, triggering colon tumorigenesis.

Our results demonstrate yet another level of the function of thyroid hormones signalling in the intestinal epithelial physiology and pathology. They also highlight a new role of a nuclear receptor in the control of cell division mechanisms.

Keywords: Cell Division, Intestinal epithelium, Thyroid hormone.

SUN-026

How curcumin induces senescence of vascular smooth muscle cells – searching for the mechanism

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Curcumin, a natural polyphenol has documented anti-inflammatory and anti-oxidant properties. A plethora of studies revealed its potential in therapy of many diseases with inflammatory origins – cancer and cardiovascular disease. Curcumin, consumed in a diet, is believed to be safe even in high doses (8–12 mg/day). Plasma concentrations of this compound do not exceed 2 μM. Recently, we showed that curcumin can induce, not only cell death, but also senescence of cancer cells.

The aim of this study was to investigate if curcumin can also induce senescence of normal cells and if so to find a mechanism of this action. Vascular smooth muscle cells (VSMCs) isolated from human aorta were relatively sensitive to curcumin. Already 2.5 μM curcumin inhibited proliferation, 5 μM was cytostatic and concentrations higher than 10 μM induced cell death. In cytostatic concentration of curcumin cells exhibited several features characteristic for senescence: increased activity of senescence-associated-β-galactosidase (SA-β-gal); senescence associated secretory phenotype (SASP) manifested by an elevated level of proinflammatory cytokines – IL-6, IL-8; an increased level of the marker specific for VSMCs senescence – AGTR1 (receptor for angiotensin II). Moreover a significant number of cells was arrested in the G2/M phase of the cell cycle and had a slight increase in cell granularity. We showed that neither prolonged mitotic arrest nor DNA damage were the causes of curcumin induced senescence. What's more, in cells undergoing senescence upon curcumin treatment a lower number of DNA DSB was observed. However the DNA damage response (DDR) pathway was activated. It is believed that curcumin apart from its antioxidative properties can act in the first phase of its action as a prooxidant. Therefore we analyzed if increased reactive oxygen species (ROS) production can lead to curcumin-induced senescence. To this end, we pre-treated cells with ROS scavengers, N-acetylcysteine as well as trolox, before adding curcumin, however none of them was able to prevent or ameliorate curcumin induced senescence.

Our results suggest that VSMCs are very sensitive to curcumin and cytostatic doses can induce senescence but the mechanism has not been elucidated.

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Keywords: Curcumin, senescence, vascular smooth muscle cells.

SUN-027

Influence of TP53 mutations on hematopoiesis in myelodysplastic syndromes (MDS) and acute myeloid leukemias (AML)

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Patients with MDS and complex karyotype have a short median survival of under 12 months and a high risk of transformation into AML. *TP53* mutations are associated with complex karyotype, resistance to chemotherapy, short survival, disease progression and poor outcome. However, the pathogenesis of the *TP53* mutations on the hematopoietic stem cells (HSCs) and their cell fate is poorly understood. Thus, we established a cell culture model and investigated the overexpression of wild-type *TP53* and four different hot spot mutations in human CD34+ cells isolated from cord blood. The CD34+ cells were transduced, sorted and co-cultured with irradiated stromal feeder cells for 6 weeks and half the cells were harvested weekly for examination. As a control we analyzed cells transduced with an empty vector and non-transduced cells. To better understand the influence of different *TP53* mutations on HSCs, we analyzed the colony-forming capacity, rate of apoptosis and proliferation. We also analyzed the *TP53* expression (RT-PCR and Western blot), the *BAX* expression as a marker for the apoptosis pathway triggered by *TP53* and *CDKN1A* expression regulated by *TP53* as a marker for the cell cycle arrest.

Using the colony-forming cell assay, we identified fewer colonies in all transduced cells, especially those overexpressing wild-type, R273H and R175H *TP53* variants. Although the ability to induce erythroid differentiation is affected in all cells during follow-up, cells overexpressing wt*TP53* and cells carrying the R273H mutation already showed impaired erythroid differentiation at an early timepoint.

We detected a higher rate of apoptosis in the cells with overexpression of wt*TP53* and cells expressing the mutations than in the cells carrying the empty vector. In concordance with these data, we identified a *BAX* overexpression in all cells with higher apoptosis. We could also show reduced cell proliferation due to the overexpression of wt*TP53* or *TP53* mutations. An elevated expression of *CDKN1A* confirms these data.

To better understand the influence of *TP53* on the development of complex karyotypes, we also performed cytogenetic banding analyses of all long-term cell cultures during follow-up. We saw an increased number of chromosomal breakages but no development of stable clonal abnormalities.

In summary, overexpression of wt*TP53* and its mutations leads to impaired hematopoiesis with decreased erythroid differentiation, increased apoptosis and decreased proliferation. However, the mutation alone did not lead to the development of complex karyotypes. In conclusion, a *TP53* mutation seems to need additional passenger mutations in order to lead to MDS or AML with complex karyotype.

Keywords: Chromosomal instability, Hematopoietic stem cells, TP53 gene.

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SUN-028
Inhibition of oncoprotein Cdc25 and G2/M transition of the cell cycle by hydrogen sulfide

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The hydrogen sulfide (H_2S), a modulator of the cellular proliferation, is a member of the gazomessenger family, including the nitric oxide and the carbon monoxide. The increase of the level of H_2S inhibits the proliferation either by blocking the cells in G1 on lung fibroblasts (MRC-5, IMR-90 and WI-38) or G2/M for breast cancer cells (MCF-7). The inhibitory effect of H_2S on the proliferation is associated to an increase of cells entering into apoptosis. The apoptotic mechanisms induced by H_2S are widely studied but the action of H_2S on cell cycle remains unexplored. H_2S may influence the progression on the cycle by a direct inhibition of the component of the cell cycle or by an indirect mechanisms depending on the DNA damage induced by the genotoxic effect of H_2S. We used the paradigm of the xenopus oocyte that doesn't show a response to the DNA damage to study the G2/M transition without genotoxic effects. We have shown that in this model, NaHS (H_2S donor) has no effect on apoptosis. Our preliminary experiments allowed us to determine Cdc25 (Cell Division Cycle 25) as a target of H_2S.

The NaHS induces a reduction or a total inhibition of the progesterone induced meiosis resumption associated with an inactive MPF (Cdc2 phosphorylated). Micro-injection of active MPF in a prophase I oocyte triggers Cdc25 activation which in turn activates pre-MPF (inactive stock of MPF) by dephosphorylation and permits oocyte maturation. It's the auto-amplification loop of MPF. The increase of H_2S totally inhibits this maturation suggested that H_2S targeted the mechanisms of auto-amplification loop of MPF. The inhibition of the phosphatase Cdc25 could be the target of H_2S. This hypothesis was tested by measuring the activity of a GST-Cdc25C protein in a cell-free environment in vitro by the colorimetric technique OMFP (O-MethylFluoroPhosphate). In the presence of NaHS, a decrease of the activity of GST-Cdc25C protein was observed in a dose-dependent manner.

Cdc25 presents, in its catalytic domain, an essential cysteine amino acid for its activity. In the presence of SOD (superoxide dismutase) and catalase, which remove ROS (Reactive Oxygen Species), the activity of NaHS on cell cycle is suppressed. Thus, ROS may alter the activity of Cdc25 via an action on the cysteine, as it does for some phosphatases. However, it is not exclude that sulfonation is also involved in this mechanism.

Keywords: Cdc25 phosphatase, Cell cycle, G2/M transition.

SUN-029
Inhibitor-3 ensures bipolar spindle attachment by limiting association of SDS22 to kinetochore-bound PP1

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Faithful chromosome segregation during mitosis is tightly regulated by opposing activities of Aurora B kinase and protein phosphatase-1 (PP1). PP1 function at kinetochores has been linked to SDS22, but the exact localization of SDS22 and how it affects PP1 is controversial. Here, we confirm that SDS22 is required for PP1 activity, but show that SDS22 does not normally localize to kinetochores. Instead, SDS22 is kept in solution by formation of a ternary complex with PP1 and inhibitor-3 (I3). Depletion of I3 does not affect the amount of PP1 at kinetochores but causes quantitative association of SDS22 to PP1 on KNL1 at the kinetochore. Such accumulation of SDS22 at kinetochores interferes with PP1 activity and inhibits Aurora B threonine-232 dephosphorylation, which leads to increased Aurora B activity in metaphase and persistence in anaphase accompanied with segregation defects. We propose a model in which I3 regulates an SDS22-mediated PP1 activation step in solution that precedes SDS22 dissociation and transfer of PP1 to kinetochores, and which is required for PP1 to efficiently antagonize Aurora B.

Keywords: Aurora B, Mitosis, protein phosphatase-1.

SUN-030
Interaction of IFI16 and various DNA substrates

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IFI16 is a member of interferon inducible HIN-200 protein family. These proteins are involved in tissue differentiation, apoptosis, cell proliferation, senescence and they also play role in tumor growth and autoimmunity. They can also interact with various target proteins like signaling proteins, tumor suppressor proteins, oncoproteins and others. IFI16 can interact with BRCA1 and p53 via ...binding regions' and it can stimulate p53 binding to DNA. IFI16 itself can also interact with DNA via DNA binding domains localized in the HIN repeats and it can interact with p53 and cMYC promoters.

We investigated interaction of IFI16 protein with DNA by electromobility shift assay with oligoinucleotides in aPAGE gels and with long DNA targets on agarose gels and confocal microscopy. We used various DNA substrates including different structures frequently located in gene promoters that influence binding of transcription factors like cruciform, quadruplex and triplex. Our preliminary results show that p53 interact the best with cruciform structure.

Keywords: IFI16, cruciform.

SUN-031
Interplay between ROS, DNA damage, p38MAPK and mitochondria is required for premature senescence of endometrial stem cells

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Recently, we have shown that endometrium-derived human mesenchymal stem cells (hMESCs) under sublethal oxidative stress enter the premature senescence but underlying mechanism remains unknown. Herein, we for the first time examine the signaling pathways which provide establishing and maintaining of H_2O_2-induced premature senescence of hMESCs. Understanding of the interplay between various signaling pathways should be highly useful to determinate the strategy for the reversal of cellular senescence. Our results demonstrate that the induction of senescence includes a prompt activation of DNA damage response (DDR) and following signal transduction via p53/p21 and p38/MAPKAPK-2 (MK-2) pathways which are necessary and sufficient to establish the irreversible cell cycle arrest that is typical of senescence. In senescent cells, we observed the prolonged induction of p21, as well as the concurrent activation of p38/MK-2 that may be indispensable to maintain the persistent proliferative block. Moreover, there was a correlation between

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the persistence of DDR signaling and permanently elevated ROS, presuming that the feedback loop between DDR and ROS production is necessary to stabilize the senescent growth arrest. In senescent cells, the specific inhibition of p38/MK-2 activity with SB203580 (SB) had a pronounced negative effect on the intracellular ROS production while the mitochondrial ROS production was diminished just in part. Similarly, mitochondrial mass and mitochondrial membrane potential were partially decreased. In senescent cells preserving the metabolic activity, we revealed the significant increase in the amount of functional mitochondria which might be responsible, at least in part, for long-term ROS production. These findings suggest that activated p38/MK-2 may regulate ROS generation via functional mitochondria. In this study, the inhibition of p38/MK-2 activation, as well as ROS scavenging with antioxidant N-acetylcysteine (NAC) was considered as a possible strategy for senescence prevention. Blocking of p38/MK-2 activity abrogated H_2O_2-induced cell enlargement and flattened morphology, but did not produce any significant effect on SA-β-Gal activity. At the same time, SB treatment allowed to avoid an irreversible cell cycle arrest in response to H_2O_2 however the recovery of proliferation was incomplete. On the other hand, the contemporary cell treatment with H_2O_2 and NAC was sufficient to essentially block the elevated ROS levels, to suppress the H_2O_2-induced senescent phenotype and to recover proliferation. Thus, suppression of p38 kinase activity as well as ROS scavenging with NAC can rescue hMESCs from the growth arrest and entering H_2O_2-induced premature senescence.

Keywords: Oxidative stress, senescence, stem cells.

SUN-032
Interplay between ROS, DNA damage, p38MAPK and mitochondria is required for premature senescence of endometrial stem cells

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Recently, we have shown that endometrium-derived human mesenchymal stem cells (hMESCs) under sublethal oxidative stress enter the premature senescence but underlying mechanism remains unknown. Herein, we for the first time examine the signaling pathways which provide establishing and maintaining of H_2O_2-induced premature senescence of hMESCs. Understanding of the interplay between various signaling pathways should be highly useful to determinate the strategy for the reversal of cellular senescence. Our results demonstrate that the induction of senescence includes a prompt activation of DNA damage response (DDR) and following signal transduction via p53/p21 and p38/MAPKAPK-2 (MK-2) pathways which are necessary and sufficient to establish the irreversible cell cycle arrest that is typical of senescence. In senescent cells, we observed the prolonged induction of p21, as well as the concurrent activation of p38/MK-2 that may be indispensable to maintain the persistent proliferative block. Moreover, there was a correlation between the persistence of DDR signaling and permanently elevated ROS, presuming that the feedback loop between DDR and ROS production is necessary to stabilize the senescent growth arrest. In senescent cells, the specific inhibition of p38/MK-2 activity with SB203580 (SB) had a pronounced negative effect on the intracellular ROS production while the mitochondrial ROS production was diminished just in part. Similarly, mitochondrial mass and mitochondrial membrane potential were partially decreased. In senescent cells preserving the metabolic activity, we revealed the significant increase in the amount of functional mitochondria which might be responsible, at least in part, for long-term ROS production. These findings suggest that activated p38/MK-2 may regulate ROS generation via functional mitochondria. In this study, the inhibition of p38/MK-2 activation, as well as ROS scavenging with antioxidant N-acetylcysteine (NAC) was considered as a possible strategy for senescence prevention. Blocking of p38/MK-2 activity abrogated H_2O_2-induced cell enlargement and flattened morphology, but did not produce any significant effect on SA-β-Gal activity. At the same time, SB treatment allowed to avoid an irreversible cell cycle arrest in response to H_2O_2 however the recovery of proliferation was incomplete. On the other hand, the contemporary cell treatment with H_2O_2 and NAC was sufficient to essentially block the elevated ROS levels, to suppress the H_2O_2-induced senescent phenotype and to recover proliferation. Thus, suppression of p38 kinase activity as well as ROS scavenging with NAC can rescue hMESCs from the growth arrest and entering H_2O_2-induced premature senescence.

Keywords: oxidative stress, senescence, stem cells.

SUN-033
Investigation of EGF A61G gene variation and serum EGF level on gastric cancer susceptibility and clinicopathological parameters

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Gastric cancer is one of the most common malignancies and the second most common cause of cancer-related death worldwide. Carcinogenesis process including gastric cancer, are known to arise through multiple genetic and epigenetic alterations and these molecular changes eventually affect the expression of cancer-associated genes, such as oncogenes and tumor-suppressor genes. Epidermal growth factor (EGF) is an important member of the epidermal growth factor superfamily, which acts several roles in the growth, proliferation and differentiation of numerous types of cells and cancers. In present study we have examined EGF A61G gene polymorphism and EGF serum level as a marker of tumour risk and progression in gastric cancer. We used polymerase chain reaction- restriction fragment length polymorphism (PCR, RFLP) and gel electrophoresis techniques to detect EGF A61G gene variation in 84 gastric cancer patiens and 146 healthy controls. EGF serum levels in patients and control subjects were examined by ELISA method. The distribution of EGF A61G genotypes were different between gastric cancer patients and controls ($p = 0.039$). The frequencies of the AA, AG and GG genotypes were 20.2%, 53.6%, 26.2% in cases and 32.9%, 52.1%, 15.1% in control group respectively. According the our study the variant GG genotype was associated with a near 1.7-fold higher risk of gastric cancer. When the analyses were stratified we didn't observed any significant association between prognostic parameters and EGF genotypes. EGF serum levels in gastric cancer cases were significantly lower than those in controls $p = 0.012$). Our results have suggested that EGF A61G gene variation might be associated with the risk of gastric cancer.

Keywords: gastric cancer, gene,serum level.

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SUN-034
Is there any relation between CDKN2 p16 and MDM2 variants and the development of primary brain tumors?

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Primary brain tumors, classified as glioma, meningioma and intracranial neoplasms, composed of different cell types. With the advent in neuroradiological imaging, the natural course of these tumors are now partially described. However, maybe because of low and different incidances according to ethnic/geographic origin and high mortality rate their etiology and molecular mechanism remains unclear. There is a set of data about the importance of tumor suppresor p53 pathway and cell cycle regulatory genes such as MDM2 and p16 (CDNK2) in brain tumors. p16 plays a pivotal role in G1/S-transition by regulating p53 pathway which was regulated by a nuclear phospho-oncoprotein, mouse double minute 2 (MDM2). Two adjacent polymorphism of p16 gene, 540C>G (rs11515) and 580C>T (rs3088440) were identified. Both polymorphisms were located in the 3′UTR region of exon3 and shown to affect the activation of p16 and contribute to cancer development, prognosis and the tumor aggressiveness. A single nucleotide polymorphism in the promoter of MDM2, SNP309T>G (rs2279744) has shown to alter protein expression and p53 activity which regards possible roles in carcinogenesis. The aim of this study is to investigate the association of p16 540C>G, p16 580C>T and MDM2 SNP309T>G polymorphisms with the risk of brain tumor development. 67 primary brain tumor patients and 71 control were included in this study. The gDNA was isolated by salting-out procedure. p16 and MDM2 polymorphisms was analyzed by PCR-RFLP based techniques. The allelic or genotypic frequencies of MDM2 SNP309T>G and p16 580C>T were balanced among the study groups. The frequency of p16 540GG genotype was lower in primary brain tumor patients and 540G allele frequency (GG+CG) was higher in controls. Thus it was found that possesing p16 540CC genotype ~2fold increases the risk of primary brain tumor development. In contrast, possesing p16 540G allele and GG genotype ~2.3fold and ~7fold increases the prevention from meningioma and glioma, respectively. Although this report was the first one to determine the relation between p16 540C>G, p16 580C>T or MDM2 SNP309T>G polymorphisms and primary brain tumors: glioma and meningioma, it was found that CDKN2 p16 540C>G may more risky for the development of brain tumors. Further studies with large cohorts are necessary to determine the genetic factors affectting the development and prognosis of brain tumors and to promote the effective therapy strategies.

Keywords: Brain tumors, MDM2, p16.

SUN-035
Methionine sulfoxide reductase B3 deficiency inhibits cell growth through the activation of p53-p21 and p27 pathways

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Methionine sulfoxide reductase B3 (MsrB3) is an oxidoreductase in the endoplasmic reticulum that catalyzes the stereospecific reduction of methionine-R-sulfoxide to methionine. Here, we report the critical role and mechanisms of MsrB3 in cell proliferation. The deletion of MsrB3 led to a significant decrease in cell proliferation in mouse embryonic fibroblast (MEF) cells. MsrB3-knockout MEF cells showed increased p53 protein levels, compared to wild-type MEF cells, which subsequently elevated the protein level of cyclin-dependent kinase inhibitor p21. In addition, MsrB3 deficiency enhanced the protein level of p27, another cell cycle regulator, and caused cell cycle arrest at the G1 stage. The inhibitory effect of MsrB3 deficiency on cell proliferation through the activation of p53-p21 and p27 pathways was also confirmed in primary human dermal fibroblasts. Collectively, the data suggest that MsrB3 is a regulator of cell growth through the p53-p21 and p27 pathways.

Keywords: Cell cycle, Endoplasmic reticulum stress, methionine sulfoxide.

SUN-036
Nek7 is possibly involved in UV damage response

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The Neks are a family of vertebrate protein kinases similar to fungal NIMA, which activity is essential for the G2/M checkpoint progression and mitosis entry. The NIMA protein has been implicated in chromatin condensation, microtubule dynamics and cell division regulation. In mammals, there are 11 Neks, of which only Nek 2, 6, 7 and 9 have been directly implicated in mitotic regulation. Futhermore, the importance of the Nek1, 4, 10 and 11 in the DNA damage response, checkpoint control, mitosis and progression and regulation of the cell cycle, has been underscored in several experimental systems. Specificy, Nek7 regulates mechanisms of centriole duplication and microtubule nucleation from centrosomes. Studies show the involvement of Nek7 is over-expressed in breast, colorectal, laryngeal and lung cancer and non-Hodgin lymphoma. Thus, the employment of functional and cellular studies aiming to unravel the Nek7 signaling pathway in regulating cell division, is necessary to clarify the function of Nek7 in carcinogenesis. Based on all these data, HeLa and Hek293 cells were transfected or not with Flag-Nek7 for protein over-expression or silenced using shRNA specific for Nek7, and exposed or not to UV light. Treated cells were submitted to apoptosis assays based on flow cytometry. Our results suggest that Nek7 plays a role in protecting cells of such damage, since the cultures over-expressing Nek7, showed a decrease in the number of cells undergoing apoptosis, after exposure to UV light. On the other hand, in cells in which Nek7 was silenced, an increase of apoptotic cells was verified. Thus, our experiments suggest a role of Nek7 in the maintenance of apoptosis mechanisms. Confirmative assays are underway to explore this potential new function in molecular detail.

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Keywords: Apoptosis, Cell cycle, Proteins Kinase.

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SUN-037
New benzothiophene-3-carboxamide based Aurora kinase inhibitors

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The serine-threonine Aurora kinases A and B were proven to play an important role in regulating normal cell division. Aurora A regulates centrosome maturation, division and required for the spindle checkpoint. Aurora B plays role in the chromosome condensation, segregation and crucial for cytokinesis. It is well known, that deregulation of Aurora kinases fosters malignant transformation, while their inhibition promotes apoptosis in experimental models. Indeed, Aurora gene amplification and/or protein overexpression is commonly found in human tumors and correlates with poor prognosis; therefore Aurora kinases emerged as potential targets in personalised cancer therapy.

In the last decade several small molecule inhibitors of Aurora A or B were published, however most of them failed in clinical experiments and there is still no Aurora inhibitor drug in the market. The aim of our work was to design and synthesise new ATP analogue inhibitors endowed with Aurora kinase inhibition properties.

Screening the Nested Chemical Library© of Vichem Ltd.'s using recombinant Aurora A kinase assay we identified a benzothiophene-3-carboxamide based compound with nanomolar inhibition potency on Aurora kinases. Further 35 analogues were synthesised, showing even better enzyme inhibition, however only a subset exhibited cell viability inhibition as well. These later compounds generated multinucleated cells stucked in G2/M and induced apoptosis according to flow cytometry, equipotent to VX-680 pan-Aurora inhibitor. Western blot analysis confirmed that these new molecules indeed diminish Aurora A and B autophosphorylation and histone H3 phosphorylation (a well known aurora B substrate).

In all, we present a family of new, patentable benzothiophene-3-carboxamide derivatives as potent, pan-Aurora kinase inhibitiors.

Keywords: Aurora, cell signaling, kinase inhibitor.

SUN-038
Nobiletin, a polymethoxy flavone, suppresses glioma cell growth through the inhibition of cell cycle

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Nobiletin is a polymethoxy flavone extracted from citrus. Although nobiletin has a potent antitumor activity against several types of human cancers, its inhibitory effects on glioma cells remains incompletely understood. In this study, we systemically examine the inhibitory effects of nobiletin on U87 glioma cells. Our data revealed that nobiletin (20–100 μM) could inhibit glioma cell proliferation detected by MTT. Nobiletin arrested cell cycle at the G0/G1 phase detected by propidium iodide (PI) staining. Using the PI-Annexin V staining, we found that nobiletin did not cause glioma cell apoptosis. These observations suggest that nobiletin inhibit glioma cell growth possibly via regulating cell cycle, but not apoptosis. In the western blot experiments, our results showed that nobiletin significantly reduced the expression of cyclin D1, CDK2, CDK4, and E2F1. In addition, nobiletin inhibited the phosphorylation of Akt and mitogen-activated protein kinases (MAPKs), including Erk, JNK, and p38. In conclusion, our findings suggest that nobiletin may suppress glioma cell proliferation by inhibiting cell cycle and MAPK signaling pathway. Thus, nobiletin may represent a high therapeutic potential for treatment or prevention of glioblastoma multiforme.

Keywords: Cell cycle, glioma cells, nobiletin.

SUN-039
Nucleolar GTP binding protein (NGP) promotes cell cycle progression through activation of p53-p21 pathway

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Nucleolar GTP binding protein (NGP) is a putative nucleolar GTPase belonging to the HSR1_MMR1 family of GTPases. Other proteins of the same family such as Nucleostemin are known to regulate cell proliferation in stem cells as well as various cancer cells. NGP has been shown to be overexpressed in breast cancers and colon cancers. Nevertheless, the function of this protein remains unknown. In this study, we show that NGP modulates cell proliferation in MCF-7 cells. Ectopic expression of NGP resulted in increased G2/M phase of cell cycle, whereas knockdown of NGP caused G1/S arrest. Surprisingly, in both instances the tumor suppressor p53 was upregulated. The NGP induced p53 was found to be functional in a luciferase based reporter assay system. Furthermore, our data suggests that NGP overexpression results in upregulation of p53 target genes such as p21 and MDM2. It is interesting to note that overexpression of NGP results in cell cycle progression despite upregulating canonical cell cycle inhibitory proteins, p53 and p21. p21 is a CIP/KIP inhibitor through which p53 mediates its tumor suppressor function. p21 binds and inactivates the G1-cyclin–CDK kinase complexes. However, increasing evidence from literature show the existence of a stoichiometric model of p21 function, where p21 acts as a positive regulator of cell cycle at lower than inhibitory concentration in cells by aiding the assembly of cyclinD-CDK 4/6 complexes. Our data suggests that NGP upregulates the cyclinD-CDK 4/6 kinase activity resulting in elevated levels of phosphorylated Rb (S780) subsequently activating E2F target genes such as cyclin A, cyclin E, E2F etc., thereby promoting cell cycle progression. Additionally, knockdown of NGP upregulates p53 resulting in cell cycle arrest further strengthening the hypothesis that NGP regulates the p53 mediated p21 stoichiometry, which determines the fate of the cell.

Keywords: Cell cycle, NGP, p53.

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SUN-040
Phosphorylation of barrier-to-autointegration factor by the vaccinia related kinase 3 and its role in cell cycle

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Barrier-to-autointegration factor (BAF) is an essential nuclear envelope protein which link the nuclear envelope and with the chromatin. Phosphorylation and dephosphorylation of BAF are required for disassembly and reassembly of the nuclear envelope during mitosis. Mitotic phosphorylation of BAF by the vaccinia related kinase 1 (VRK1) has been reported. However, BAF is also phosphorylated on Ser4 during interphase and the kinase mediating this role remains elusive. Here we demonstrate that the vaccinia related kinase 3 (VRK3) also phosphorylates BAF on Ser4. The expression of VRK3 is increased during interphase whereas VRK1 is enriched in late G2 phase. Ectopic expression of VRK3 induces the relocalization of BAF from the nucleus to the cytoplasm. In addition, depletion of VRK3 decreases the population of proliferating cells. These data suggest that VRK3-mediated phosphorylation of BAF may facilitate DNA replication or gene expression by weakening the association between nuclear envelope proteins and chromatin during interphase.

Keywords: None.

SUN-041
Proliferation control of tetraploid cells

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Most eukaryotic cells are diploid: they contain two sets of chromosomes. However, exceptions can be found. Tetraploid cells that contain four haploid sets of chromosomes can arise as a final differentiation stage in specialized tissues. In contrast, erroneous tetraploidy may lead to aneuploidy and chromosomal instability (CIN), which are frequently associated with cancer. Tetraploid cells usually undergo a p53-dependent cycle arrest after the first tetraploid mitosis, but the triggers of this arrest are not understood. Using high-throughput RNAi-based screen, we identified 140 candidate genes that restrict proliferation of tetraploid cells after abnormal mitosis. Many of these candidates are components of signaling pathways or are involved in cancer. We are currently validating the candidates and determining the mechanisms of their effects on tetraploid cell proliferation.

Remarkably, around 1% of tetraploid cells escapes the arrest and proliferates further. These post-tetraploid cell lines share some features frequently observed in cancer: they have a near-triploid karyotype and show high CIN. We found that post-tetraploid cells are more tolerant to mitotic defects than progenitor diploid or the newly formed tetraploid cells. Remarkably, we found that the regulation of the p53 pathway is altered in post-tetraploids. In particular, the nuclear accumulation of p53 after abnormal mitosis is diminished in post-tetraploids compared to diploids. The identified changes may be at least partially responsible for the tolerance. The mechanisms of cellular tolerance to mitotic defects and the contribution of the candidate genes identified in our screen will be discussed. Taken together, our results show that the deregulation of the p53-dependent cell cycle arrest contributes to survival and CIN in tetraploid progeny. In addition, our genome-wide screen data suggest that alternative mechanism responsible for tetraploid cell cycle arrest might exist.

Keywords: Cell cycle arrest, tetraploidy.

SUN-042
Protein tyrosine phosphatase PTPN13 and its interaction partner SDCCAG3 are involved in the regulation of cytokinesis

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Cell division is a vital step in cytokinesis, failure of which is believed to contribute to the development of cancer. PTPN13, a protein tyrosine phosphatase, is previously implicated in the regulation of cytokinesis, carcinogenesis and tumor aggressiveness. Besides it N-terminal phosphatase domain, PTPN13 also contains an N-terminal KIND domain, followed by a FERM domain and five PDZ domains. Here we demonstrate that the serologically defined colon cancer antigen-3 (SDCCAG3) is a new interaction partner to the FERM domain of PTPN13.

We show that SDCCAG3 is a novel endosomal protein, primarily localized at the early/recycling endosomal compartment. It undergoes dynamic localization during cell division with strong accumulation at the midbody during cytokinesis. Altered SDCCAG3 expression, either overexpression as well or downregulation, increases the number of multinucleated cells. Furthermore, we identify interaction of SDCCAG3 with the ArfGAP protein GIT1. Overexpression of an ArfGAP negative version of GIT1 or downregulation of GIT1 have a similar phenotype as the SDCCAG3, leading to the formation of multinucleated cells. Thus, we suggest that PTPN13, SDCCAG3 and GIT1, possibly through the formation of a complex, play a regulatory role in cytokinesis.

Keywords: cytokinesis, endosomal protein, midbody.

SUN-043
Replication aberrations linked to genome plasticity: modeling DNA re-replication across a complete genome

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DNA replication in eukaryotes initiates from hundreds of sites along chromosomes, called origins of replication, ensuring complete and accurate duplication of the genome during S-phase. DNA replication along the genome is complex and uncertain: a small fraction of putative origins is selected to fire in each cell in a population and the progression of DNA replication along the genome is unique in every cell. Aberrations in the control mechanisms ensuring once per cell cycle replication allow re-firing of origins within the same S-phase, leading to genome overreplication, which can result in genomic instability, closely connected to tumorigenesis and cancer.

To capture how re-replication progresses along the genome, we have developed a stochastic hybrid model of DNA re-replication which permits genome-wide analysis of re-replication kinetics. The model accurately portrays the interplay between discrete dynamics (origin states), continuous dynamics (movement of the replication forks) and stochastic events (uncertainty in firing events) and permits insight into unknown mechanisms underlying DNA re-replication at the single cell level. We have used experimental data on origin locations and efficiencies from fission yeast to simulate re-replication along the complete fission yeast genome. Monte-Carlo simulations permit analysis of re-replication kinetics genome-wide. Comparison of simulated to experimental

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population-level data on copy-number variations shows a good correlation along the genome, validating our approach. Sensitivity analysis and implementation of different model variations have permitted insight into the key parameters affecting re-replication dynamics. Analysis of simulated data allows us to visualize how different regions along the genome respond to over-replication at the single cell level. Our analysis reveals great variations in copy number along the genome from cell to cell, and a unique pattern of over-replication events in each cell in a population. Sites of increased copy-number along the genome are affected by intrinsic properties of each locus, cis effects from adjoining loci and trans effects from distant loci. Single-cell visualization of copy-number variations in multiple loci along the genome in live fission yeast cells have been used to validate model predictions.

Our analysis shows that cell-to-cell heterogeneity is inherent in re-replication and can lead to a high degree of genome plasticity. It also permits us to define principles governing rereplication genome-wide.

Keywords: Cell cycle, modeling, Replication.

SUN-044
Stimulation of GDNF production by tricyclic compounds in retinal cells

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Retinal degeneration and vision lost is one of the important problems of medicine. Probably one of best way of resolving this problem is a range of prophylactic measurements against retinal cell degeneration process. Glial derived neurotrophic factor (GDNF) is a member of the transforming growth factor-beta family. It is secreted not only in various classes of neuronal and glial but in other types of cells. GDNF shows antiapoptotic properties, reduces damage from oxidative stress, increases cell viability and takes part in providing cell proliferation in retina. Application of various compounds is responsible for direct and indirect impact and protection of retinal cells by stimulating GDNF secreting.

We tested 2 tricyclic (Amitriptyline, Cyclobenzaprine) and 2 tetracyclic compounds (Amoxapine and Loxapine succinate) for stimulation of GDNF production in neuronal and glial cells (ARPE-19 and SVG respectively) and human retinal progenitor cells (hRPC). GDNF content was evaluated by ELISA, cell growth rate by CyQuant and compounds toxicity by MTT. GDNF intracellular level measured in cell lysate, and GDNF secretion was evaluated by GDNF content in supernatant.

We observed significant increasing of GDNF level inside of ARPE-19 after 10–25 μM Amitriptyline, SVG after 0.5–50 μM Amitriptyline, 0.5–50 μM Cyclobenzaprine and 1.0–50 μM Amoxapine. hRPC was non-sensitive to 3 abovementioned compounds, but 100 μM Loxapine succinate increased GDNF intracellular level. Studying of GDNF secretion showed what 0.5–5 and 25–50 μM Amitriptyline stimulated GDNF secretion in ARPE-19, 0.5–50 μM Cyclobenzarpine, 1–100 μM Amoxapine and 10–100 μM Loxapine succinate in SVG (Amitriptyline hasn't been tested for SVG), 1–100 μM Amoxapine and 10, 50–100 μM Loxapine succinate in hRPC. Thus glial cells are more sensitive to tri- and tetracyclic antidepressants, antipsychotics and muscular relaxants. Interestingly, increasing of GDNF intracellular level and its secretion was registered even at toxic and subtoxic concentrations of applied compounds (10–50 μM dependently of compound and cell line). It could be a reason of prediction what at toxic concentrations of abovementioned compounds GDNF didn't self-bound by cellular specific receptors. Stimulation of cell growth after application of Amitriptyline to hRPC without increasing of GDNF secretion and increasing of GDNF secretion level in ARPE-19 without stimulation of cell growth could be indirect evidence of GDNF involvement of autocrine loop and this loop disruption at high concentrations of the compounds.

Keywords: cell growth, GDNF, tricyclic compounds.

SUN-045
Structural and functional investigation of the role of the transmembrane domain in the VEGF receptor 2 activation

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Vascular Endothelial Growth Factor receptors, VEGFRs, regulate blood and lymphatic vessel development and homeostasis and belong to receptor tyrosine kinase (RTK) family of integral membrane proteins. Transmembrane signaling by RTKs entails ligand-mediated dimerization and structural rearrangement of the extracellular domain. RTK activation also depends on specific orientation of the transmembrane domain (TMD) helices, as suggested by pathogenic, constitutively active RTK mutants. Such mutants carry polar amino acids in the TMD, promoting stable transmembrane helix dimerization, which is essential for kinase activation. Here we investigated the spatial structure of the TMDs and the activity of VEGFR-2 constructs, where we mutated the wild-type sequence by replacing specific amino acids in the TMD with glutamate residues. Our data revealed that single-point mutations V769E, I767E and L768E are capable to activate the VEGFR-2 receptor with deleted extracellular domain, but not the full-size receptor. On the other hand, double mutations G770E/F777E and T771E/F778E triggered the ligand-independent activation of the full-size VEGFR-2.

With this respect we then investigated the spatial structure of the dimers and the dimerization propensities for three transmembrane peptides, corresponding to the wilt-type TMD of VEGFR-2 and to V769E and G770E/F777E mutant TMDs with solution NMR spectroscopy. As a result we found that three major aspects distinguish the G770E/F777E TMD dimer from the wild-type: (1) dimerization propensity is enhanced; (2) dimerization occurs via a completely different interface; (3) the spatial structure of this dimer is flexible. On the other hand, we found that V769E substitution in the VEGFR-2 TMD does neither substantially enhance its propensity for dimerization nor change the preferred dimeric conformation. This mutation creates additional surface for helix-helix interactions, which is manifested in the observed trimer formation by the mutant TMD. Further molecular dynamics simulations confirmed the structural interpretation of the NMR data.

The obtained structural and functional data on the VEGFR-2 TMD, both wild-type and carrying various mutations, were then summarized to propose the new mechanism for the VEGFR-2 activation, involving the ligand-induced reorientation of TMDs in the pre-formed VEGFR-2 dimers.

'**Keywords:** activation mechanism, membrane domain, VEGFR.

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SUN-046
Studies of acrylodan labeled cAMP-dependent protein kinase A catalytic subunit (PKAc-acr) conformational changes

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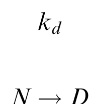
PKAc-acr fluorescence spectrum is sensitive to the environment that surrounds the fluorescent label, and this property can be used to study conformational changes of the protein. Significant change in fluorescence can be monitored if native (N) PKAc-acr goes through to denatured state (D) (Figure 1).

The process of protein denaturation can be described by a simple reaction scheme, where k_d denotes kinetic constant:



$$N \xrightarrow{k_d} D$$

Since ligands bound to PKAc affect rate of this reaction, it was possible to study ligand binding by measuring the rate constant k_d. Using this method, binding of ATP and inhibitory peptide PKI [5–24] were studied and the allosteric effect of their simultaneous binding was analyzed. Kinetic measurements were performed at different temperatures and enthalpic and entropic components of the allosteric effect were calculated.

Keywords: fluorescence based assay, High throughput screen, Ligand binding, allostery.

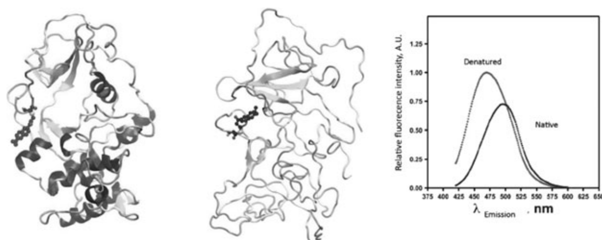


Fig. 1. Computer models of PKAc-acr in native (left panel) and denatured (middle panel) conformations. Fluorecencent label acrylodan was noted as ball-and-stick model. It can be seen from these structures that environment around the acrylodan dye is different in native and denatured states. This change was also detected from changes in fluorescence spetra (right panel). Computer models of PKAc-acr were prepared by A. Kuznetsov.

SUN-047
Suppression of tumorigenesis in mitochondrial NADP+-dependent isocitrate dehydrogenase knock-out mice

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The tumor host microenvironment is increasingly viewed as an important contributor to tumor growth and suppression. Cellular oxidative stress resulting from high levels of reactive oxygen species (ROS) contributes to various processes involved in the development and progress of malignant tumors including carcinogenesis, aberrant growth, metastasis, and angiogenesis. In this regard, the stroma induces oxidative stress in adjacent tumor cells, and this in turn causes several changes in tumor cells including modulation of the redox status, inhibition of cell proliferation, and induction of apoptotic or necrotic cell death. Because the levels of ROS are determined by a balance between ROS generation and ROS detoxification, disruption of this system will result in increased or decreased ROS level. Recently, we demonstrated that the control of mitochondrial redox balance and cellular defense against oxidative damage is one of the primary functions of mitochondrial $NADP^+$-dependent isocitrate dehydrogenase (IDH2) that supplies NADPH for antioxidant systems. To explore the interactions between tumor cells and the host, we evaluated tumorigenesis between IDH2-deficient (knock-out) and wild-type mice in which B16F10 melanoma cells had been implanted. Suppression of B16F10 cell tumorigenesis was reproducibly observed in the IDH2-deficient mice along with significant elevation of oxidative stress in both the tumor and the stroma. In addition, the expression of angiogenesis markers was significantly down-regulated in both the tumor and the stroma of the IDH2-deficient mice. These results support the hypothesis that redox status-associated changes in the host environment of tumor-bearing mice may contribute to cancer progression.

Keywords: apoptosis, redox status, tumorigenesis.

SUN-048
The Cep192-organized Aurora A-Plk1 cascade is essential for centrosome cycle and bipolar spindle assembly

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Centrosomes are non-membrane-bound organelles of animal cells that function as the major microtubule (MT)-organizing centers (MTOCs) and participate in spindle assembly, cell division, polarity, and motility. Centrosomal abnormalities, both numerical and functional, have been linked to cancer and other diseases.

Centrosomes consist of one or two (depending on the cell cycle stage) centrioles surrounded by pericentriolar material (PCM). Prior to mitosis onset, the two centrosomes separate and grow dramatically, each forming a nascent spindle pole that nucleates a radial array of MTs. Centrosome growth (and the associated microtubule nucleation surge), termed maturation, involves the recruitment, via a hitherto unknown mechanism, of additional PCM components. Among them, the most prominent is the γ-tubulin ring complex (γ-TuRC), which serves as a MT template.

In *Xenopus* egg extracts, we found that the key regulator of centrosome biogenesis, Cep192, via its N-terminal domain, binds the two major mitotic serine/threonine kinases, Aurora A (AurA) and Plk1, and organizes them in a multistep signaling cascade that underlies centrosome maturation and culminates in MT assembly. Specifically, following their pericentrin-mediated recruitment to centrosomes, Cep192 complexes undergo local oligomerization/clustering, which promotes AurA activation via T-loop autophosphorylation. AurA then phosphorylates Plk1 in its T-loop, turning it on and facilitating its docking onto a conserved threonine in the N-terminus of Cep192. The active, Cep192-bound Plk1, in turn, phosphorylates Cep192 at several serine residues to generate the attachment sites for γ-TuRC and, possibly, other PCM components, thus promoting their recruitment and subsequent MT nucleation. Cep192 mutants lacking the binding sites for AurA, Plk1, or γ-TuRC failed to promote centrosome maturation and

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MT assembly in Cep192-depleted cycling egg extracts. Our Cep192 siRNA knockdown/rescue experiments in mammalian cells corroborated these results and demonstrated that the Cep192-organized AurA-Plk1 cascade is conserved in vertebrates and is essential for: i) centrosome maturation, ii) kinesin 5-mediated centrosome separation, iii) bipolar spindle assembly, and iv) equal centrosome/centriole segregation into daughter cells.

Our study unveils a Cep192-organized signaling cascade that underlies centrosome maturation and that is integral to the centrosome cycle and to bipolar spindle assembly in vertebrates. It also offers a framework for further dissection of the mechanisms of mitotic MTOC formation in a cell-free system.

Keywords: Cell Division, Centrosome cycle, Kinases.

SUN-049
The evaluation of cell viability and anticancer properties of calcium ions transported by electroporation into human breast adenocarcinoma cell lines: MCF-7/WT (wild type) and MCF-7/DX (resistant type)

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Calcium is an important factor that can play different functions in human organism, both systemically and within a single cell. It is both a primary and a secondary messenger which participates in many processes.

The main objective of the presented work was evaluation of cell viability and the investigation of anticancer activity of calcium introduced to malignant cells with electroporation into breast adenocarcinoma cell lines: wild type and doxorubicin-resistant type.

Two human adenocarcinoma cell lines were used: MCF-7/WT and MCF-7/DX. To electroporation following parameters were selected: 800, 1000, 1200 and 1400V/cm; by 8 pulses of 100 μs, pulse duration 1 Hz. The following calcium concentrations were applied: 0.25, 0.5, 1 and 5 mM. Due to viability analysis MTT assay. CLSM method was used to evaluate the effect of calcium and EP on cytoskeleton proteins: α-actin and β-tubulin.

The cytoskeleton studied indicated that cellular morphology after EP exposition only remained unchanged. The changes in the cytoskeleton and the nucleus were observed only when cells are treated with calcium solution and electroporated (weaker fluorescence signal actin and tubulin).

The best anticancer effect was observed after the application of EP + Ca^{2+}. The cell survival rate was lower in comparison with the method using only one. EP with calcium stimulated antitumor responses: after 24 h even 80% cells were eliminated (both cell lines). After 48 h the number of viable cells decreased significantly; in resistant-type cells survival reached 10%. The satisfactory results were obtained at higher voltages-1200 and 1400 V/cm (independent of the concentration of calcium). At lower voltages marked decline in survival occurred with 5 mM solution of calcium. Lower survival rate was observed after 48 h, especially at high voltages.

The application of EP does not cause changes in the cytoskeleton; even high intensity electric field (1400 V/cm) does not affect destructive to the cells. Only after the addition of calcium observed microtubules system disorders, and thus a weaker fluorescence signal.

EP or Ca^{2+} application alone did not cause such a strong antitumor effect. EP with a voltage 1400 V/cm induced changes in cellular viability especially after 48 h. MCF-7/WT cell lines were more sensitive to EP. EP assisted by calcium solution induced decrease of the amount of surviving cells even with 800 V/cm. In these conditions more sensitive occurred the line MCF-7/DX.

The obtained results present a preclinical study of electroporation application to introduce calcium as an efficient anticancer drug in cancer cells.

Keywords: calcium, cell cytoskeleton, electroporation.

SUN-050
The HERCulean E3 ligase HERC2 acts as a fate switch controlling the RB/E2F signaling network

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The retinoblastoma tumor suppressor protein (RB) plays an integral role as a regulatory node controlling proliferation, quiescence and differentiation, the G1-S checkpoint control and consequently is a frequent target for inactivation in cancer. Post-translational modifications (PTM) play a critical role in modulating RB activity. Although multisite phosphorylation of RB by CDKs has been extensively studied, activities regulated by other PTMs remain largely enigmatic. Understanding the molecular mechanisms and effects of RB modulation by PTMs is therefore imperative.Here we identified HERC2, a giant (0.5 MDa) and highly-conserved member of the eukaryotic HECT E3 ligase family, acting as a fate switch by regulating the RB/E2F signaling network. We found that HERC2 associates with RB via it's RCC domain and modulates levels of RB in a cell cycle and phosphorylation dependent manner. Mechanistically, in quiescent (G0) cells the retinoblastoma tumor suppressor RB becomes a target of hypophosphorylated HERC2, which consequently leads to binding and subsequent proteasome dependent degradation of RB: low HERC2 activity results in accumulation of both RB and hyperphosphorylated RB (pRB) and therefore renders cells unable to exit the cell cycle and properly transition into quiescence. By contrast, high levels of HERC2 correlate with loss of RB/pRB. Furthermore, gene expression profiling and global proteome analysis reveal that loss of HERC2 concomitantly leads to a fundamental change in the transcriptional program downstream of RB/E2F with detrimental consequences for critical cell cycle licensing factors. Thus our findings suggest that HERC2 activity plays a pivotal role in regulating the balance of RB and therefore E2F mediated transcriptional control, cell cycle progression and cellular quiescence.

Keywords: Cell cycle, transcriptional and post-translational regulation, Tumor suppressor.

SUN-051
The impact of CAFFEINE mediated Cdc25 STABILIZATION on cell cycle kinetics in *Schizosaccharomyces pombe*

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Caffeine has generated much interest, by virtue of its ability to override the replication and DNA damage checkpoints in both *Schizosaccharomyces pombe* (*S. pombe*) and mammalian cells. In *S. pombe*, cell progression and mitosis are regulated by Cdc2.

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Mik1 and Wee1 negatively regulate Cdc2 by phosphorylating Tyr15. Conversely, Cdc2 is positively regulated by Cdc25 which dephosphorylates Tyr15. Proper activation and enforcement of checkpoints thus requires inhibition of Cdc25 and activation of Mik1 and or Wee1. Following its activation by stalled replication or DNA damage, Rad3 activates the downstream kinases Cds1 and Chk1. These kinases in turn, inhibit Cdc25 by phosphorylation and simultaneously activate Mik1 and Wee1. Previous studies suggested that caffeine ov errides checkpoints by inhibiting Rad3, the major regulator of the replication and DNA damage checkpoints in *S. pombe*. We recently demonstrated however, that caffeine induces the stabilization of Cdc25 contributing to the checkpoint override. Paradoxically, Caffeine also activates Sty1 leading to the phosphorylation and partial inhibition of Cdc25. To better understand how caffeine- induced Cdc25 stabilization contributes to checkpoint override, we compared its effect on the wt isoform of the phosphatase and a 12A mutant lacking all 12 inhibitory phosphorylation sites. We have also investigated the effect of caffeine on Mik1 and Wee1 stability. Additionally, we investigated the effects of Mik1 and Wee1 on the ability of caffeine to modulate Cdc25 activity during the normal cell cycle and following activation of the replication or DNA damage checkpoints.

Keywords: Caffeine, Cdc25 phosphatase, cell cycle.

SUN-052
The impact of tubulin heterogeneity on the biophysical properties of the microtubule cytoskeleton

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Microtubules are subject to extensive heterogeneity due to expression of multiple tubulingenes and to posttranslational modifications. Our present understanding of how the diversity ofthe tubulin molecules affects microtubule functions is very limited. The bottleneck is the absence of experimental system that would allow direct measurements of the impact of tubulin heterogeneity on microtubule properties and functions. Thus, it is urgent to develop a system where recombinant tubulin can be generated and be used for functional and biophysical studies. Here we develop fission yeast as amodel system to express recombinant tubulin. Using this system, we will first focus onthe roles of tubulin C-terminal tails (CTT), their heterogeneity and posttranslational modifications. To get insights into the microtubule functions that are sensitive to tubulin heterogeneity, we will replace the yeast CTTs with different mammalian counterparts by expressing tubulin chimera, and study the microtubule phenotypes by live microscopy. We will also study the behavior of a set of microtubule interacting proteins in these cells.CTTs are major sites of posttranslational modification in higher eukaryotes and thus CTTs are critical regulator for microtubule function.We would like to explore the specific function of different regions CTTs in vivo using a systematic collection of point mutated tubulin in conjunction with tubulin-modifying enzymes in yeast cells, to (i) study the specificity of the enzyme, and (ii) investigate the potential roles of these enzymes in the regulation of microtubule functions.In parallel, we will optimize the yeast expression system to purify recombinant chimeric tubulin for *in-vitro* biophysical experiments.

Keywords: Fission Yeast, Recombinant Tubulin, Tubulin posttranslational modification.

SUN-053
The interaction between ELK-1 and mitotic kinases

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Elk-1 is a member of the ETS domain superfamily of transcription factors and involved in many biological processes, such as cell growth, differentiation, apoptosis, survival, angiogenesis and cancer. Activation of the Elk-1 requires phosphorylation, in particular on Serine 383 and Serine 389 residues within the activation domain, by ERK, JNK or p38 pathways and this activation was shown to induce its binding to DNA. However, when the Elk- 1 protein sequence was bioinformatically analyzed, a number of other potential phosphorylation motifs were identified, including consensus motifs for mitotic kinases Aurora and Plk. Mitosis is tightly regulated to prevent improper segregation of sister chromatids as any errors in this process leads to chromosomal instability, aneuploidy or even tumorigenesis. Mitotic kinases have pivotal roles during regulation of this mechanism as well as cell cycle progression, centrosome maturation and microtubule dynamics, which is pivotal for the formation of bipolar mitotic spindle and accurate segregation of chromosomes. It has been previously demonstrated in our laboratory that Elk-1 interacts with neuronal microtubules as well as motor proteins, dynein and kinesin, in a serum dependent manner. Furthermore, Elk-1 was shown to colocalize and interact with Aurora kinase. Such an interaction of Elk-1 with mitotic apparatus brings to mind a possible role during mitosis. In this study, we have shown that Elk-1 phosphorylated in some of these potential motifs was also colocalized with the mitotic spindle, using phospho-specific antibodies. We will discuss our findings on the interaction of Elk-1 with Plk and Cdk kinases.

Keywords: Elk-1, Kinases, Mitosis.

SUN-054
The p97-Ufd1-Npl4 ATPase complex ensures robustness of the G2/M checkpoint by facilitating CDC25A degradation

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The p97-Ufd1-Npl4 ATPase complex is associated with the response to DNA damage and replication stress, but how its inactivation leads to manifestation of chromosome instability is unclear. Here, we show that p97-Ufd1-Npl4 has an additional direct role in the G2/M checkpoint. Upon DNA damage, p97-Ufd1-Npl4 binds CDC25A downstream of ubiquitination by the SCF-βTrCP ligase and facilitates its proteasomal degradation. Depletion of Ufd1-Npl4 leads to G2/M checkpoint failure due to persistent CDC25 activity and propagation of DNA damage into mitosis with deleterious effects on chromosome segregation. Thus, p97-Ufd1-Npl4 is an integral part of G2/M checkpoint signaling and thereby suppresses chromosome instability.

Keywords: checkpoint, DNA damage, ubiquitin.

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SUN-055
The role of central carbon metabolism in the regulation of human DNA replication

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DNA replication is one of fundamental and important processes occurring inevery living cellular organism. The process of DNA synthesis has been thesubject of many works and is relatively well studied. However, the controlmechanisms of the regulation of this process remain poorly understood.Perturbations in the regulation of DNA replication lead to theaccumulation of harmful mutations, often leading to severe diseases, including cancer. Therefore, it seems important to investigate themechanisms that regulate this fundamental biological process. Experimentsperformed with bacterial models indicated that there is a direct linkbetween central carbon metabolism and regulation of DNA replication.Impairments in particular genes coding for enzymes involved in centralcarbon metabolism (CCM) influenced DNA replication.The aim of this study was to understand the global regulatory processesthat control DNA replication in eukaryotic organisms, and to answer thequestion whether such mechanisms are universal. Since glycolyticmetabolism of glucose is a major pathway for the generation of energy, andits intermediates serve as precursors for biosynthetic processes of thecell, our work was focused primarily on connection between glycolysis andDNA replication.The expression of particular isoforms of genes of the glycolysis pathwaywas reduced at the level of up to 95% by using siRNA. Then, thequantification of cell proliferation, based on the measurement of BrdUincorporation during DNA synthesis, was performed. Additionally, the cellcycle was analyzed with particular regard to the transition of the cellsinto the S phase. The most pronounced difference in the transition ofcells treated with siRNA into the S phase was observed in the case of thegene encoding glyceraldehyde 3-phosphate dehydrogenase (GAPDH), itamounted to about 50%. This result was also confirmed by the use of thecell proliferation assay. In the case of other glycolytic enzymes, however, the difference was also observed in some particular cases.Surprisingly, silencing the expression of the gene encoding the enzyme GPIresulted in enhancement of the replication phase.These results facilitate understanding of the correlation betweenmetabolic processes and DNA replication in eukaryotic cells. This can bepractically used in studies on carcinogenesis and its prevention as wellas in biotechnological use of cell cultures.

Keywords: DNA replication, glycolysis.

SUN-056
The role of HSP90 in quercetin-induced apoptosis in human papillary thyroid (B-CPAP) cancer cells

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As in many other cancers, genetic alterations that occur in genes encoding for key proteins of major signalling pathways are the driving force for the tumorigenesis of thyroid cancer. HSP90 is an emerging therapeutic target of interest for the treatment of cancer and is responsible for modulating cellular response to stress by maintaining the function of signalling proteins. In this study, we have worked with B-CPAP, a thyroid papillary cancer cell line which is known to have BRAF V600E mutation. We have administered the flavonoid *Quercetin*, which is known to induce apoptosis by inhibiting HSP production on various cancer cell lines, at different concentrations (between 10 and 75 μM) for 24 hours. We have measured cell viability using WST-1 assay. We found that the viability decreases in a dose dependent manner. Then, we chose *Quercetin* concentrations between 10–75 μM for 24 hours, for the apoptosis and cell cycle analysis in flow cytometry by Annexin V and propidium iodide. We have also applied Hoechst (33342) stain to cancer cells for visualizing them on fluorescence microscopy and confirmed apoptosis with the presence of apoptotic signs in nuclei of cancer cells. Finally, we used Western blotting to compare HSP90 and Cleaved-PARP levels in cells treated with *Quercetin* and the control group. In the light of the obtained data, our results suggest that in future studies it would be useful to investigate the apoptotic mechanisms of *Quercetin* and use combinational therapies on BCPAP cells.

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Keywords: HSP 90, Quercetin, human papillary thyroid (B-CPAP) cancer cells.

SUN-057
The *S. pombe* mitotic exit scaffold protein regulates mitotic entry

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Cdk1/CyclinB is the key regulator for mitotic entry. Cdk1/CyclinB is controlled primarily by the coordinated action of the Wee1 kinase and Cdc25 phosphatase. While the molecular basis of Cdk1/CyclinB activity is well characterised, how the initial activation is triggered is less clear. There is increasing evidence that events at the centrosome may play an important role. Work performed in fission yeast has shown that artificially activating Plo1 or Cdk1 at the yeast centrosome drives mitotic entry, overcoming some of the barriers to mitotic entry.

The fission yeast spindle pole body (SPB), analogous to the eukaryotic centrosome acts as a platform to regulate events that occur throughout the mitotic cell cycle. The SPB component, Cut12 play a key role as a regulator for mitotic entry because mutations disrupting its PP1 phosphatase binding site result in the bypass for the requirement for Cdc25 phosphatase activity for mitotic entry. Conversely, enhancing PP1 affinity for Cut12 with the loss of function mutant *cut12.1* blocks mitotic progression where only the older SPB is activated resulting in monopolar spindles. This monopolar phenotype can be rescued by increasing Cdc25 phosphatase activity. In contrast, the SPB component Sid4 is involved with signalling pathways that regulates mitotic exit. Mutations that disrupt Sid4 function such as *sid4.SA1*, leads to defects in cytokinesis resulting in multi-nucleate cells. Both Cut12 and Sid4 are scaffold proteins that act as a platform where a variety of proteins can be recruited in a context depend manner. Scaffold proteins are important as they allow for the integration of signals from multiple signalling pathways. We now report that a Sid4 loss of function mutant *sid4.SA1* rescues the mitotic commitment monopolar spindle phenotype caused by *cut12.1*. More importantly, we have isolated Sid4 mutants that rescue *cut12.1* monopolar spindle defect but are not defective in mitotic exit.

This suggests that Sid4 may play a role in mitotic entry and cross-talk involving Cut12 and Sid4 is important for the formation of a bipolar spindle.

Keywords: mitosis, Spindle pole body.

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SUN-058
The stability of the binding of p53 protein to DNA. The importance of binding domains of p53

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The tumor suppressor protein p53 is a transcription factor involved in regulation of crucial processes, like cell cycle and programmed cell death. It plays critical roles in preventing malignant transformation and maintaining genomic integrity. The function of p53 is closely related to its ability to bind DNA in sequence-specific manner via its core domain. The C-terminal domain has a regulatory function towards the sequence-specific DNA binding and is responsible for the ability of p53 to bind DNA structure-selectively.

We studied the interactions of full-length wtp53 protein and deleted p53 constructs (cΔ30 p53 (without last 30 amino acids), core domain, C-terminal domain) with different DNA substrates (supercoiled, sc or linear, lin DNA containing or lacking specific p53 target sequence – p53CON) towards increased salt concentrations using an immunoprecipitation assay on protein G magnetic beads. We observed an importance of binding domains of protein p53 and an influence of different antibodies on the stability of protein complexes with DNA. The preformed immunocomplexes of p53-DNA with DO-1 antibody (mapping to N-terminus, aa 21–25) and antibody mapping to C-terminus (Bp53-10.1, aa 375–379; Bp53-6.1, aa 381–390; PAb421, aa 371–380; ICA-9, aa 388–393) were exposed to various salt concentrations. The complexes were prepared in two ways: ad1) immunocomplexes of p53 with antibody were incubated with DNA followed uptaking by magnetic beads or ad2) p53 was incubated with DNA followed uptaking by magnetic beads with bound antibody.

We found differences between stability of p53-DNA complexes formed in different ways. The immunocomplexes of DO1-p53 (full lenght and cΔ30) with scDNA are stable at high concentration of salt. The blocking of C-terminus by antibody results in a destabilizing of p53 complexes with DNA at higher concentration of salt maybe due to conformational changes in p53.

Keywords: p53, binding stability, immunoprecipitation.

SUN-059
Theoretical studies and pharmacological characterization of a new antagonist 'C9' of vasopressin receptors

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G protein coupled receptors (GPCRs) are involved in a wide variety of physiological disorders. The vasopressin receptors are classified into V1, V2 and V3 subtypes. All these types of receptors are expressed in the central nervous system. However at the periphery, V1aR is mainly expressed in vascular smooth muscle cells and and hepatocytes, whereas V1bR is exclusively located in pituitary corticotrophs, and V2R is mainly expressed in the kidney.

Vasopressin receptor antagonists (VRAs) are drugs that block these receptors to treat diseases like hyponatremia, congestive failure, etc.

The synthesis of a new putative antagonist of vasopressin receptors (1-[(4-methyl phenyl)sulfonyl]-5-oxo-2,3,4,5-tetrahydro-1*H*-1-benzazepine-4-carbonitrile, named C9 was carried out in our group. Such compound was characterized through ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and spectrometric mass.

Due to C9 has a moiety core similar to vaptans, theoretical studies were achieved using this compound on V1aR, whose three-dimensional (3D) model was built, refined through molecular dynamics simulations and validated through docking studies. With such studies we could determine the free binding energy values of this compound on such vasopressin receptor and the key amino acids involved in their molecular recognition.

Moreover, radio-ligand binding assays were performed to determine its pharmacological properties on the different vasopressin receptors: V1aR, V1bR and V2R to study selectivity of such compound. Firstly, saturation binding assays were carried-out using membranes expressing the human vasopressin receptors in order to obtain the maximun number or receptors (Bmax) and the affinity of the tritiated vassopresin (^{3}H AVP) for the different receptors (Kd). Furthermore, competition experiments were carried out to determine the inhibition constant (Ki) of this compound on the different vasopressin receptors. At the end of these assays we could determine that the affinity of C9 for the different vasopressin receptors is as follows: V2R>V1aR>V1bR.

Our studies will serve as basis for the design of new selective antagonists for the vasopressin receptors, for example by making some modifications on C9 compound in order to improve its affinity for any of the vasopressin receptors.

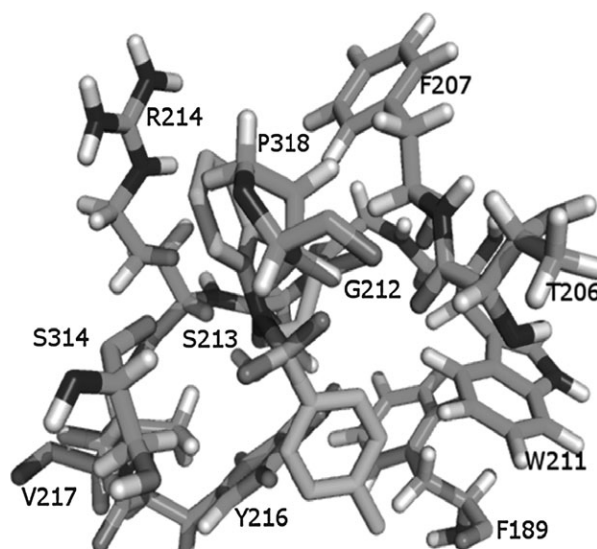

Fig. 1. Residues involved in the binding of C9 compound on V1aR.

Keywords: antagonist, drug design, vasopressin receptors.

SUN-060
Tumor suppressor miRNA let-7b promotes mitotic errors and targets Aurora B kinase

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Let-7 is involved in regulation of many cellular processes, such as proliferation and differentiation. In addition, let-7 has been iden-

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tified as a tumor suppressor miRNA in various human tumor types. Excessive proliferation rate of tumor cells is often associated with chromosomal instability and mitotic defects. Spindle assembly checkpoint (SAC) works to maintain genomic balance by preventing chromosome segregation in the presence of mitotic spindle errors and chromosome misalignment. In cancer cells, SAC function may become compromised allowing chromosome missegregation and aneuploidy in daughter cells. In our study, we found that excess let-7b affects mitosis and SAC signalling in cancer cells by targeting Aurora B kinase. Let-7b binds to Aurora B kinase 3′UTR leading to suppression of the kinase mRNA and protein levels. The reduced Aurora B activity in let-7b overexpressing HeLa cells can lead to premature inactivation of SAC indicated by forced exit from microtubule drug induced mitotic arrest. In non-perturbed culture conditions, excess of let-7b resulted in multipolarity and aneuploidy. Interestingly, let-7b had an additive effect on the rate of polyploidy induction in cells together with a chemical Aurora B inhibitor. In vivo, significant negative correlation was observed between let-7b and Aurora B kinase expression in different breast cancer tumor grades. Aurora B was overexpressed in grade 3 tumors whereas let-7b was downregulated in grade 3 tumors as well as in the most aggressive breast cancers determined by clinicopathological markers. Our data suggests that increased let-7b expression can lead to reduction of Aurora B levels and thereby may suppress tumorigenesis.

Keywords: Aurora B, let-7b, mitosis.

SUN-061
Ubiquitin receptor protein UBASH3B determines mitotic localization of Aurora B

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Mitosis ensures equal segregation of the genome to daughter cells, and defects in mitotic pathways can lead to aneuploidy and polyploidy, frequently observed in cancers. Ubiquitin attachment to substrate proteins regulates fidelity of mitotic division through proteolytic and non-proteolytic mechanisms. However, it remains unexplored how the fate of ubiquitylated substrates is determined during mitosis and what are the mitotic roles of intracellular ubiquitin receptors in mammalian cells. Using high content visual siRNA screening approach, we identified ubiquitin-binding domain (UBD) protein UBASH3B that critically regulates chromosome segregation, acting as a receptor for the key mitotic kinase Aurora B. Essential functions of Aurora B in chromosome segregation are dependent on its dynamic localization to centromeres and midzone microtubules. UBASH3B directly binds Aurora B, and this interaction is dependent on CUL3 E3-ubiquitin ligase and on ubiquitin recognition. Unlike known UBDs, transferring substrates for the proteolytic degradation, UBASH3B does not regulate protein levels of Aurora B. Instead, UBASH3B localizes to the mitotic spindle and is both required and sufficient to transfer Aurora B to microtubules. Our data also show that redistribution of Aurora B from centromeres to microtubules controls timing and fidelity of chromosome segregation and thereby euploidy of cells. Thus, our findings explain how ubiquitin attachment regulates localization and function of Aurora B, linking receptor-mediated ubiquitin signaling to mitosis.

Keywords: Aurora B, Cell cycle, mitosis.

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CSI-02 – Inflammation & Disease

SUN-063
Memantine changes lipids spectrum and lipid peroxidation in animal brain and plasma of patients with Alzheimer's disease

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Alzheimer's disease (AD) leads to a dramatic decline in cognitive abilities and memory. Amyloid beta-protein (Abeta) and oxidative stress induced by proinflamatory citokines is thought to be main primary factors causing neurodegeneration in AD. At the moment the attention of many studies of neurons death mechanisms at Alzheimer's dementia concentrate on the function of lipids which is supposed to play a key role in amyloidogenesis and neuronal disfunction. There has been increasing interest in studying the effect of drugs using for AD treatment on lipid metabolism.

The main goal of our study was detection of influence of memantine – inhibitor of NMDA receptors- on changes in molecular species of phospholipids (phosphatidylcholine, lysophosphatidylcholine, sphingomyelin and phosphatidylethanolamine) and rate of lipids oxidation in blood plasma during treatment of patients with AD and changes in phospholipids and ceramide contents and level of lipids peroxides products in brain regions of mice (hippocampus, cerebellum and cortex).

This study included 18 pathients with AD. The plasma levels of total phospholipids and their molecular species were measured in plasma AD patients that were untreated (control) and were orally treated with memantin in dose of 20 mg daily during 6 months by chromato- electrospray ionization mass spectrometry screening.

Our results have shown that memantine considerably differentiated influence on phospholipids with various set of fatty acids and decrease oxidation rates of plasma lipids of AD patients in compare with untreated patients. It was shown that memantine obtains antioxidant properties. Given results reflect the ability of memantine to inhibit oxidative stress, induced in AD.

Changes in the level of phospholipids (phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, sphingomyelin) and ceramides in hippocampus, cerebellum and cerebral cortex of mice brain within 4 and 24 hours after injection of memantin in doses of 20 mg per mice were detected by HPTLC. Maximal changes in sphingomyilins and ceramides after single administration of memantine were found in the hippocampus, and were less expressed in the cerebral cortex and cerebellum after 4 and24 hours. Memantine decreased level of ROS products in brain regions after 24 hrs. According to our results lipids may serve as a new target for memantine and may interfere with lipid metabolism in AD patients.

Keywords: Alzheimer's disease, ceramides, memantine.

SUN-064
1,25-dihydroxyvitamin D3 inhibits macrophage-induced inflammatory response in human adipocytes via NF-κB and MAPK pathways

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Introduction: Obesity is characterised by the accumulation of macrophages in adipose tissue. As adipose tissue makes up the largest endocrine organ in the body, the increased accumulation of macrophages in adipose tissue in obesity is now known to be a major contributor of chronic systemic inflammation. Higher circulating levels of inflammatory mediators are strong risk factors for the development of metabolic disorders. 1,25-dihydroxyvitamin D_3 is the active metabolite synthesised from vitamin D_3 obtained from the diet or skin exposure to UVB radiation. It is suggested that 1,25-dihydroxyvitamin D_3 may have immunoregulatory effects and may reduce inflammation in adipose tissue. This study investigated the effects of 1,25-dihydroxyvitamin D_3 on macrophage-elicited inflammatory response in adipocytes and the signalling pathways involved.

Method: Human primary adipocytes were pre-treated with 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) (10 nM) for 48 h or vehicle control and then cultured with THP-1 (a human myelomonocytic leukaemia cell line) macrophage-conditioned (MC) medium (12.5% or 25% with adipocyte maintenance media) for 24 h. Cytokine release was measured by ELISAs, intracellular signalling proteins were determined by western blotting and monocyte migration was assessed using a chemotaxis cell migration assay.

Results: MC medium dose-dependently increased secretion of all cytokines and chemokines tested (27–368-fold; all $P < 0.001$) and all secreted levels were reduced by $1,25(OH)_2D_3$: IL-6 (29%, $P = 0.019$ and 34%, $P < 0.001$), IL-8 (43% and 26%, $P < 0.001$), MCP-1 (36%, $P < 0.001$ and 36%, $P = 0.002$) and RANTES (54% and 50%, $P < 0.001$) compared with controls. Monocyte migration elicited by the culture media of adipocytes treated with $1,25(OH)_2D_3$ was also decreased (21%, $P < 0.001$). $1,25(OH)_2D_3$ inhibited macrophage-induced activation of the NF-κB signalling pathway, increasing IκBα (2.7-fold, $P = 0.005$) and reducing phosphorylated NF-κB p65 (by 68%; $P < 0.001$). MAPK signalling activated by MC medium was also decreased by $1,25(OH)_2D_3$, with a downregulation of phosphorylated p38 MAPK (32%, $P = 0.005$) and phosphorylated p44/42 ERK (49%, $P = 0.001$).

Conclusion: The results suggest that 1,25-dihydroxyvitamin D_3 may be anti-inflammatory in adipose tissue, decreasing macrophage-induced release of proinflammatory cytokines by adipocytes and monocyte migration. The inhibitory effect of 1,25-dihydroxyvitamin D_3 on NF-κB and MAPK signalling pathways suggests there may be therapeutic advantages to vitamin D supplementation and enhancing its bioavailability within tissues.

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Keywords: Adipose tissue, Inflammation, Macrophages.

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SUN-065
Inflammatory signal pathway changes in midkine silenced and overexpressed rat alveolar macrophages

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Evidence obtained in intact animals and in primary cell cultures indicate that alveolar macrophages activated by hypoxia release mediator(s) into the circulation. Molecular mechanisms of the inflammatory reaction in hypoxia-induced lung injury are not well defined. Midkine is one of the molecules playing a significant role in the control of inflammatory processes and promotes cellular migration, proliferation and survival. In this study we aimed to evaluate effect of midkine in p38 MAPK and NFkb related signal pathways in alveolar macrophages in normoxia and hypoxia. Midkine expression in rat alveolar macrophage cell line NR8383 was silenced using midkine siRNA sequence primers and overexpressed. Quantitative RT-PCR assay was performed to quantify the mRNA expression of midkine. Cells were exposed to *K. pneumonia* lipopolysaccaride (LPS) in normoxic and hypoxic conditions to induce inflammation. Cytokine secretion, p38MAPK and NFkb levels were evaluated. Cell proliferation rate was higher in midkine overexpressed cells. Basal and LPS induced cytokine secretion levels were found increased in midkine overexpressed cells and decreased in midkine silenced cells. Our results showed that midkine is important for cytokine secretion functions and survival of alveolar macrophage cells.

This study was supported by TUBITAK-BMBF (SBAG108S262).
Keywords: alveolar macrophages, hypoxia-inducible factor-1 (HIF-1), Midkine.

SUN-066
2S Albumin from *Jatropha curcas* L.: structural characterization, and mapping of allergenic epitopes

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Increasing energy demand has contributed to the use of bioenergy consciousness. *Jatropha curcas* L., (physic nut) an oilseed, not edible, is mentioned as one alternative resource. This oilseed Euphorbia contains allergens belonging to the family of 2S albumin that need characterization. The 2S albumins are storage proteins present in seeds of various plants and the recognition of allergen epitopes is of fundamental importance to the development of new strategies for the elimination of allergens and the development of new drugs for the treatment of allergy. The aim of this study was to elucidate possible IgE-binding epitopes in Jat c 1, a 2S albumin allergen from *J. curcas*. Jat c 1 protein from *J. curcas* seeds was isolated by size exclusion chromatography (Sephadex G-50) followed by reversed-phase chromatography in a C_2C_{18} column. The partial primary structure of the 2S albumin was elucidated employing the automatic sequencing and its complete sequence was obtained by search in protein databank. From this primary structure were synthesized peptides (P1, P3, P4, P5, P6 and P8). Based on the prior knowledge that, the 2S albumin from *Ricinus communis* Ric c 1 and Ric c 3 posses amino acids (Glutamic [E] or Aspartic [D]) in the constitution of their allergenic epitopes, we seek the same amino acids in the primary structure of *J. curcas*. Synthetic peptides were treated with Woodward's Reagent K (WRK) a compound that reacts with the carboxylic acid grouping of these amino acids and their potential allergenic properties were investigated by rat mast cells degranulation assay. To prove the role of these amino acids, peptides were synthesized change Glu by Leucine residue (P1Leu, P3Leu, P5Leu, P6Leu e P8Leu). It was observed that the modified peptides and samples treated with WRK had a lower degranulation percentage compared to the initial sample. Immunoassays (ELISA) were done and allowed to detect and quantify the binding capacity of specific antibodies against Jat c 1. Molecular modeling studies were employed to identify the peptides in the three-dimensional structure of Jat c 1. We observed the presence of at least two glutamic acid residues in these peptides and demonstrated the fundamental role of these negative amino residues, in the IgE –binding. The sequences LEKQLEEGEVGS, P5 peptide and sequence VGSEDEARR, part of peptide P6, composes a random loop that is located in the most exposed part of the protein, probably this region is the most important epitope to the outbreak of allergic response.The recognition of allergen epitopes could be employed for the development of future vaccines and pharmacological agents for alergy terapy.
Keywords: 2s albumin, Allergy, *Jatropha curcas*.

SUN-067
5Alpha-tetrahydrocorticosterone: mechanisms of action of a new selective anti-inflammatory drug

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Glucocorticoids (GCs) are a highly effective treatment for inflammatory conditions. However, prolonged use causes serious side effects and more selective anti-inflammatory drugs are needed. A promising candidate is 5α-tetrahydrocorticosterone (5αTHB), a metabolite of the endogenous glucocorticoid, corticosterone (B). In studies in mice we have shown that 5αTHB is as potent as hydrocortisone in reducing inflammation in a model of irritant dermatitis, while not causing skin thinning. Here, the mechanisms of action of 5αTHB as a topical anti-inflammatory drug were investigated.

Right ears of C57Bl/6 male mice were treated for 6 or 24 h with the irritant croton oil alone (CO, 300 μg) or together with B or 5αTHB. Left ears were untreated. Swelling was assessed at cull by weighing ears. B was applied at its EC50 dose (6 h, 10 μg; 24 h, 5 μg) and the dose-response to 5αTHB (5–50 μg) was explored. Ears were frozen for myeloperoxidase (MPO) assay or qPCR, or formalin fixed for haematoxylin & eosin staining. Data are mean ± SEM expressed as % of response induced by CO (set to 100%); *P < 0.05 versus CO.

At 6 h, 5αTHB did not reduce swelling at any concentration. At 24 h 5αTHB decreased swelling to a similar extent to B, but at a five times higher dose (25 μg 5αTHB 65 ± 8%* versus 5 μg B 57 ± 4%*). Cellular and molecular responses were investigated at 24 h, comparing equipotent doses of steroids.

Under these conditions, B (5 μg) and 5αTHB (25 μg) reduced cellular infiltrate in ears (52 ± 4%* and 58 ± 4%* respectively), and decreased MPO activity, a measure of granulocyte infiltration (68 ± 4%* and 39 ± 3%* respectively). B and 5αTHB reduced amounts of mRNA encoding the pro-inflammatory cytokines IL1β and INFγ (B 25 ± 5%* and 51 ± 7%*, 5αTHB 31 ± 13%* and 59 ± 10%* respectively), and decreased abundance of *Vegfα*, *Icam1* and *Pecam1* mRNAs (B 55 ± 5%*, 67 ± 6%* & 68 ± 5%*; 5αTHB 68 ± 5%*, 65 ± 3%* and

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67 ± 5%* respectively), encoding factors that modulate vasculature permeability and inflammatory cell recruitment.

Classical GC anti-inflammatory effects are mediated by the glucocorticoid receptor (GR). To establish if 5αTHB effects are mediated via GR, the experiment was repeated in adrenalectomised mice given a subcutaneous injection of RU486 (a GR antagonist; 0.5 mg/mouse), 15 min before topical application of CO or CO + steroid. RU486 limited the effect of B on ear swelling (5 μg, B 40 ± 9% versus B+RU486 82 ± 17%; $p < 0.05$) but did not prevent the effect of 5αTHB (25 μg, 5αTHB 51 ± 4% versus 5αTHB+RU486 54 ± 9%).

In conclusion, B and 5αTHB target similar pathways to reduce skin inflammation. However, 5αTHB acts more slowly than B and is not antagonised by RU486. These differences may underpin the selective actions of 5αTHB and indicate an alternative mode of action from conventional GCs.

Keywords: glucocorticoids, Inflammation, Metabolism.

SUN-068
8-hydroxy-2′-deoxyguanosine, malondialdehyde and protein carbonil levels as indicators of oxidative stress in patients with sickle cell anemia

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Background: Hemoglobinopathies are the most common genetic diseases in Cukurova region, south of Turkey. Sickle cell anemia and beta-thalassemia are prevalent in this region. The oxidative phenomena play a significant role in its pathophysiology. This study was performed in order to determine 8-hydroxy-2′-deoxyguanosine (8-OHdG), Malondialdehyde (MDA) and protein carbonyl (PC) levels as indicators of oxidative stress from serum samples of SCA patients.

Methods: Patients, who suffer from SCD (n = 45), and healthy controls (n = 38) under the age of 18 were included the study. 8-OHdG levels were measured by ELISA method, MDA and PC levels were measured by spectrophotometric method from serum samples.

Results: 8-OHdG ($p < 0.05$) PC and MDA ($p < 0.001$) levels were higher in SCA group when compared with the control group.

Conclusion: Elevated 8-OHdG (forms as a result of oxidative modification of guanine in DNA structure), MDA (the product of lipid peroxidation) and PC (occurs as a result of protein oxidation) levels in patients with SCA support the agreed evidence that oxidative stress is very crucial for pathophysiology of SCA.

Keywords: 8-hydroxy-deoxyguanosine, Oxidative Stress, Sickle Cell Anemia.

SUN-069
A (S)-(+)-decursin derivative, (S)-(+)-3-(3,4-dihydroxy-phenyl)-acrylic acid 2,2-dimethyl-8-oxo-3,4-dihydro-2H,8H-pyrano[3,2-g]-chromen-3-yl-ester, attenuates the development of atopic dermatitis-like lesions in NC/Nga mice

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(S)-(+)-decursin is a biological coumarin compound isolated from *Angelica gigas Nakai*. (S)-(+)-decursin and its analogue have a variety of pharmacological activities. In the present study, the anti-inflammatory effect of a (S)-(+)-decursin derivative, (S)-(+)-3-(3,4-dihydroxy-phenyl)-acrylic acid 2,2-dimethyl-8-oxo-3,4-dihydro-2H,8H-pyrano [3,2-g]-chromen-3-yl-ester (Compound 6, C6), on in vitro and in vivo atopic dermatitis was investigated. C6 suppressed the secretion of IL-6, IL-8, and monocyte chemotactic protein-1 increase by the house dust mite extract in the eosinophilic leukemia cell line and THP-1 cells. C6 inhibited the production of TARC, IL-6, and IL-8 increase by IFN-γ and TNF-α in the human keratinocyte cell line. In the in vivo experiment, NC/Nga mice were sensitized to 2,4-dinitrochlorobenzene, and then C6 or dexamethasone (Dex) were orally and dorsally administered for three weeks. C6 treatment reduced the skin severity score compared with that of the control group. C6 inhibited the thickening of the epidermis and inflammatory cell infiltration into the dermis by evaluating the histological examination. The serum immunoglobulin E (IgE) level decreased in the C6–treated group compared with that of the control group. The inhibitory effect of C6 on IgE concentration was similar to that of Dex. Taken together, C6 may attenuate atopic dermatitis-like lesions through its anti-inflammatory effect, such as inhibition of IgE and inflammatory cytokines, and it may be valuable as a therapeutic drug for the treatment of atopic dermatitis.

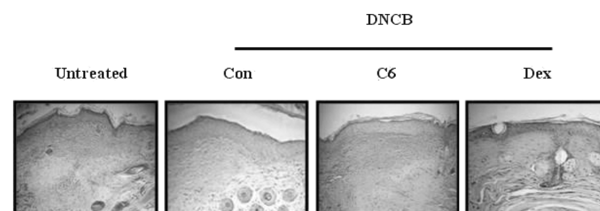

Fig. 1.

Keywords: (S)-(+)-decursin derivative, atopic dermatitis, anti-inflammatory effect.

SUN-070
A farnesyl switch regulates the dynamic membrane binding of human guanylate binding protein 1

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Guanylate-binding proteins (GBPs) are interferon-inducible large GTPases of the dynamin superfamily. Recent data show that human and murine GBPs mediate antimicrobial resistance against

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intracellular pathogens at multiple levels. Several GBPs are subjected to C-terminal isoprenylation. In the case of human GBP1 the CaaX motif serves as a signal for farnesylation, which is necessary for the recruitment to cellular membranes. In addition to that, our research revealed that the lipid modification changes the nucleotide binding and GTP hydrolysis activity of hGBP1.

Both factors, i.e. the lipid modification as well as the GTPase activity are important for the association with membranes, which only occurs in the activated state. Therefore, we have established a fluorescence assay to monitor this transient membrane association of lipid-modified hGBP1 during GTP hydrolysis in real time. Using this assay we show that solvent exposure of the farnesyl anchor is significantly increased in the activated state and during GTP hydrolysis enabling transient membrane binding of hGBP1. Inversely, this interaction of the protein with membranes has an influence on the hydrolytic activity. We therefore conclude that the farnesyl anchor is switchable and regulated by the nucleotide state of the GTPase domain.

Furthermore, fluorescence microscopy of hGBP1 together with several endo- and phagolysosomal markers revealed that human GBP1 localizes to diverse endo-lysosomal compartments and is recruited to phagosomes during the uptake of latex beads. Transient and fast dynamics could also be observed on the intracellular level. Applying Fluorescence recovery after photobleaching (FRAP) revealed a fast and constant activation-dependent exchange of hGBP1-molecules on membranes along the phagolysosomal compartment.

Keywords: Antimicrobial activity, GTPase, Membrane interaction.

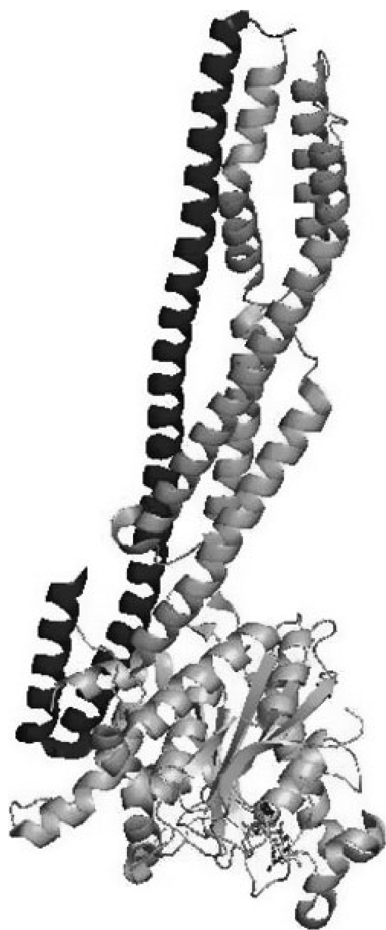

Fig. 1.

SUN-071

A longitudinal study to examine the exome of a good responder CML patient under imatinib at diagnosis and under remission

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Very few genomic studies have looked at the progression of cancer with treatment. We have undertaken a longitudinal case study by carrying out the exome sequencing of a patient with Chronic Myeloid Leukemia (CML). We attempt to understand from a genomics perspective, the changes in the exome of a CML patient in response to imatinib. This will enable us to look at the genetic makeup of CML patients that make them sensitive to lower dosage. Ultimately this will help in better prognosis and precision medicine for this category of patients who are better responders to imatinib.

Our study involves a patient with BCR–ABL positive CML who had been diagnosed in the blast stage. Subsequent to treatment with Imatinib, the subject is now responding to a dosage as low as 200 mg per a day, lower than the recommended 400 mg/day. At the end of three years, there has been no progression of disease with the bone marrow aspirate and biopsy being normal and the BCR-ABL transcript being below detectable levels. We have the used the bone marrow aspirate of this unique responder CML patient at the stage close to diagnosis and two years post treatment (remission) for exome sequencing. Matched skin biopsy was used as a control. This study has been approved by the Institutional Ethical Review Board of St. John's Medical college and Hospital.

We observe genome instability with time in terms of single nucleotide variants (SNVs) and insertions and deletions (indels). The SNVs picked up by exome sequencing were contrasted at the stage of diagnosis and post treatment and compared to dbSNP, allowing retention of only novel SNPs. This finally gave rise to three discreet sets of SNVs to consider: Unique in the genome of the patient at 1) Diagnosis 2)Post treatment, and 3)Persisting throughout the longitudinal study. The non-synonymous coding mutations with high confidence of call of SNP were shortlisted for validation by Sanger sequencing. These shortlisted ones were prioritized based on their relevance in CML via involvement in apoptosis, cancer signaling and cell differentiation. The novel mutations were mapped on the protein structure where possible to provide insight from a structural basis for the deleterious effects of such mutations. These mutations, after validation by Sanger sequencing will be tested in a cohort with similar prognosis to be validated as a probable marker for screening patients. We hence attempt to understand this clinical case with an integrated view involving basic experimental biology, bio-informatics and structural biology.

Keywords: Chronic Myeloid Leukemia, longitudinal analysis, next generation sequencing data.

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SUN-072
A prospective role of citrate treatment for PKC β II inhibition in hyperglycemia-damaged endothelial cells

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Introduction: Organic acids present in *Morus alba* (Moraceae) possess multiple biological effects. Scientific reports have been focused on flavonoids, which have always been considered to be the most crucial rich elements responsible for the pharmacological effects in *M. alba* leaves, while low molecular weight organic acids (LMWOA) were forgotten.

Background: Endothelial dysfunction is a potential contributor to the pathogenesis of cardiovascular disease. Chronic hyperglycemia is an important factor which plays a major role in induced vascular endothelial damage. Elevated glucose levels lead to increase the content of diacylglycerol (DAG), an activator of the protein kinase C (PKC), which plays a key role (particularly PKC β II) in the etiology of diabetic vascular complications [1]. Some recent studies demonstrated the crucial function of citrate treatment of cardiovascular-damaged cells (cardioprotective, anti-inflammatory, anti-platelet aggregation) but predominantly on the hyperglycemia-damaged endothelial cells (HDEC). Residual reports informed that citric acid may be responsible in HDEC for decreasing PKC β II expression down to background level and mitigates process of apoptosis and necrosis [2].

Obtained Results: The objective of our study were to evaluate the therapeutic efficacy of aqueous extracts from various genotypes of *M. alba* leaves for hyperglycemia-damaged cardiovascular endothelial cells. Such extracts are used in some countries as a pharmaceutical materials possessing cardioprotective and antidiabetic activity and known as a rich source not only phenolic compounds but also LMWOA. Thus, our assays included determination of *M. alba* metabolites, antioxidant activity (IC_{50}), the total flavonoids and polyphenols content. Our research [3] revealed that citric acid is the predominant constituents among LMWOA (17.05 mg/mL), representing 25.68% all of them. Furthermore, we determined that citric acid occurs in higher quantities than the total flavonoids (12.25 mg/mL) and polyphenols content (17.46 mg/mL). The antioxidant ability of *M. alba* leaves extract scavenging the DPPH radical measured as IC50 varied significantly from 0.80 to 1.16 mg/mL. Carried studies so far are extremely promising and they are required to be continued.

Future Experiment: The last stage of our experiment will be an assessment of protective and anti-inflammatory activity of citric acid obtained from *M. alba* leaves carried on the Human Aortic Endothelial Cells (HAEC) lines.

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Keywords: None.

SUN-073
A rapid and reproducible standardized procedure for MALDI-TOF profiling of gingival crevicular fluid

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Gingival crevicular fluid (GCF) has captured an increasing interest for its potential diagnostic and prognostic value in periodontal diseases. Comparative MALDI-TOF MS analysis of GCF between healthy and periodontal diseases subjects may help in monitoring changes of expression patterns of disease specific peptides and proteins. However, the sub-microliter volumes of GCF obtained from healthy subjects limit significantly proteomic analysis. As a part of an ongoing project aimed to identify disease-related biomarkers in biological fluids [1–3], in this study we describe a rapid and standardized procedure to obtain reproducible MALDI-TOF MS peptidome profiles from the limited volume of GCF obtained from clinically healthy gingival tissue. We carefully evaluated analytical variables during sample collection and sample processing which could significantly influence the quality and the reproducibility of MALDI-TOF profiles. GCF was collected from the four maxillary incisors in 4 healthy subjects according to two common sampling techniques based on the use of paper strips and paper points in a sampling time of 30 seconds. To measure the amount of GCF we introduce the use of analytical balance as a method for the differential weighing of the device before and after the fluid collection. Contrary to staining techniques, that may induce fluid evaporation end errors in measurements [4], this approach is able to estimate very small amounts of fluid with higher accuracy and, unlike dental instrument such as the electronic measuring device Periotron®, the analytical balance is easily available in many research laboratories, thus making this protocol feasible in a larger number of laboratories. Peptides and proteins were extracted by centrifugal elution in different acidic solutions with and without protease inhibitor cocktail. MALDI-TOF MS analysis was performed varying matrix composition. We found that MALDI-TOF fingerprints of GCF were most largely influenced by extracting solution and matrix composition. In conclusion, we proposed a rapid and an optimized procedure enabling the generation of qualitative and reproducible MALDI-TOF fingerprints from limited volume of GCF which could be useful for high-throughput screening for the identification of peptide-biomarkers of gingival inflammation.

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Keywords: Biomarkers, Gingival crevicular fluid, inflammation.

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SUN-074

A recombinant human IgM crosses blood brain barrier, promotes survival and protects spinal cord anterior horn cells in two transgenic murine models of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a severe progressive, neurodegenerative disease. There is increasing evidence that inflammation accompanies the death of motor neurons in ALS. However, anti-inflammatory strategies to treat ALS yielded no results, suggesting that targeting just inflammation is not sufficient to improve ALS phenotype. An elusive goal of neuro-protection is a reagent effective across multiple diseases. Our laboratory discovered human monoclonal antibody (sHIgM12) that binds to the surface of neurons and promotes remarkable neurite outgrowth. The recombinant version, rHIgM12, binds to the ganglioside GT1b with high affinity by TLC. We demonstrated a therapeutic role for rHIgM12 in a murine model of demyelination with axon loss. Here we expand the use of this natural human IgM as a strategy to treat murine models of ALS. SOD1*G86R and SOD1*G93A transgenic mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). We first verified that rHIgM12 crosses the blood-brain barrier (BBB). To this end we generated ^{35}S-labeled rHIgM12 antibody and treated wild type and SOD mice. ^{35}S-rHIgM12 was found in the CNS in both 90-day old SOD1 mice and wild type mice. In sick SOD1 transgenic animals, a 0.03%> to 0.2% >dose was detected in CNS 20 hr after administration. In a blinded study, 8-week animals received i.p., 200 µg of rHIgM12, or isotype-control human IgM that does not bind to neurons or saline. Interestingly, the single 200 µg dose of rHIgM12 treated before the onset of deficits increased survival in the two independent genetic-based mutant SOD1 mouse strains by 8 and 10 days (Fig. 1). Importantly, the increased lifespan correlated with improved function (for 16 days) assessed by continuous monitoring of spontaneous activity. Finally, rHIgM12 preserved spinal cord axons and neurons of the anterior horn. We validated the specificity of rHIgM12 to gangliosides presented in a membrane platform by nanohole SPR. rHIgM12-GT1b SPR-binding curves were fit to a biphasic exponential model. The apparent dissociation constant (K_D), calculated for rHIgM12 to GT1b was 24.8 ± 7.9 nM, values suggestive of a strong binding. To our knowledge, this is the first demonstration that a single dose of a fully recombinant human monoclonal neuron-binding antibody is efficacious in a model of demyelination with axon loss and in increasing survival of two transgenic models with an ALS phenotype.

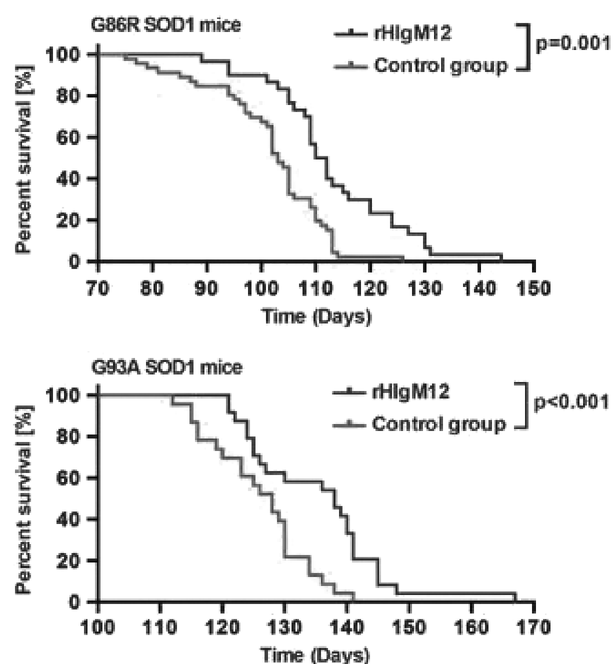

Fig. 1.

Keywords: ALS-linked SOD1 mutants, antibody, Inflammation.

SUN-075

Abnormal correlation between COX activity and prostaglandin E2 level in colon tissue of rats fed diet containing bioactive substances

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Inflammation and colon cancer are very important problem in human public health. One of the most effective methods of colon diseases prevention is diet and regular physical exercises. Cyclooxygenase (COX) is an enzymatic complex responsible for formation of important biological mediators called prostanoids, such as prostaglandins, prostacyclin and thromboxane. Prostaglandin E2 is one of the most important prostanoids taking part in modulation of inflammation process.

The aim of the present study was to evaluate the influence of long-term complex supplementation of diet with bioactive compounds on COX activity and prostaglandin E2 (PGE2) level in rats' colon tissue. The following sources of bioactive substances were used: puree from pumpkin (*Cucurbita maxima*) as a source of -carotene and prebiotics fiber, hydrolysed water extract from linden (*Tilia cordata*) inflorescence as a source of flavonoids, rapeseeds oil, salmon fat as a source of DHA, EPA and other n-3 and n-6 fatty acids and two strains of bacteria with proven probiotic properties: *Lactobacillus acidophilus* LA-5 and *Bifidobacterium animalis* ssp. lactis BB-12.

8-weeks old Spraque–Dawley rats were fed for 14 months with standard semi-synthetic diet (control), which was enriched with above mentioned sources of bioactive substances (experimental group). After 14 months first group was sacrificed and examined.

The enzyme-linked immunosorbent assay (ELISA) analyses revealed that the diet supplementation with investigated bioactive substances caused statistically significant decrease of COX activity (t-test, $p < 0.05$), but the level of PGE2 in that same tissue significantly increased (t-test, $p < 0.05$).

The presented study assessed the potential protective effect of diet supplemented with bioactive compounds against inflammation in colon tissue. Data received showed decreased COX activity and decreased levels of other investigated inflammation markers (data not presented) in rats receiving the supplemented diet, which confirms its protective activity. The observed increase in PGE2 concentration in colon tissue was surprising, because PGE2 is synthesized via biotransformation of arachidonic acid by COX enzymatic complex. Available literature data confirm our findings, showing that all used bioactive substances have the ability to decrease COX activity. We hypothesize that the observed unexpected increase in PGE2 concentration and

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reversed correlation between COX activity and PGE2 level may results from interactions between bioactive compounds, but the mechanisms of this process remains unknown, and requires further research.

Keywords: bioactive substances, COX, rat.

SUN-076

Activation of TLR4 induces VEGF expression via Akt pathway in nasal polyps

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Background: Nasal polyposis is characterized by tissue remodelling and oedematous nasal mucosa. Vascular endothelial growth factor plays a significant role in the regulation of remodelling in nasal polyps. TLR4 activation is associated with VEGF expression in murine macrophages and odontoblasts.

Objective: This study aimed to evaluate whether lipopolysaccharide, an inducer of TLR4, stimulates VEGF expression and to determine the mechanism underlying VEGF production in nasal polyps.

Methods: Nasal polyp-derived fibroblasts were isolated from 10 patients with nasal polyps and exposed to LPS. LPS from Rhodobacter sphaeroides was used to inhibit the expression levels of TLR4, MyD88 and VEGF. Messenger RNA expression levels of TLRs, MyD88 and VEGF were determined by gene expression microarray and semiquantitative reverse transcription-PCR. Protein expression levels of TLR4 and VEGF were analysed using western blot, immunofluorescence staining and enzyme-linked immunosorbent assay. Activation of MAPKs and Akt was examined using western blot analysis. The expression level of VEGF was measured by ELISA and western blot analysis in ex vivo nasal polyp organ culture.

Results: The protein expression level of VEGF was increased in nasal polyp tissues compared with inferior turbinate tissues. LRS inhibited the mRNA and protein expression of TLR4, MyD88 and VEGF in LPS-stimulated NPDFs. LPS-activated MAPKs and Akt signals, whereas MAPK inhibitors did not inhibit VEGF expression, and only Akt inhibitor blocked VEGF production. LRS reduced the production of VEGF in LPS-stimulated ex vivo organ culture.

Conclusions and Clinical Relevance: These results suggest that LPS stimulates the production of VEGF through the TLR4-Akt signalling pathway in nasal polyps. LPS may be involved in the pathogenesis of nasal polyp remodelling.

Keywords: nasal polyposis, TLR, VEGF.

SUN-077

Adenosine affects the slit diaphragm and podocyte cytoarchitecture

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Introduction: Diabetic Nephropathy (DN) continues being the major cause of terminal kidney disease worldwide, and it remains incurable. It is well known that DN progresses with alterations of the podocyte cells function. The most remarkable feature is the deterioration of the filtration barrier leading to proteinuria. Interestingly, the nucleoside adenosine increases locally during progression of diabetic glomerulopathy, most likely affecting podocyte cells function. Our aim was to determine the role of adenosine on the physiology of this cell type.

Methods: Glomeruli were isolated from male rats by a differential sieving method. Podocytes were primary cultured and used at passage 2. Glomeruli or podocytes were exposed to adenosine 10 μM and MRS1754 50 nM, a selective antagonist of adenosine A_{2B} receptor subtype. Fluorescence microscopy was used to evidence cell type specific markers and evaluate fibrillar actin staining using phalloidin-FITC. Activated RhoA was measured using a pull-down coupled to western blot system. The expression of adenosine receptors and integrin α3 were measured using western blots. Differences in protein composition of slit diaphragm-enriched fractions were determined by comparative shotgun proteomics using spectral count data and Quasi-Likelihood modeling. Experimental diabetes was induced in rats using streptozotocin (65 mg/kg). Following two months, diabetic rats were treated with MRS1754 (i.p. 0.2 mg/kg) or vehicle for 4 weeks. Urinary proteins and creatinine were quantified by the Pyrogallol red-molybdate method and Jaffe reaction, respectively, and proteinuria expressed as the ratio.

Results: Exposure of podocytes to adenosine 10 μM triggered a biphasic response consisting of a transient induction of actin stress fibers and the activation of RhoA GTPase, whiles long term exposure led to a loss of fibrillar actin and cell transition to an elongated morphology. Correspondingly, long term exposure to adenosine decreased the expression of the adhesion integrin α3 and increased adenosine A_{2B} receptor subtype. All these effects were blocked using a selective antagonist of the adenosine A_{2B} receptor. Furthermore, the protein composition in a slit diaphragm-enriched fraction derived from glomeruli was changed following exposure to adenosine, suggesting altered interaction with cytoskeleton. In vivo blockage of adenosine A_{2B} receptor inhibited the increased levels of proteinuria in diabetic rats.

Conclusions: Adenosine mediates the induction of a motile phenotype in podocytes in vitro. This effect affects the integrity of the glomerular filtration barrier because in vivo intervention using an adenosine A_{2B} receptor antagonist ameliorates the proteinuria in diabetic animals.

Funded by grant FONDECYT 1130414 from CONICYT-Chile.

Keywords: adenosine, diabetic nephropathy, podoccytes.

SUN-079

Age-linked spontaneous rat prostate inflammation is concomitant with an increase in the expression of several specific pro-inflammatory cytokines

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Age-linked spontaneous prostatitis (ASP) is an inflammation of the prostate. This disease in men is characterized by irritative voiding symptoms, and sexual dysfunction. It is known that histological prostate inflammation analysis shows neutrophils, lymphocytes, macrophages and plasma cells infiltrates. In fact, this type of inflammation is in itself of low risk. However, its importance lies in the fact that a few epidemiological studies indicate that ASP is associated with increased risk of prostate cancer. Taking this into account, our main aim was to determine if the appearance of ASP is concomitant with changes in the presence of any tissue inflammatory marker. For this purpose, prostatic tissues from healthy rats (Wistar rats are a good model for studying and comparing prostate inflammation disease) were taken and a mini-array analysis of 21 specific cytokines was carried out. Rats were divided into two groups. The first group com-

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prises 5 animals with a body weight of 187.4 ± 9.8 g (young adult rats). The second group was formed with 8 rats with a body weight of 371.8 ± 21.8 g (adult rats). Simultaneously, a standard histological analysis of prostatic tissue was performed in order to determine the presence of ASP. Histological analyses revealed that all of the analysed adult rats, but none of the young adult ones, showed the presence of ASP in their prostate samples. Concomitantly, prostate from adult rats showed a significant ($P < 0.05$) increase in the specific expression of cytokines PDGF-AA (increase of about 90%), TIMP-1 (increase of about 80%), Cd86 (increase of about 55%), NGF (increase of about 45%), CINC-3 (increase of about 50%) LIX (increase of about 60%) and VEGF (increase of about 60%). These results indicate that ASP is associated with and specific and significant increase in the expression of several important cellular inflammatory mediators.

Keywords: Inflammation, prostate, age.

SUN-080

AGE-RAGE induced oxidative alterations associated with diabetes complications are modulated by heat shock proteins

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Diabetes complications are generally accompanied by up-regulated expression of receptor for advanced glycation end products (RAGE). This acts as a mediator of AGEs deleterious effects.

Our aim was to identify a link between the heat shock proteins (Hsp) levels and oxidative changes leading to diabetic nephropathy.

Human embryonic kidney cells were exposed for 12, 24 and 48 h to 200 μg/ml glycated-bovine serum albumin (AGE-BSA). RAGE expression augmented in a time -dependent manner, with maximal increases of 2.25 fold in mRNA levels and 2.01 fold in protein expression after 48 h. RAGE activation is known to trigger pro-inflammatory signaling pathways and reactive oxygen species (ROS) production. Consequently, the reduced glutathione pool depleted by 70% after 48 h, hindering the cellular anti-oxidative defenses.

The superoxide dismutase (SOD) activity increased in a time dependent manner by 18%, 30% and 33% after 12 h, 24 h respectively 48 h. SOD zymography revealed that after 12 h of exposure, Cu,Zn SOD contribution to the total SOD activity was more significant than Mn SOD one, whereas after 48 h the two isoenzymes contributed equally. After 24 and 48 h advanced lipid peroxidation end products (MDA) raised by 75% and 129% respectively. Catalase activity increased over 200% after 12 h and remained over 50% higher than controls thereafter. In contrast, glutathione peroxidase activity increased in a time dependent manner, after 48 h, being higher by 50% compared to control, probably due to its involvement in MDA detoxification. Glutathione reductase and glucose 6-phosphate dehydrogenase activities increased only after 48 h by about 35%, probably under Hsp 70 chaperone action.

The Hsp70 maximal mRNA levels of 2.07-fold and protein of 2.78-fold were registered after cells exposure to AGE-BSA 200 μg/ml for 48 h, and seemed to be associated with several altered biochemical parameters. Hsp 27 gene and protein expressions increased after 12 h of treatment by 1.93 and 1.52-fold, but decreased to 0.45 and 0.16-fold after 48 h. Hsp 60 had a similar profile, suggesting that the exposure of human embryonic kidney cells to AGE-BSA up to 48 h could activate pro-apoptotic pathways.

Our data suggest that RAGE activation increased ROS, and consequently antioxidant enzyme activities were modulated. Possibly the oxidative damage of proteins could not be avoided completely. Although protective and antiapoptotic Hsps can improve some of the ROS deleterious effects, therapeutic approaches based on Hsp overexpression must be addressed with caution, as apoptosis inhibition could invoke cancerous transformation.

Keywords: advanced glycation end products, heat shock proteins, oxidative stress.

SUN-081

Aggregation prone human interferon gamma and its mutant show native-like solubility and biological activity after sumo tag removal

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The *E. coli* expression system is a preferable choice for production of therapeutic proteins due to the low cost and simplicity of cultivation. The high expression level and lack of glycosylation, however, often lead to increased aggregation of the target protein in the form of 'inclusion bodies' (IBs) where the protein is biologically inactive. Protein purification out of IBs requires additional technological steps such as solubilisation and refolding, which are crucial for the properties and the yield of the final product.

The aim of our study is to develop a strategy for production of biologically active, soluble and stable in solution recombinant proteins. To this end two highly prone to aggregation proteins were studied: human interferon gamma (hIFNγ) and its mutant analogue K88Q (Q substitution for K in position 88). The therapeutic applications of hIFNγ, an antiviral proinflammatory cytokine, have challenged scientists for years to develop and optimize methods for its production, which are however mainly based on purification from IBs. Our previous results show that when constitutively expressed in *E. coli* LE 392 at 37°C 60–70% of hIFNγ and up to 95% of K88Q aggregate in IBs. Whereas hIFNγ has been successfully recovered from IBs, the mass recovery of K88Q showed to be ineffective upon numerous conditions. In order to avoid the renaturation step and to increase the share of the soluble fraction, we constructed fusion expression vectors bearing three different solubility tags: N-terminal His-FLAG, C-terminal RTX(protease)-His-tag and N-terminal His-SUMO (small ubiquitin-like modifier) tag. The first two expression systems showed no sufficient increase in solubility and/or major difficulties in tag cleavage, while His-SUMO-tag demonstrated high efficiency for both hIFNγ and K88Q proteins. The expression system thus selected was further optimized towards growth conditions and *E. coli* host strain. Expression in the strain BL21(DE3)pG-KJE8, co-expressing two chaperone systems, at 24°C lead to significant increase in solubility of both target proteins from 1.5-fold for hIFNγ up to 8-fold for K88Q. Two-step chromatography (affinity and ion-exchange) with on-dialysis His-SUMO cleavage was applied for protein purification from the cytosolic fraction. It led to a high yield of 99% pure products demonstrating completely preserved biological activities as estimated by kynurenine bioassay.

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Keywords: aggregation, human interferon gamma, soluble expression.

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SUN-082
Alterations in liver gene expression profile in streptozotocin-induced diabetic rats

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Microarray technology has been applied to study the genome-wide gene expression levels in streptozotocin (STZ) induced diabetic liver tissues, which plays a key role in glucose metabolism and homeostasis. The rats were fed with a standard diet and were randomly divided into two groups: (1) non-diabetic control group (2) diabetic group received STZ (55 mg/kg). After STZ injection, rats whose blood glucose concentration above 300 mg/dl was considered as diabetic. After four weeks of diabetes, rats were decapitated and liver tissues were removed for microarray analysis.

Among significantly expressed probe sets, fold change of at least two was considered as differentially expressed probe sets. Principal components analysis revealed clustering of the two different groups and it has been found that there were 273 genes with at least ±2-fold significantly altered expression (90 increases, 183 decreases). Gene ontology groups with significant over-expression with respect to control were responsible for cellular catalytic activities (35 genes), oxidation-reduction reactions (11 genes), co-enzyme binding (7 genes) and terpenoid biosynthesis (3 genes). Whereas; genes responsible for cellular carbohydrate metabolism (11 genes), regulation of transcription (9 genes), cell signal transduction (9 genes), calcium independent cell-to-cell adhesion (3 genes) and lipid catabolism (3 genes) had at least two fold decreased expression. Microarray data were also validated using Real-time PCR for the gene expression of catalase (CAT), ubiquitin specific peptidase 2 (Usp2), insulin-like growth factor binding protein 2 (Igfbp2), cytochrome P450 8B1 and 1A1 isoforms (CYP8B1 and CYP1A1). Real-time PCR confirmed the directionality of changes in expression of the genes tested.

As a conclusion, global gene expression in the liver tissues is affected by streptozotocin induced diabetes in several specific areas, including catalytic activities, oxidation-reduction reactions, co-enzyme binding, cellular carbohydrate metabolism, regulation of transcription and signal transduction. The present data suggest the presence of several processes which contribute and possibly interact to impair liver function in type 1 diabetes, several of which are potentially amenable to therapeutic interventions.

Keywords: Diabetes, Microarray, Gene expression.

SUN-083
Altered activation of monocytes from the patients with antiphospholipid syndrome induced by LPS and ATP

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The antiphospholipid syndrome (APS) is an acquired autoimmune disorder of unknown etiology. Growing evidences support the involvement of monocytes in APS pathogenesis. Inflammatory activation of monocytes promotes thrombus formation and other APS complications. However, mechanisms underlying their activation are poorly investigated. We therefore determined spontaneous and induced by LPS (10 ng/ml) and LPS+ATP (100 μM) expression of IL-1β, IL-6, IL-23, TNFα and CCL2 in circulating monocytes isolated from APS patients (n = 10) and healthy (n = 7) subjects using comparative qRT-PCR method. Spontaneous expression of all candidate genes ($p < 0.05$) in APS monocytes was significantly lower than in healthy cells. LPS and LPS + ATP stimulation had no significant effect on gene expression in healthy cells, while in APS monocytes LPS and ATP had profound effects. LPS significantly increased expression level of all genes ($p < 0.05$). Exposure of APS monocytes with LPS + ATP decreased mRNA levels of IL-1β and CCL2 compared with untreated cells ($p < 0.05$) and levels of IL-1β, IL-23 and CCL2 compared with LPS induction ($p < 0.05$). LPS-induced mRNA levels of IL-23, CCL2 were significantly higher in diseased cells than those in healthy cells ($p < 0.05$), in opposite LPS+ATP induced expression of IL-1β, IL-6, CCL2 was higher in healthy controls than those in APS monocytes ($p < 0.05$). Thus, present study revealed an increased sensitivity of APS monocytes to bacterial LPS. Presence of low concentrations ATP appears to diminish LPS-induced inflammatory state of diseased monocytes, suggesting that ATP-mediated inhibition of inflammatory processes in monocytes may provide protection and limit tissue injury in autoimmune APS.

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Keywords: antiphospholipid syndrome, ATP, gene expression.

SUN-084
Altered production of reactive oxygen species and release of extracellular traps by neutrophils under the influence of selected mucolytic, anti-inflammatory, anti-histamine and cardiovascular drugs

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Introduction: Neutrophils, the first line of the host defense against pathogens, circulate in the bloodstream and can also infiltrate other tissues and organs, e.g., the lungs. These defense cells exploit various mechanisms for pathogen killing, including the phagocytosis, the degranulation with a release of numerous microbicidal agents and the formation of neutrophil extracellular traps (NETs). NETs play important role in capturing pathogens and are also responsible for the increase of the mucus viscosity in the lungs as well as for intravascular clot formation by binding morphotic blood elements. Many of drugs commonly used in the therapy of various diseases can interact with neutrophils, with modulatory effects on the NET formation. Mucolytic drugs help to reduce mucus viscosity, e.g., in cystic fibrosis, cardiovascular drugs improve the condition of the heart as well as of the circulatory system, likewise anti-inflammatory and anti-histamine drugs extinguish the immune response.

Objective: Determination of the effects of mucolytic (N-acetylcysteine), anti-inflammatory (ketoprofen, hydrocortisone), anti-histamine (clemastine) and cardiovascular (etamsylate, dopamine) drugs on the ability of neutrophils to produce reactive oxygen species (ROS) and release NETs.

Results: In the response to contact with pathogens, neutrophils produce high amounts of ROS. With the use of the chemiluminescence and flow cytometry methods, we found that this activity of neutrophils was markedly reduced after their pretreatment

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with N-acetylcysteine, ketoprofen, etamsylate and dopamine. Selected drugs seemed also to modulate the NET production, with N-acetylcysteine, etamsylate and dopamine inhibiting this process, in contrast to ketoprofen which markedly promoted the NET release.

Conclusions: The decrease of ROS production by neutrophils under the influence of anti-inflammatory and anti-histamine drugs helps to prevent the development of inflammation. Similarly, the inhibition of ROS generation by mucolytic drugs protects the lung against the adverse action of neutrophil antimicrobial factors on the host tissue. On the other side, the inhibition of ROS production by cardiovascular drugs seems to exert the adverse effect in systemic infection (sepsis) where these drugs are very often applied. Inhibition of NET release by mucolytic drugs helps to reduce mucus viscosity; however, the same action of cardiovascular drugs can cause development of infection (dopamine administration during sepsis) or reduce the ability of clot formation (etamsylate treatment in bleeding).

This work was supported by the National Science Centre, Poland (grant No. UMO-2012/05/B/NZ1/00003 to M.R-K.).

Keywords: Neutrophil extracellular traps, Neutrophils, Reactive Oxygen Species.

SUN-085
Ameliorative effects of Naltrexone on CCl4-induced liver cirrhosis in an animal model study

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Introduction: Hepatic cirrhosis is a common pathological feature of progressive chronic liver disease. Naltrexone (NTX), (a μ-opioid antagonist) attenuates hepatocellular injury. Since there is evidence about the role of oxidative stress in this disease, we aimed to determine if NTX affects on CCl4-induced cirrhotic rats by ameliorating oxidative stress.

Material and Methods: In this study, 124 male Wistar rats were divided into six groups (7rat/group) and the groups received drugs and reagents (i.p.) as follows: 1- Control, 2- 150 mg/kg CCl4 every other day, 3- 1 ml Mineral oil (as a carrier of CCl4) every other day, 4- NTX 10 mg/kg daily, 5- Nal+CCl4, and 6- NTX+Mineral oil as described. After 2, 6 and 8 weeks, the animals were sacrificed and the blood samples were collected. Total antioxidant capacity of plasma (FRAP), and the activity of alkaline phosphatase (ALP), alanine aminotransaminase (ALT), aspartate aminotransaminase (AST) and gamma-glutamyl transferase (GGT), were assessed in plasma. Metabolites of nitric oxide were evaluated in plasma as well. Also, erythrocyte membrane composition i.e. protein carbonyl and MDA were assessed in RBC ghosts.

Results: The plasma enzyme activites were significantly higher in CCl4 group when compared to the other groups ($P < 0.05$) showing the progression of liver injury ($P < 0.05$). NTX severely diminished carbonyl content of erythrocytes of CCl4-induced cirrhotic rats after 8 weeks of injection, but increased it significantly after 2 and 6 weeks when used as control ($P < 0.05$).

Discussion: The results show that upon treatment with CCl4, antioxidant capacity of erythrocytes is depleted and oxidative stress markers especially protein carbonyl content of RBC membrane increase at the early stages of cirrhosis. It seems that NTX could ameliorate these alterations.

Acknowledgments: This work was supported by Research Council of Tehran University of Medical Sciences (Grant No. 15244).

Keywords: cirrhosis, Naltrexone, Oxidative stress.

SUN-086
Analysis of GOLPH3 depletion in human glioblastoma multiforme T98G cells

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Glioblastoma multiforme (GBM) is the most common and most aggressive malignant primary brain tumor in humans. Despite enormous advances on our understanding of this type of brain cancer, little is known about the different aspects of the secretory pathway in GBM tumorigenesis, and in particular the contribution of the Golgi apparatus is poorly understood. GOLPH3 is a highly conserved phosphoprotein of the Golgi apparatus originally identified in proteomic analyses. Importantly, GOLPH3 is overexpressed in several tumor types, including breast, lung, melanoma, ovarian, prostate, and GBM, but its function in malignant cells is little known. GOLPH3 is a peripheral membrane protein enriched at the trans-Golgi network that has been implicated in several cellular functions, such as in the retrograde transport of Golgi sugar transferases, or in the maintenance of the structure of the Golgi apparatus through its interaction with the actin cytoskeleton. To understand the possible role of GOLPH3 in GBM tumorigenesis, we analyzed the effect of GOLPH3 depletion in the GBM cell line T98G. Depletion of GOLPH3 by RNAi resulted in a distinct change in morphology of T98G cells, accompanied by a reorganization of the actin cytoskeleton. Depletion of GOLPH3 also resulted in a different steady-state distribution of several transmembrane proteins, suggesting an effect on protein trafficking. Strikingly, the analysis of the protein expression pattern in cells depleted of GOLPH3 showed either downregulation or upregulation of several proteins. We propose that overexpression of GOLPH3 is related to the tumorigenic phenotype of T98G cells by means of reorganization of gene expression that affect membrane trafficking.

Funded by FONDECYT 1130710, and DID-UACh.

Keywords: Glioblastoma Multiforme, Golgi, GOLPH3.

SUN-087
Annexin A8 controls leukocyte recruitment to activated endothelial cells via cell surface delivery of CD63

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Proinflammatory activation of endothelium releases the bleukocyte receptor P-selectin from Weibel-Palade bodies (WPB) to the endothelial cell surface, with CD63 acting as a stabilizing co-factor. we find that loss of annexin A8 (anxA8) in human umbilical vein endothelial cells (HUVEC) strongly decreases cell surface presentation of P-selectin, with a concomitant reduction in leukocyte rolling and adhesion. We confirm the compromised leukocyte adhesiveness in inflammatory-activated endothelial venules of anxA8-deficient mice. We report that WPB of

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anxA8-defient HUVEC contain less CD63. This is caused by improper tranport of CD63 from late multivesicular endosomes to WPB, with CD63 being retained in intraluminal vesicles. Consequently, reduced CD63 cell surface levels cause enhanced P-selectin re-internalization. Our data support a model in which anxA8 affects leukocyte recruitment to activated endothelial cells by supplying WPB with sufficient amounts of the P-selectin regulator CD63.

Keywords: late endosomes, leukocyte recruitment, P-selectin.

SUN-088
Annexin V and anti-Annexin V Antibodies in acute myocardial infarction

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Background: Myocardial infarction is the combined result of environmental and personal factors. Prothrombotic factors might play an important role in this phenomenon. Annexin V (ANV) is a calcium-dependent glycoprotein widely present in various tissues exerting a potent anticoagulant effect in vitro by reducing plaque adhesion and aggregation. Anti-annexin V antibodies (aANVAs) are detected in various diseases like rheumatoid arthritis, systemic lupus erythematosus and anti-phospholipid antibody syndrome. The study of ANV in Acute Myocardial Infarction (AMI) might shed light on hypercoagulability mechanisms in the pathogenesis of acute coronary syndromes. This study was conducted to investigate the association of plasma ANV, aANVAs and anti-cardiolipin antibodies (aCLAs) with AMI.
Methods: This study recruited 45 patients with the diagnosis of AMI according to WHO criteria in their first 24 hours of admission. 36 matched individuals were studied as the control group with normal coronary artery angiography. Plasma levels of ANV, aANVAs and aCLAs were determined by enzyme-linked immunosorbent assay and the results were compared.
Results: Plasma ANV levels in the patients with AMI on admission were significantly lower than those in the control group ($p = 0.002$). Positive test for aANVAs were found to be present in a significant number of our patients ($p = 0.004$). The studied groups were similar in their rate of patients with positive aCLAs tests. ANV, aANVAs and aCLAs were not correlated with hypertension, diabetes mellitus, hyperlipidemia, sex, age and smoking.
Conclusion: Our findings suggest that low plasma ANV levels along with positive aANVAs tests in patients with AMI are indicative of hypercoagulable state that is not related to the traditional cardiovascular risk factors.
Disclosure of interest: None declared.

Keywords: annexin, jnfarction.

SUN-089
Antibodies against neurotrophin receptor p75 and the prion protein protect memory impairment in mouse model of Alzheimer's disease

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Alzheimer's disease is characterized by beta-amyloid binding with cell surface proteins in the brain of patients which leads to pathological changes inside the cells followed by Alzheimer's disease progression. p75 receptor involved in cell balance support and the prion protein are both the targets for toxic beta-amyloid. We proposed that prevention of beta-amyloid binding with the extracellular proteins such as neurotrophin receptor p75 and the prion protein by means of induction of antibodies against the proteins can be a promising approach towards development of new anti-Alzheimer's disease treatment. We investigated the effect of induction of antibodies against p75 or prion in preventing the development of several Alzheimer's disease features in the mouse model of the disease. Four potentially immunoactive fragments of p75 and one fragment of the prion protein were chosen and chemically synthesized. Investigation of immunoprotective effect of the peptide fragments was carried out in mice with experimentally induced form of Alzheimer's disease and revealed that immunization with two fragments of p75 (155–164 and 167–176) conjugated with a carrier protein and the prion fragment (17–33) in a conjugated and non-conjugated form induced anti-peptide antibody formation and effectively preserved murine memory from impairment. Results obtained by ELISA biochemical analysis showed that only immunization with fragment p75 155–164 conjugated with a carrier protein led to significant decrease in beta-amyloid level in the brain of the experimental mice. We also demonstrated that immunization with prion fragment 17–33 in a conjugated and non-conjugated form significantly improved morphofunctional state of neurons in the experimental mice. To reveal the role of antibodies against the two proteins, the level of the antibodies in patients with Alzheimer's disease is currently under investigation. Thus, immunization with both fragments of p75 receptor and the prion fragment provides a new insight into anti-Alzheimer's disease drug design.

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Keywords: Alzheimer's disease, neurotrophin receptor p75, synthetic peptides.

SUN-090
Anti-obesity effect of *Allomyrina dichotoma* (Arthropoda: Insecta) larvae on high-fat diet induced obese mice

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We tested whether *Allomirina dichotoma* larvae (AD) prevents obesity in high fat diet (HFD) induced obese mice and further investigated the underlying mechanisms for its body weight lowering effect. All of the mice were maintained on normal diet (ND) for 1 week and then fed with ND, HFD, or HFD + AD (100 mg/kg and 3000 mg/kg) for 6 weeks, respectively. The body weights of HFD-induced obese mice were monitored after daily oral administration of AD for 6 weeks. Blood lipid profile and plasma leptin levels, as well as expressions of genes involved in lipogenesis were examined. The results showed that AD significantly reduced body weight and fat mass in HFD-fed mice. Moreover, mice fed with HFD + AD had lower concentrations of triglyceride in their blood and lower serum leptin levels compared with the HFD-fed mice. Real-time PCR analysis showed that the expression levels of peroxisome proliferators-activated receptor γ (PPAR γ) and CCAT/enhancer binding protein α (C/EBP α) genes in the epididymal fat tissue of mice fed with the HFD+AD (3000 mg/kg) were reduced approximately 73% and 37%, respectively, compared to mice fed with HFD only. Additionally, HFD + AD oral administration significantly decreased the mRNA expression of lipoprotein lipase (LPL) as compared with the mRNA levels in the HFD group. These results sug-

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gest that AD has a possibility of development as alternative medicine for preventing obesity in the future.

Keywords: High fat-diet (HFD), Lipogenesis, Obesity.

SUN-091
Antioxidant compounds suppress diamine oxidase activity

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Dysregulated signalling via biogenic amines is associated with the development of several human pathologies. The biochemical routes for the interconversion and elimination of biogenic amines share high similarities among different species. Oxidative degradation of biogenic amines is catalysed by two types of amine oxidases: quinoprotein copper-containing amine oxidases (CAO) and flavoprotein polymamine oxidases (PAO).

Diamine oxidase, which belongs to CAO, catalyzes the oxidative deamination of diamines like putrescine and histamine to the corresponding aldehyde via Schiff base formation of the oxidized topaquinone (TPQ) cofactor with the substrate amine [1]. Histamine is a potent mediator of inflammation and allergic reactions. The large amount of histamine in certain foods is suggested to be responsible for the symptomatology of histamine intolerance (HIT) or sensitivity, a food intolerance affecting about 1% of the population [2]. In healthy people, DAO provides an enzymatic barrier function to prevent histamine resorption in the intestine. Inefficient degradation gives rise to high intestinal histamine, however there is equivocal information regarding the role and diagnostic relevance of DAO in HIT [3].

Recent studies already discuss the involvement of exogenous antioxidants and 'antioxidative stress' in the development of allergies [4]. By using DAO as a model enzyme we analysed the influence of selected food-contained antioxidants such as preservatives and colorants on the enzymatic activity by applying radiolabelled putrescine as a substrate. Exposure to most antioxidant compounds resulted in a dose-dependent reduction of enzyme activity. If this in vitro effect on DAO can be extrapolated to in vivo, it opens another possibility how overload with antioxidants may interfere with biogenic amine metabolism. In addition, although in vitro only, our results emphasize an additional effect to the suppression of Th1-type immune reactions and cytokines by antioxidant compounds, which was demonstrated earlier for food preservatives, colorants, phytochemicals and drugs [5], thus promoting a shift of the Th1-Th2-type immune balance towards Th2-type immunity.

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Keywords: antioxidant, diamine oxidase, histamine intolerance.

SUN-092
Anti-phosphatidylethanolamine antibodies in acute myocardial infarction

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Background: Acute myocardial infarction (AMI) is a clinical manifestation of coronary atherothrombosis and is the important causes of death. Many factors play a role in AMI. Anti-Phospholipid (aPL) antibodies may act in the induction of immunological response leading to the development of AMI. Anti-Phosphatidylethanolamine (aPEA) antibody has been detected in various autoimmune diseases and anti-phospholipid antibody syndrome. The study of aPEA antibody in AMI might shed light on etiologic mechanisms in the pathogenesis of coronary atherothrombosis and AMI. This study was aimed to evaluate whether prevalence of aPEA antibodies, in patients with AMI and to analyze their relationship with traditional cardiovascular risk factors.

Methods: The prevalence of aPEA IgG and IgM in a well characterized group of patients with AMI as a case group and in age and sex matched healthy subjects as a control group. Sera from two groups were tested to evaluate the presence of aPEA IgG and IgM isotypes by ELISA method.

Results: The frequencies of positive test for aPEA IgG were 12.22% and 2.22% among patients and controls respectively with significant difference ($P = 0.007$).

The aPEA IgM frequencies were 3.33% and 0.00% in patients and the controls, with significant difference ($P = 0.005$).

Conclusion: According to the results of this study, aPEA antibodies have a role in AMI, independent risk factors for AMI, which may represent a link between autoimmunity and coronary atherothrombosis. Further studies with larger sample size of patients and healthy people are needed to explore the role of aPEA antibodies in coronary atherothrombosis.

Keywords: antibody, infarction, phospholipids.

SUN-093
Anti-phosphatidylserine antibodies in acute myocardial infarction

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Acute Myocardial Infarction (AMI) is the combined result of environmental factors and personal predispositions. Many factors play a role in AMI including anti-Phospholipid (aPL) antibodies, that may act in the induction of immunological response leading to the development of AMI. Anti-Phosphatidylserine (PS) antibody is detected in various diseases like rheumatoid arthritis, systemic lupus erythematosus and anti-phospholipid antibody syndrome. The study of anti-PS antibody in AMI might shed light on etiologic mechanisms in the pathogenesis of acute coronary syndromes. This study was conducted to evaluate whether prevalence of anti-PS antibodies, in patients who had AMI and to analyze their relationship with traditional cardiovascular risk factors. The prevalence of anti-PS IgG and IgM in a well characterized group of patients with AMI as a case group and in age and sex matched healthy subjects as control group. Sera from two groups were tested to evaluate the presence of IgG and IgM isotypes to anti-PS by ELISA method. The frequencies of positive test for anti-PS IgG were 26.70 and 8.90% among patients and controls respectively with significant difference ($p = 0.003$). The anti-PS IgM frequencies were 12.20 and 1.10% in patients and the controls, with significant difference ($p = 0.005$). The findings of this study suggest that anti-PS antibodies seemed to play a role in AMI, independent risk factors for AMI, which may represent a link between

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autoimmunity and atherosclerosis in patients with AMI. Further studies with bigger sample size including patients with AMI and healthy people are recommended to explore the exact role of anti-PS antibodies in AMI.

Keywords: antibody, infarction.

SUN-094
Apoptotic and pro-/antioxidant processes depends on the vitamin D3 availability in the liver of diabetic mice

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Precise mechanism by which oxidative stress could facilitate and accelerate the development of hepatic lesions in diabetes is not fully clarified. The present study was performed to determine the relationship between 25-hydroxyvitamin D_3 ($25(OH)D_3$) availability, apoptosis and *pro-/antioxidant* profile in liver of diabetic mice.

Type 1 diabetes was induced in male C57BL/J6 mice (weighing 25.0 ± 1.5 g) by i.p. injection of multiple low dose streptozotocin (40 mg/kg b.w.). Control and STZ-diabetic mice were treated with or without vitamin D_3 (15 IU/mouse per os, for 8 weeks). Serum $25OHD_3$ was assessed by ELISA. The levels of Casp3, poly(ADP-ribose)polymerase 1 (PARP-1), poly-ADP-ribosylated *and nitrosylated proteins* were *measured* by Western-blot analysis. Intracellular reactive oxygen and nitrogen species (ROS and RNS) production were detected by 2′,7′-dichlorofluorescin (DCF) and 4,5-diamino-fluorescein diacetate (DAF-DA) fluorescence respectively using flow cytometry. Activities of pro-/antioxidant enzymes in liver were measured spectrophotometrically.

Serum level of $25OHD_3$, the main circulating metabolite of D_3, was shown to be reduced to 23.8 ± 1.9 in diabetes versus 39.7 ± 2.9 nmol/l in control, that reflects reliably vitamin D_3 deficiency ($p < 0.05$). As a strong evidence of diabetes-induced oxidative stress that may lead to liver lesions, increased hepatocytes ability to oxidize the fluorogenic substrate DCF and DAF was found. These changes were accompanied by a significant rise in the levels of protein nitrotyrosine, carbonyl groups and poly-ADP-ribose by 42, 38, and 61% respectively versus control, $p < 0.05$. Diabetes also caused more than 1.67-fold increase in the level of 89 kDa apoptotic cleavage fragment of PARP, as well as 1.51- and 2.04-fold overactivation of Casp3 cleavage fragments respectively as compared to control ($p < 0.05$), indicating connection between proapoptotic process and oxidative stress. The diabetes-associated increase in the activities of key pro- and antioxidant enzymes in the liver was also established. Vitamin D_3 treatment completely restored blood serum $25OHD_3$ level, partially decreased PARP-1 and Casp3 activity and counteracted diabetes-induced abnormalities of pro-/antioxidant profile in liver tissue. Normalization of vitamin D_3 availability strongly correlated with a significant decrease in ROS and RNS generation in hepatocytes as compared with diabetic mice.

The findings indicate that diabetes-associated vitamin D_3 insufficiency can be related, at least in part, to increased proapoptotic and prooxidant status of liver cells. Our data suggest a potential role of vitamin D_3 treatment in the regulation of impaired oxidative metabolism in diabetes.

Keywords: diabetes mellitus, vitamin D3, liver, oxidative stress, apoptosis.

SUN-095
Assessing immunogenicity of enzymatically cross-linked beta-lactoglobulin using dendritic-cell derived endolysosomal degradome

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The growing number of novel protein derivatives with potential applications in food industry or for the treatment of allergic diseases imposed a dramatic increase in the demand for assessment of immunogenicity of novel proteins. Since efficient antigen processing is of crucial importance for immunogenicity of proteins we subjected cow's milk beta-lactoglobulin, BLG, and its laccase cross-linked derivative, CL-BLG, to proteolysis by endolysosomal enzymes isolated from dendritic cells derived from bone marrow cells of BALB/c mice.

SDS-PAGE electrophoresis of digested samples revealed that BLG was degraded slowly in time with half-time >48 h, but also that some proteolysis of CL-BLG occurred. BLG peptides identified in the samples were used for generation of peptide maps that provide insight into peptide profile and identification of potential T-cell determinants. Peptides from both BLG and CL-BLG appeared in seven nested clusters (start-end amino-acid): 1–19, 20–42, 42–57, 58–81, 83–103, 123–145 and 146–162. Peptides from one cluster share common core and have variable lateral regions. The most abundant peptide cluster for BLG was 123–145, while peptides from C-terminus (146–162) where the least present. Although CL-BLG releasing peptides share common clusters with BLG, their overall distribution is different. When compared to BLG, CL-BLG provided higher number of peptides in clusters 42–57 (dominant), 20–42 (abundant) and 146–162. For prediction of core sequences of peptides binding to H-2 class II molecules IAd and IEd that are MHC II molecules expressed in BALB/c mice we used RANKPEP and IEDB analysis resource SMM-Align. Peptides from clusters 1–19, 20–42, 58–81 and 123–145 were listed as possible candidates.

Finally, we investigated whether cross-linking of BLG interfered with antigen presentation by DCs in vitro. Peptides occurring from CL-BLG endolysosomal digestion when presented with MHC II complex on the surface of DCs induced stronger activation and Th2 response of BLG-specific $CD4^+$ T cells. Therefore, in agreement with previously published results, we report that cluster 42–57 for which CL-BLG provided constantly higher number of peptides is probably T-cell immunodominant epitope. Also, after aligning our data with theoretical predictions, we propose that peptides in the cluster 20–42 may (co-) determine higher immunogenicity of CL-BLG.

From our experience, when examining differences in immunogenicity between two structurally different proteins, except for monitoring resistance to intracellular cleavage by endolysosomal proteases, it is important to analyze and identify released peptides.

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Keywords: beta-lactoglobulin, endolysosomal degradome, immunogenicity.

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SUN-096
Assessment of TNF-α genotypes and serum level of patients with cardiac syndrome X

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Introduction: Patients undergoing the non-invasive tests for angina typical showed ischemia with normal coronary angiograms, these patients may feature cardiac syndrome X (CSX) or microvascular angina. The pathogenesis of cardiac X syndrome is still unresolved, although in the presence of culprit factors like inflammation, endothelial dysfunction, increased oxidative stress, diffuse atherosclerosis and microvascular dysfunction. It is a well-known fact that inflammation plays an important role in endothelial dysfunction and leads to the development of microvascular dysfunction. In the present study we aimed to investigate the relationship between cardiac X syndrome and inflammation.

Materials and Methods: The present study was conducted on 111 patients diagnosed with stable angina pectoris and 97 control patients registered with chest pain and exercise stress test was negative for ischemia, with Framingham Risk Score lower than 10% for 10-year cardiovascular risk. DNA was isolated from peripheral blood samples to study the TNF-α genotype polymorphism using PCR and RFLP techniques. ELISA technique was performed to determine the serum TNF-α levels in 78 patients with CSX and 80 healthy individuals.

Results: There was no significant difference between CSX and control group in terms of age, sex, BMI, diabetes mellitus, hypertension, hyperlipidemia, family history, cardiovascular drugs in use, fasting plasma glucose, creatinine, total cholesterol, HDL, LDL, triglycerides, GGT, white blood cell count, hemoglobin, platelet count, mean platelet volume and C-Reactive Protein levels ($p > 0.05$). TNF-α -308 G> A polymorphism genotype in patients with CSX were GG (85.6%), GA (14.4%) and AA (0%) whereas GG (88.7%), GA (9.3%) and AA (2.1%) in control group. There was no significant difference in genotype distribution of TNF-α between patients and control groups ($p = 0.510$). The serum levels of TNF-α in CSX patients and control group were 83.1 ± 2.3 pg/mL and 84.9 ± 3.5 pg/mL respectively. TNF-α levels of control group were significantly higher than that of the patients ($p < 0.05$).

Conclusion: Inflammation plays an important role in endothelial dysfunction and leads to the development of microvascular dysfunction. However, the present study exhibited contradictory result such as low concentration of serum TNF -α in patients with respect to control. The underlying cause of low levels of TNF-α can be relatively small number of patients included in the study. In addition, local microvascular inflammation thought to play major role in the development of cardiac x syndrome than that of systemic inflammation.

Keywords: Cardiac Syndrome-X, Inflammation, Polymorphism.

SUN-097
Association of Lp-PLA2-mass, vitronectin and PAI-1 activity with maternal endothelial dysfunction in patients with preeclampsia

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Although the cause of pre-eclampsia remains largely unknown, the leading hypothesis strongly rely on abnormal physiologic transformation of spiral arteries, anti-angiogenic state, uthero-placental ischemia, excessive intravascular inflamation and disruption of endothelial and haemostatic function. Links between abnormal activation of haemostatic and inflammatory systems in preeclampsia may help to elucidate some questions in pathophysiology of the disease.

Lipoprotein associated phospholipase A2 (Lp-PLA2) is an enzyme produced by inflammatory cells and degrades oxidatively modified phospholipids in oxidized LDL and oxidized lipoprotein (a), leading to formation of proinflammatory and cytotoxic products. Plasminogen activator inhibitor-1 (PAI-1) is inhibit fibrinolysis in the blood by blocking this plasminogen activators. Antifibrinolytic activity of PAI-1 facilitated by vitronectin that binds the inhibitor and may regulate its activity by the stabilizing the active PAI-1 conformation. In addition, several studies have demonstrated that PAI-1 play a key role in the regulation of local inflammatory process.

The aim of the present study; was determining, correlating and comparing the plasma Lp-PLA2, vitronectin, tissue-type plasminogen activator (t-PA) and PAI-1 activity levels in early-onset preeclampsia, late-onset preeclampsia and in control pregnant women.

A total of 79 individuals, 30 early-onset, 22 late-onset preeclamptic and 27 control pregnant women were included into the scope of this study. Enzyme-linked immunosorbent assay (ELISA) procedure was used to determine the serum Lp-PLA2 and plasma vitronectin, t-PA antigen and PAI-1 activity levels.

Major findings of this study; 1) In patients with preeclampsia, Lp-PLA2, PAI-1, t-PA, blood pressures and urine protein levels were increased ($p < 0.001$) and correlated with each other. 2) Vitronectin levels was decreased ($p = 0.045$) but not correlated with other parameters in preeclamptic patients. 3) Biochemical data obtained from early-onset and late-onset preeclamtic patients are not statistically significant.

We think that increased Lp-PLA2 levels may partially contribute to endothelial dysfunction by the progression of inflammation. In addition, high levels of Lp-PLA2 may also as a result of endothelial dysfunction due to impaired levels of the angiogenic and increased levels of the anti-angiogenic factors in eary stage of disease. Moreover, increased t-PA and decreased vitronectin levels may the results of compensatory mechanisms against disease progression.

Keywords: Lp-PLA2, PAI-1, Vitronectin.

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SUN-098
Association analysis of GSTO2 Asn142Asp genetic polymorphism and ischemic stroke risk in Turkish population

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Aim: Stroke is described as an interruption or severe reduction of the blood supply to cerebral arteries of brain. Free radicals are continuously produced during normal biochemical reactions and are known to cause oxidative stress in the body. Oxidative stress has an important role in initiation and pathogenesis of atherosclerosis leading to ischemic stroke. Glutathione S-transferases (GSTs) are a superfamily of polymorphic enzymes catalyzing the detoxification of metabolites produced by oxidative stress. The polymorphisms in genes coding GSTO2 have been shown to cause reduction in enzyme activity. Therefore, this study was aimed to investigate the possible association between GSTO2 Asn142Asp polymorphism and ischemic stroke risk in Turkish population.

Materials and Methods: Genomic DNA was isolated from whole blood samples of 239 ischemic stroke patients and 130 controls and the genotypes of subjects were determined by using PCR-RFLP technique.

Results: For the GSTO2 Asn142Asp (A→G) polymorphism, the genotype frequencies of wild type (AA), heterozygous genotype (AG) and homozygote polymorphic genotypes (GG) found in patient and control groups were found as 40.6%, 44.8%, 14.6% and 38.5%, 42.3%, 19.2%, respectively. The frequency of the wild type allele, A, was 0.63 and 0.596 and the frequency of the polymorphic allele, G, was 0.37 and 0.404, respectively in patients and controls. The polymorphic allele was not significantly associated with ischemic stroke in this study (OR = 0.866, $P = 0.118$ and CI: 0.724–1.037). This polymorphism was also analyzed in subgroups. Presence of the polymorphic allele increased stroke risk for diabetics and smokers.

Conclusion: No significant difference was found between patient and control groups with respect to A and G allele frequencies in GSTO2 Asn142Asp polymorphism. Therefore, it could be concluded that Asn142Asp polymorphism may not play an important role in the pathology of ischemic stroke in the studied Turkish population.

Keywords: GSTO2, ischemic stroke, polymorphism.

SUN-100
Association between TLR-3 gene polymorphism rs3775291 and progression of hepatitis C virus infection

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Background: Hepatitis C virus (HCV) is a major global health problem with about 210 million people infected worldwide, and constitute the most important cause of chronic liver disease. HCV is an enveloped positive-strand RNA virus belonging to the genus *Hepacivirus* of the family Flaviviridae. During the viral replication cycle, double-stranded RNA (dsRNA), produced as an intermediate, is sensed by several pattern recognition receptors (PRRs) of the innate immune system including Toll-like receptors (TLR). TLRs constitute a family of receptors playing a key role in innate and adaptive immune response, among them TLR3,-7 and -8, which are expressed on endosomal membrane, and have been suggested to play an important role in antiviral immune responses based on their recognition of dsRNA and single-stranded RNA (ssRNA). Single nucleotide polymorphisms (SNPs) may shift balance between pro- and anti-inflammatory cytokines, contributing to successful resistance to infection or leading to chronic inflammation and cancer. The aim of this study was to investigate the association between the TLR-3, -7 and -8 polymorphism and the outcome of HCV infection.

Materials and Methods: 517 patients were enrolled in the study and genotyped for the TLR3, -7 and -8 SNPs. Logistic regression was used to assess the association between the polymorphisms and the outcome of the infection.

Results: A significant association between TLR-3 SNP at rs3775291 and risk of advanced liver disease was identified. The rs3775291-A/A genotype was more common in subjects with advanced liver disease than subjects with mild chronic hepatitis C (OR = 3.81; 95% CI, 2.16–6.72; $p = 0.000004$) and this difference was higher with healthy controls (OR = 5.34; 95% CI, 2.70–10.58; $p = 0.000002$).

Conclusions: Our findings indicate that a TLR-3 SNP rs3775291 is associated with progression of HCV infection to cirrhosis and hepatocellular carcinoma.

Keywords: Functional Polymorphism, Hepatitis C Virus, Toll-Like Receptor-3.

SUN-101
Association of insulin resistance with serum interleukin-6 level during normal pregnancy

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Objectives: The purpose of this study was to evaluate the role of interleukine-6 (IL-6) in insulin resistance (IR) during normal pregnancy.

Approach: This cross sectional study was carried out on 86 healthy pregnant women including 26, 23 and 37 individuals in the 1st, 2nd and 3rd trimesters, respectively, and in 21 healthy non pregnant women. Serum IL-6 concentration was measured by Enzyme Linked Immunosorbent Assay (ELISA) method.

Results: There were significant differences between serumIL-6 level in pregnant women as compared with maternal healthy controls. There was significant correlation between gestational age and Body Mass Index (BMI) ($r = 0.28$, $P = 0.01$). There was no significant correlation between gestational age and insulin resistance (IR). We also did not find correlations between IR and TNF-α and IR and IL-6 in pregnant women.

Conclusion: In conclusion, our findings suggest that IL-6 are not greatly contributed to pregnancy induced insulin resistance in normal pregnancy.

Keywords: il-6, ir, pregnancy.

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SUN-102
Association of single nucleotide polymorphism rs10896449 on prostate cancer in Turkish population

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Prostate cancer is one of the most prevalent of all human malignancies and it is the second cause of death because of cancer among men in Turkey. Its incidence is rising rapidly in most countries. Age, ethnicity, diet, environment, lifestyle, family history, and genetic susceptibility are risk factors for the development of prostate cancer. It is well known that genetics play important roles in susceptibility to almost all human cancers. Recently, several single nucleotide polymorphisms (SNPs) associated with prostate cancer have been identified in genome-wide association studies. However, the effect of these SNPs on prostate cancer risk in Turkish population is largely unknown. In this study, genotyping of rs10896449 was conducted using the hME/iPLEX assay in study group which consists of prostate cancer patients and healty controls (total 175 individual). Per-allele odds ratio (OR) and 95% confidence interval (CI) were calculated between cases and controls. Associations between genotypes of rs10896449 and clinico-pathological variables of cases such as age at diagnosis, family history, PSA level, Gleason score, smoking and alcohol consumption were determined by using chi square test and Fisher's exact test. Results showed that rs10896449 variant significantly associated with prostate cancer and genotypes of rs10896449 were significantly associated with smoking in prostate cancer cases ($p \leq 0.05$).

Keywords: Prostate cancer, single nucleotide polymorphisms (SNP), SNP genotyping.

SUN-103
Autoantibodies in rheumatoid arthritis may arise from immunogenicity of peroxynitrite-modified IgG

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Peroxynitrite (PON) is a potent oxidizing and nitrating agent with a biological half life of approximately 10 ms. It is produced in vivo by diffusion-controlled reaction of nitric oxide and superoxide anion. It can oxidize and/or nitrate many amino acids causing changes in protein structure and function. The changes may lead to the pathogenesis of several inflammatory diseases including Rheumatoid Arthritis (RA). In this study, IgG was isolated from healthy subjects' sera on protein A-agarose affinity column and PON was synthesized by rapid quenched flow method. PON-modified IgG (PON-IgG) was prepared by incubating IgG with PON at 37°C for 30 min and maintaining pH at 10–11. Physicochemical alterations in PON-modified IgG were monitored by UV, fluorescence, CD and FT-IR spectroscopy, and SDS-PAGE. Oxidation and aggregation were assessed as free thiols, protein carbonyls, and thioflavin T and congo red binding. Nitrotyrosine, dityrosine and nitrotryptophan were also quantified. Formation of 3-nitrotyrosine was verified by LC-MS and HPLC, and attachment of PON to IgG was elucidated by MALDI-TOF mass spectrometry. PON-modified IgG exhibited hyperchromicity at 278 nm along with bathochromic shift and appearance of a new peak at 420 nm, decrease of tryptophan and tyrosine fluorescence, loss in β-sheet and appearance of new peak in FT-IR, compared to native IgG. SDS-PAGE results revealed concentration dependent decrease in the band intensity of PON-IgG compared to native IgG. Experimentally induced antibodies against PON-IgG showed high titre antibodies and specific binding may be due to generation of highly immunogenic neo-epitopes on PON-IgG. PON-IgG binding with circulating autoantinbodies of RA patients were compared with the experimentally induced antibodies against PON-IgG and its significance was analysed using biostatistical tools. The results so far suggest likely involvement of PON-IgG as an autoantigen in the induction and/or progression of RA.

Keywords: autoantibodies, peroxynitrite, rheumatoid arthritis.

SUN-104
Autotaxin and adiponectin expression in neuroinflammation and its effects on microglial cells

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Many chronic neurodegenerative diseases such as Amyotrophic lateral sclerosis, Alzheimer and Parkinson diseases have been associated with inflammation in the Central Nervous System (CNS). The balance between pro and anti-inflammatory factors is tightly controlled as dysregulation of this equilibrium may lead to progressive neurodegenerative disorders. Autotaxin (ATX) and adiponectin (ADIPO) have anti-inflammatory properties, but the precise mechanisms mediating this response in the CNS remains to be determined.

Here, we propose to use two distinct inflammatory stimuli to characterize the expression of ATX and ADIPO in mouse CNS. Acute intraperitoneal (ip) injection of lipopolysaccharide (LPS) (100 μg/Kg bwt) mimics gram negative bacterial infection, while acute ip injection of organometal trimethyltin (TMT) (2 mg/kg bwt), induces hippocampal neurodegeneration. Microglial cells are the major source of inflammatory factors in the brain and to investigate the role of ATX and ADIPO on these cells in inflammatory condition, we generated stable over-expressing transfectant in murine microglia BV2 cells for these two factors. BV2 and stably transfected, overexpressing clones were treated with LPS (1 μg/mL) and TMT (10 μM). The inflammation status and the expression of ATX and ADIPO in vivo and in vitro were determined by qRT-PCR.

Here, we show that ip injection of LPS in mice, results in an early response of TNFα and iNOS in the hippocampus, cortex and cerebellum with a peak at 2–4 hours, while ADIPO showed its expression peak at 6 hours only in hippocampus while no significant changes were observed for ATX. We confirmed that an acute ip injection of TMT induces an increase in TNFα mRNA (peak at 24 h). Elevated ATX and ADIPO mRNA levels in the hippocampus were demonstrated in mice 5 and 8 days respectively following the injection. ATX expression was significantly enhanced in LPS or TMT treated BV2. TNFα and IL-6 were inhibited in LPS or TMT treated BV2 overexpressing autotaxin, while IL-10 level was increased. Experiments with ADIPO overexpressing BV2 are in progress.

All together, these results demonstrated that ATX and ADIPO are expressed in vivo in the brain and that ATX inhibits microglia induced inflammation, suggesting that ATX and ADIPO could play a role in controlling neuroinflammation.

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Keywords: Adiponectin, Autotaxin, Neuroinflammation.

SUN-105
Beneficial effect of *Crocus sativus* stigma extract in amyloid-beta degradation by monocytes from sporadic Alzheimer's disease patients

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Alzheimer's disease (AD), the most common form of dementia in the elderly, is characterized by neurofibrillary tangles, extracellular amyloid-beta (Abeta) plaques, and neuroinflammation. The accumulation of Abeta in the brain of patients with sporadic Alzheimer's disease (SAD) seems to reflect a defective clearance. In this process, cells of monocytes-macrophage lineage exert a relevant role. Saffron, the dried stigma of *Crocus sativus* L., is a naturally derived plant product with several biological properties such as anti-inflammatory and antioxidant activity. The present study investigates the possible reversal effects of saffron in the reduced amyloid-beta degradation by monocytes of SAD patients.

Crocus sativus L. was collected from Umbria (Central Italy) and kindly provided by the *Associazione dello Zafferano di Cascia – Zafferano Purissimo dell'Umbria.*

Cytotoxicity of saffron extract was first assessed, and then amyloid-beta (1–42) HiLyte Fluortm 488 – labeled (FITC-Abeta42) degradation rate by monocytes after saffron treatment was analyzed by flow cytometry (Beckman Coulter EPICS XL). Monocytes from SAD subjects pre-treated with low concentration of saffron extract (5microM referred to *trans* crocine amount) showed an improved ability to degrade FITC-Abeta42 and an increased level of lysosomal protease Cathepsin B, one of the key Abeta42 degrading enzyme.

Even though more studies are needed to identify the active molecules, or their combinations, responsible for the enhanced Aβ42 degradation, these data suggest a potential beneficial effect of saffron on AD patients in terms of Aβ42 increased clearance.

Keywords: amyloid-beta, monocytes, Saffron.

SUN-106
Bioactive fractios of *Zanthoxylum acanthopodium* modulated biomarkers of obesity in 3T3-L1 preadipocytes

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Obesity is marked by chronic low-grade inflammatory response, excessive production of proinflammatory cytokines (interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α). Obesity is also linked by process called adipogenesis, which is differentiation of preadipocytes to adipocytes. Therapeutic strategy using spice-derived compounds which are naturally occurring phytochemicals and biomolecules may elicit antiobesity properties. In this study, the antiobesity effect of bioactive fractions, i.e. essential oil and polyphenol fractions, isolated from lemon pepper (*Zanthoxylum acanthopodium*) was evaluated through modulation of proinflammatory cytokines, adipogenic enzymes, and adipogenic transcription factors in 3T3-L1 preadipocytes in vitro. Bioactive fractions of essential oils and polyphenols from lemon pepper were done by using solvent extraction and identified for their contents by using chromatographic technique (pyGC-MS). Cytotoxicity of lemon pepper fractions (1–100 μg/ml) was measured using MTT assay. Bioactive lemon pepper fractions at 1–25 μg/ml were tested for their anti-obesity effects on the modulation of IL-6, IL-1b, TNF-a, leptin, adiponectin, sterol regulatory element-binding protein (SREBP)-1, and MCP (monocyte chemoattractant protein)-1 at protein and gene levels in 3T3-L1 preadipocytes by conducting ELISA and Real Time-PCR assays. ELISA profiles demonstrated that both essential oil and polyphenol fractions from lemon pepper dose-dependently upregulated adiponectin and leptin levels. Meanwhile, bioactive fractions decreased the expression of IL-6 protein. Secretion of leptin and adiponectin was tended to increase in 3T3-L1 preadipocytes when treated with essential oil and polyphenol fractions. SREBP-1c is known for its role as adipogenic transcription factor, while IL-1β, IL-6, and MCP-1 are major adipocytokines that promote inflammatory action in adipocytes. In order to know whether bioactive fractions has anti-adipogenic activity, the mRNA levels of SREBP-1c and proinflammatory cytokines after treatment were quantified. RT-PCR profiles showed that SREBP-1c mRNA was significantly decreased by lemon pepper fractions of essential oil and polyphenol. Both fractions were also found to suppress the mRNA expression of proinflammatory cytokines, including IL-6, IL-1b, and MCP in 3T3-L1 preadipocytes. In summary, these results indicate that essential oil and polyphenol fractions derived from lemon pepper can be applied for natural materials for weight reducing diet via modulating the expression of proteins and genes regulating obesity.

Keywords: 3T3-L1 preadipocytes, antiobesity effect, *Zanthoxylum acanthopodium.*

SUN-107
Biohybrid LeukoLike vector molecular characterization to improve the therapeutic efficacy and biocompatibility of current drug delivery systems

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The ultimate goal of nanomedicine is to improve the therapeutic biocompatibility and targeting while decreasing the potential side effects. In order to provide drug delivery systems with the aforementioned properties, various surface modification strategies based on antibodies, polymers, and peptides were developed. However, multiple surface modifications increased the complexity of the synthesis process and were very often incompetent in avoiding the immune response activation and the targeting of the diseased tissue. Bio-inspired approaches to develop a new generation of drug delivery system is currently being investigated to enable synthetic particles to achieve functional targeting and

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increase carrier biocompatibility. LeukoLike Vectors (LLV) are nanoporous silicon particles coated with purified leukocyte membranes. These were shown previously to inhibit immune system recognition and possess superior targeting ability towards the inflamed endothelium. The coating composition was characterized and the results unveiled self-tolerance biomarkers and targeting receptors through the Western Blotting assay and high-throughput proteomic analysis. These were performed to identify the proteins that can be purified from leukocyte membranes and consequently, transferred onto the silicon surface. We demonstrate an enrichment of leukocyte membrane proteins on the LLV surface in their intact native, active configuration with the appropriate post-translational modification and orientation. Among them, CD45 favors extended circulation time and avoids unspecific clearance, while Leukocyte Associated Function-1 (LFA-1) facilitates the targeting to and permeability of the tumor inflamed vasculature. These findings will advance the field of bio-engineering to provide a strategy to enhance the therapeutic efficacy of many drug delivery systems.

Keywords: drug delivery, naonomedicine, proteomics.

SUN-108

Biological activities of Moroccan vipers' venoms and their purified fractions

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In Morocco, ophidian envenomation are often attributed to the bites of the Moorish viper (*Macrovipera mauritanica*), the horned viper (*Cerastes cerastes*), and sometimes to snakes of the genus Bitis and Echis. The diagnosis of these types of bites is mainly based on the recognition of local or systemic signs of bleeding, an acute inflammation (pain, swelling) and necrotic complications at the bite site that can spread to internal organs.

To determine the biological activities of the venoms of Moroccan vipers, we conducted a biological and toxicological characterization of the venom of *Macrovipera mauritanica* (VMm) and *Cerastes cerastes* (VCc) and their purified fractions the venom by using chromatographic techniques and in vivo and in vitro physiological tests.

The results of this work showed a similar toxic power between the two venoms. Only fractions VCF1 and VCF2 from VCc venom and VMF1, VMF2 and VMF3 from VMm venom feature toxic and biological activities. Both venoms and their toxic fractions have shown hemorrhagic, necrotic, edematous, myotoxic, coagulant, proteolytic, hemolytic and phospholipasic activities.

Microscopic studies of intradermal and muscle section from mice envenomed by VCc and VMm venoms and their toxic fractions showed signs of intense hemorrhage and necrosis manifested by increased thickness of the epidermis due to edema, disruption of muscle fibers, the occurrence of bleeding sites and inflammatory infiltrates.

The diversity of biological effects of venoms and their toxic fractions confirms the complexity and richness of Moroccan vipers' venoms by proteolytic enzymes responsible for the pathophysiological effects observed in viper envenomation.

Keywords: biochemical characterization, Biological activity, Vipers venom.

SUN-109

Bone-marrow derived cells expressing TLR4 are important for the induction of obesity-associated hypothalamic inflammation

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Dietary long-chain saturated fatty acids are known to induce hypothalamic inflammation and dysfunction through a mechanism dependent on the activation of microglial TLR4. Diet-induced hypothalamic inflammation contributes to the progressive loss of the coordinated control of food intake and energy expenditure, resulting in obesity. Thus, characterizing the mechanisms involved in the generation of diet-induced hypothalamic inflammation may provide an important advance in the understanding of the pathophysiology of obesity. TLR4 mutant mice are protected from diet-induced hypothalamic inflammation and obesity. Transplanting bone marrow derived cells harboring a functional TLR4 to TLR4 mutant mice recapitulates diet-induced hypothalamic inflammation and obesity, suggesting that bone marrow derived cells are important components of the hypothalamic inflammation associated with obesity. Here asked if the presence of a functional TLR4 in bone marrow derived cells would modulate markers of inflammation, chemotaxis and apoptosis in the hypothalamus of mice fed on high fat diet. TLR4-mutant ($TLR4^{-/-}$) and wild-type (WT) mice were irradiated in a cobalt 60 source and submitted to bone marrow transplantation (BMT). $TLR4^{-/-}$ received BM from WT and vice-versa. After recovery, mice were fed on a HFD or standard chow for 8 weeks. To evaluate inflammation and quimiotaxy markers, RT- PCR was performed. We also investigated in microglia (BV-2) and neurons (CLU189) cell culture lineage the mechanism and temporal inflammatory process initiation after palmitic acid (PA-500uM) time-course treatment (by RT-PCR and immunoblotting). In mice, when TLR4 mutant mice received BM from WT donors, microglial cells harboring WT-TLR4 were detected in the hypothalamus after 8 weeks. Differently of TLR4 mutant mice, which are protected from diet-induced obesity (DIO), these chimeric mice presented increased body weight when fed on HFD. So, functional TLR4 expression is required to warrant of adiposity increase during HFD. Fractalkine (CX3CL1) presented highest and earliest RNAm levels in DIO mice hypothalamus. In cell culture, neurons cells produced the highest levels of the CX3CL1 RNAm after 6 h of incubation with supernatant from BV-2 cells previously treated with PA. Bax apoptotic protein expression was increased after 12 h following by decreased Bcl-2 anti-apoptotic protein expression, suggesting that microglial immune factors are quickly released in high-levels after PA stimulation. Thus, hypothalamic monocytic cells involved in hypothalamic inflammation in obesity are derived from the bone marrow and depend, at least in part from neuron-produced fractalkine.

Keywords: fatty acids, Inflammatory response, obesity.

SUN-110

Can cathepsin-D a new inflammation biomarker in detection of lysosomal storage diseases?

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Background: Lysosomal storage diseases (LSDs) comprise a group of at least 50 distinct genetic diseases and the incidence is about 1:7000. LSDs are characterized by inappropriate lipid storage in lysosomes, due to specific enzyme deficiencies or associated

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cofactors as a result of a mutation in encoding genes of lysosomal enzymes. The lysosome is commonly referred to as the cell's for intracellular digestion and recycling of macromolecules. When the lysosome doesn't function normally, excess products destined for breakdown and recycling are stored in the cell. Commonly, chitotriosidase activity measurement is a method of impression in the treatment of lysosomal storage diseases. But, approximately 6% of white race population has no plasma chitotriosidase activity as a result of a mutation and different tests may require for LSD diagnosis. In addition, chitotriosidase levels may be low in the monitoring of treatment. We aimed to investigate/compare possible markers (Cathepsin-D and chitotriosidase) in diagnosing/monitoring of two different lysosomal storage diseases (Gaucher and Niemann Pick).

Methods: Blood samples into EDTA tubes and dried blood specimens were collected from 43 subjects (between 0 and 50 years old) as 25 patient (17 Gaucher, 8 Niemann Pick) and 18 healthy individuals. Plasma chitotriosidase enzyme activities were determined fluorometrically in all subjects. Plasma chitotriosidase enzyme protein levels were also determined by using spectrophotometric ELISA kit. Cathepsin-D activities were determined by using Cathepsin-D ELISA kit. The test results were analyzed statistically by means of the SPSS 18 Program.

Results: Plasma Chitotriosidase enzyme activities and Chitotriosidase enzyme protein levels were statistically significant higher ($p < 0.001$) in patients (Gaucher disease: 6226.36 ± 4935.52 ng/mL/h, 10.63 ± 5.59 ng/mL, Niemann Pick disease: 2548.3 ± 1359.18 ng/mL/h, 11.54 ± 2.78 ng/mL) when compared with control group (83.3 ± 66.2 ng/mL/h, 0.62 ± 0.28 ng/mL). Cathepsin-D activities were also statistically significant higher in patients (Gaucher disease: 348.57 ± 130.27 ng/mL, Niemann Pick disease: 455.24 ± 201.91 ng/mL) when compared with control group (157.24 ± 30.74 ng/mL), $p < 0.001$.

Conclusions: Chitotriosidase enzyme activities were comparable with chitotriosidase enzyme proteins in plasma, The enzyme activities in plasma samples seems were more reliable than DBS samples which having high variation. While It might be suggested to measure plasma chitotriosidase activities for Gaucher patients, Cathepsin-D seems as better indicator for Niemann Pick disease. The relationship between the disease severity and the enzyme activities is subject to further research.

Keywords: Cathepsin-D, Chitotriosidase, LSDs.

SUN-111

Can CD15s (sialyl Lewis x) be used as a potential marker for neonatal sepsis? Control study. Split, Croatia

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Objective: Neonatal sepsis carries a high mortality when diagnosed late. Early diagnosis is difficult because initial clinical signs are nonspecific. Consequently, physicians frequently prescribe antibiotic treatment to newborn infants for fear of missing a life-threatening infection. Current biomarkers are not infallible, however, and do not permit neonatologists to withhold antibiotics in sick neonates with suspected infection. In response to infection, circulating leukocytes tether to the vessel wall and then roll. Only certain adhesion molecules including (E) selectins and some integrins have been found to support rolling. E-selectins bind to carbohydrate ligands on leukocytes in which the sialyl Lewis x (sLex, CD15s) moiety is of key importance. The aim of this pilot study was to determine a diagnostic value of CD15s in detection of early onset neonatal sepsis.

Methods: A total of 25 neonates born in minimum 37th gestation week and <72 hours before sampling were enrolled in the study. One group of neonates had clinical signs or obstetric risk factors suggesting neonatal sepsis. The control group included healthy neonates with physiological hyperbilirubinemia and without clinical signs of infection. The percentage of CD15s+ leukocytes was measured by flow cytometry using monoclonal antibody. Data were analyzed using WinMDI v.2.9 software. Statistical differences among the groups were analyzed by t-test using MedCalc v.12.5 software.

Results: Statistically significant differences in percentage of CD15s+ total leukocytes, monocytes and granulocytes were observed between control and case group. We found an increased percentage of CD15s+ monocytes and granulocytes in neonates with suspected sepsis ($P < 0.0001$ and $P = 0.0338$, respectively). There was no statistically significance in percentage of CD15s+ total leukocytes between groups.

Conclusions: Recent research in immunology has led to the discovery of cell surface antigens, chemokines, cytokines and acute phase proteins that can potentially be used to 'rule in' or 'rule out' sepsis. These preliminary data show that CD15s has a diagnostic potential for an early identification of neonatal sepsis. Further studies with greater number of samples are required for potential inclusion of this marker in the currently used screening panels for the early diagnosis or the infection treatment in the neonates.

Keywords: Neonatal sepsis, Biomarkers, CD15s.

SUN-112

Capparis ovata water extract (MSCov) for the potential treatment of multiple sclerosis without drug interaction: molecular evidences

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This study evaluates the effect of *Capparis ovata* water extract (*MSCov*) on the experimental animal model of multiple sclerosis at the molecular level and on the expression of drug metabolizing enzymes. For this purpose, *MSCov* was prepared by using the plant's fruit, bud and flower parts (Turkish Patent Institute, PT 2012/04 093). Animal MS model, experimental allergic encephalomyelitis (EAE) was induced in 6–8 weeks old C57BL/6 mice. *MSCov* was given to EAE-induced mice intragastrically for 21 days, at 500 mg/kg. At the end of the experimental period, the animals were sacrificed, the brain and liver tissues were isolated. The expression at the mRNA level was detected by qRT-PCR in brain and liver tissues of control, EAE and EAE+*MSCov* treated animals. Differential expression of MS marker genes such as 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP), myelin associated glycoprotein (MAG), myelin basic protein (MBP), matrix metalloproteinase-2 (MMP-2), nuclear factor kappa B p50 and p65 (NF-kB p50 and p65), proteolipid protein (PLP), peripheral myelin protein (PMP) and tumor necrosis factor-α (TNF-α) and various drug metabolizing genes such as CYP1A1, 1A2, 2A4, 2B9, 2C37, 2C39, 2D9, 2D22, 2E1, 2F2, 2J5, 3A11, 4A14, 7A1, 7B1 were measured. We have observed that the expression levels of CNP, MAG, MBP, PLP and PMP22 was reduced 20%, 45%, 40%, 23% and 32%, respectively in EAE, which was returned to control levels by 14%, 65%, 32% 60% and 72%, respectively with *MSCov* treatment. In addition, it was observed that *MSCov* treatment reduced the 17%, 45%, 13% and 62% of induced MMP-2, NF-kB p50, NF-kB p65 and TNF-α expression levels seen in EAE by 100%, 79%, 100%, and 35%, respectively. Moreover, *Capparis ovata* extract did not significantly elicit the any major CYP450 enzyme expression. These result strongly suggest that *MSCov* has high potential as an alternative or com-

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plementary therapeutics in MS treatment with no possible drug interaction.

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Keywords: multiple sclerosis, *Capparis ovata*, drug interaction.

SUN-113
Catalase, carbonic anhydrase and xanthine oxidase activities in patients with mycosis fungoides

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Mycosis fungoides (MF) is the most common form of cutaneous T-cell lymphoma. In several studies the relationship between catalase (CAT), human cytosolic carbonic anhydrases (hCA-I and hCA-II) and xanthine oxidase (XO) enzyme activities has been investigated in various types of cancers but carbonic anhydrase, catalase and xanthine oxidase activities in patients with MF have not previously been reported. Therefore, in this prelimanary study we aim to investigate CAT, CA and XO activities in patients with MF. This study was enrolled with 32 patients with mycosis fungoides and 26 healthy controls. According to the results, CA and CAT activities were significantly lower in patients with mycosis fungoides than controls ($p < 0.001$) ($p < 0.001$). There was no significant difference in XO activity between patient and control group ($p = 0.601$). Within these findings, we believe these enzyme activity levels might be a potentially important finding as an additional diagnostic biochemical tool for mycosis fungoides.

Keywords: Carbonic Anhydrase, Catalase, Mycosis Fungoides.

SUN-114
CD14-enriched microregions of the plasma membranes as places of PI(4,5)P2 accumulation in LPS-stimulated macrophages

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Lipopolysaccharide (LPS) of Gram-negative bacteria induces pro-inflammatory responses of macrophages upon binding to Toll-like receptor 4 (TLR4)/MD-2 complex. Once activated, TLR4 triggers two signaling pathways which engage Mal/MyD88 or TRAM/TRIF adaptor proteins and are initiated at the plasma membrane and after endocytosis of TLR4, respectively. Binding of LPS to TLR4/MD-2 complex is facilitated by CD14, a GPI-linked protein anchored in cholesterol- and sphingolipid-rich microdomains of the plasma membrane, named rafts. A line of studies, including those of our group, indicated also that CD14 controls internalization of LPS and TLR4.

To reveal mechanisms underlying involvement of CD14 in LPS-triggered signaling, we followed dynamics of CD14 and PI(4,5)P_2 lipid, the latter known for its versatile functions, including recruitment of Mal/MyD88 adaptor proteins. LPS-stimulation of cells led to a moderate elevation of the amounts of CD14 on the surface of J774 cells, as indicated by laser scanning cytometry analysis. Confocal microscopy analysis indicated that LPS can up-regulate trafficking of CD14 from Golgi apparatus toward the plasma membrane. In agreement, biochemical analysis revealed that CD14 was enriched in raft fractions at the onset of cell stimulation with LPS.

To follow LPS-induced redistribution of CD14, we performed ultrastructural analysis of CD14 localization in the plane of sheets of the plasma membrane obtained by mechanical cleavage of J774 cells and subjected to immunogold labeling. Stimulation of cells with 100 ng/ml LPS induced rapid and transient clustering of CD14 in the plasma membrane. Double immunogold labeling revealed that CD14 aggregates co-localized transiently with PI(4,5)P_2 and its precursor PI(4)P. Specificity of LPS action was confirmed using antibody which inhibited binding of LPS to CD14 – no clustering of CD14 or PI(4,5)P_2 was found in these conditions. To reinforce the ultrastructural data, we developed a technique of PI(4,5)P_2 measurements based on binding of a fusion protein, GST-tagged PH domain of phospholipase C, to extracts of cellular lipids. Such quantitative analysis showed that the PI(4,5)P_2 level in cells slightly rose after short time of LPS action and increased significantly after 1 hour of cell stimulation with LPS. PI(4,5)P_2 level in cells was strongly diminished by LiCl which inhibited also LPS-induces production of pro-inflammatory mediators.

Taken together, our studies indicate that during stimulation of cells with LPS, CD14-enriched regions of the plasma membrane can serve as platforms for the initial generation of PI(4,5)P_2 which, in turn, plays essential role in signaling cascades of TLR4.

Keywords: CD14, lipopolysaccharide, phosphatidylinositol 4,5-bisphosphate.

SUN-116
Changes of serum alpha2-macroglubulin levels in rats induced acute inflammation

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α_2-macroglubulin (α2M) is a typical acute phase protein in rats. We investigated the characters of α2M in rats induced acute inflammation. The relationship between intensity of inflammatory stimulation and production of α2M in rats and the synthesis of α2M in hepatopathic rats were investigated. Sprague-Dawley rats were injected with turpentine oil at doses of 0.05, 0.2 or 0.4 ml/rat. Serum levels of α2M, interleukin (IL)-6 and cytokine-induced neutrophil chemoattractant-1 (CINC-1) were measured by ELISA. Hepatopathy was induced by oral administration of acetaminophen at a dose of 1 g/kg daily for 2 weeks or a 25% solution of carbon tetrachloride (CCl4) at 2 ml/kg body weight three times per week for 7 weeks. Acute inflammation was induced by intramuscular injection of turpentine oil at a dose of 1.0 ml/kg body weight. Peak serum levels of α2M and in rats injected at 0.05 ml/rat were significantly lower than those at 0.2 or 0.4 ml/rat. However, no significant difference was observed for peak serum levels of these acute-phase proteins between 0.2 and 0.4 ml/rat. Furthermore, peak serum levels of IL-6 and CINC-1 in rats injected at 0.05 ml/rat were significantly lower than those at 0.2 or 0.4 ml/rat. Thus, the production of α2M have upper limit even if increased the strength of inflammatory stimulation. Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and total protein differed significantly between acetaminophen or CCl_4-induced hepatopathic rats and acetaminophen-control (AA-control) or CCl4-control (CC-control) rats. Furthermore, pathological examination confirmed hepatopathy in rat livers. Peak serum concentrations and area under the time-concentration curve for α2M showed significant differences between hepatopathic rats and AA- or CC-control rats. Thus, serum concentrations of α2M did not increase when compared with non-treated rats. α2M is considered to be an useful as an acute phase protein in rats.

Keywords: inflammation, rats.

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SUN-117
Characterization of a novel protein kinase D1 inhibitor using different inflammatory cell models

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Inflammatory diseases have severe effects on healthcare worldwide. Protein kinase D1 (PKD1) a member of protein kinase D family is a well characterized and validated target in inflammatory diseases. In endothelial cells PKD1 is a member of VEGFR2 signaling pathway, which has a critical role in angiogenesis and related inflammatory diseases. In neutrophils PKD1 has a positive regulatory role in superoxide production through Fcγ-receptor induced NADPH activation.

The aim of this study was to identify a novel PKD1 inhibitor drug candidate and characterize its effects on different inflammatory cellular models.

First applying recombinant kinase assay we screened a kinase specific inhibitor library against protein kinase D1 and identified some small molecule PKD1 inhibitor. We selected the best one for further investigation. Then we tested the effect of this inhibitor on different cellular assays.

Treatment with the inhibitor effectively inhibited the intracellular activation of PKD1 and also the proliferation of endothelial cells. Our compound was also an efficient inhibitor in in vitro angiogenesis assays interfering with endothelial cell wound healing and tube formation processes.

Inflammatory cellular model such as neutrophils were also applied to prove the anti-inflammatory effect of the inhibitor. The neutrophil study showed that the inhibitor could specifically diminish the immune-complex stimulated adhesion dependent neutrophil activation.

In the present study, we have identified and characterized a small molecular inhibitor of PKD1, which could efficiently inhibit pathological inflammatory pathways involving PKD1.

Keywords: Inflammation, inhibitor, PKD1.

SUN-118
Characterization of PERK-directed endoplasmic reticulum stress response to lipids

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Pathways that lie at the interface of metabolism, inflammation and stress have a profound influence on Cardiometabolic Syndrome (CMS). Metabolic overload initiates a chronic inflammatory response that promotes the complications of CMS. However, the molecular mechanisms linking metabolic stress to immune activation remain elusive. Earlier studies demonstrated metabolic overload leads to endoplasmic reticulum (ER) stress and activate the unfolded protein response (UPR). The UPR is essentially an adaptive signaling emanating from the ER to cope with cellular stress. In addition to its protective responses and role in ER biogenesis, the UPR can also impinge on inflammatory signaling. Prolonged ER stress is detrimental and contributes to obesity, diabetes and atherosclerosis in part by activating pro-inflammatory and pro-apoptotic pathways. Moreover, the existence of inflammatory mediators, chemotactic cytokines, reactive oxygen species and other stress signals in obesity make it even more challenging for the ER to establish and maintain a healthy metabolic equilibrium. Clarifying the regulatory mechanisms and novel players in a metabolically-driven ER stress response is needed to develop specific, new therapeutic strategies for CMS. Here, we investigated the UPR that can sense excess lipids and couple to inflammation, and whether its unique operation under metabolic stress can be suitable for therapeutic exploitation in CMS. We adapted a chemical-genetic approach to probe the full range of substrates of a proximal UPR kinase, protein kinase RNA-like endoplasmic reticulum kinase (PERK), during metabolic stress. This method, which yields a monospecific inhibitor or activator of a kinase involves targeting of a conserved 'gatekeeper' amino acid residue in the ATP binding pocket to a small amino acid, enlarging the pocket sufficient enough to accommodate bulky ATP analogs. Then PERK's potential substrates are identified by coupling this approach with proteomics. Furthermore, by using the same approach to specifically modify PERK kinase activity in macrophages, we investigated its direct contribution to inflammation. Our findings show that PERK is a critical modifier of lipid-induced ER stress and inflammation and substantiated through studies using complementary approaches to block PERK activity such as RNAi-directed knock-down and a specific inhibitor. The results of this study demonstrate the individual contribution of the PERK branch of the UPR to lipid-induced inflammation and underscore the importance of delineating specific UPR targets that could be therapeutically modified to treat CMS.

Keywords: cardiometabolic syndrome, Endoplasmic reticulum stress, inflammation.

SUN-119
Cigarette smoke extract induces expression of HSP27 and HSP32 but not HSP70 in A549 alveolar epithelial cells

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Heat shock proteins (HSPs) are highly conserved proteins involved in folding of native and damaged cell proteins. HSPs participate in cellular responses to stress and can be induced by various stress conditions, including high and low temperatures, reactive oxygen species, hypoxia, infection, inflammation etc. Cigarette smoke is known to contain many different oxidants and reactive oxygen species, thereby causing oxidative stress, chronic inflammation and severe damage to proteins in the lungs of smokers and patients with chronic obstructive pulmonary disease (COPD).

The aim of this study was to find out whether exposure of A549 alveolar epithelial cells to increasing concentrations (1.25; 2.5; 5; 10 and 20%) of cigarette smoke extract (CSE) during 4, 6, 8, 24 and 48 h activates cellular defense mechanisms, including expression of HSP27, HSP32 (also known as heme oxygenase 1) and HSP70 in response to accumulation of damaged proteins.

Our study shows that exposure of A549 cells to 20% CSE for 24 h produced considerable damage to cellular membranes with increased permeability and leakage of LDH into the incubation medium, as compared to untreated cells ($P = 0.01$). Metabolic activity explored by MTT test was reduced at the same time point ($P < 0.01$). In addition, AnnexinV – propidium iodide double staining confirmed that 20% CSE during 24 h is causing primarily necrotic cell death ($P < 0.001$). However, cellular defense mechanisms were only partially activated. Expression of HSP32

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was induced 8 h after incubation with 20% CSE ($P = 0.029$) and 24 h after incubation with 10% and 20% CSE ($P = 0.019$). HSP27 was induced with 20% CSE only after 48 h ($P = 0.012$). Unexpectedly, we could not detect significant changes in HSP70 expression ($P > 0.05$).

The unchanged level of HSP70 expression might reflect lack of induction, or increased release of HSP70 from the damaged, necrotic cells.

We conclude that CSE-induced stress produces only limited activation of HSPs response indicating disturbed cellular defense mechanisms. It is possible that chronic exposure to CSE leads to the depletion of HSP-related defense mechanisms and contributes to the abnormal inflammatory response observed in lungs of smokers and COPD patients.

Keywords: chronic obstructive pulmonary disease, cigarette smoke extract, heat shock proteins.

SUN-120
Circulating biomarkers assessment for early diagnosis of vascular calcification in chronic kidney disease

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Background: Chronic kidney disease (CKD) describes the gradual loss of kidney function that typically evolves over many years due to its clinically silent behavior. CKD is associated with an increased inflammatory condition, which involves complex interactions among immune cells and soluble proteins. Accelerated vascular calcification (VC) is an important and devastating complication of CKD and contributes to the high mortality in these patients.

The aim of the present study is to assess a novel biomarker panel particularly useful for identification of VC early phases in CKD patients, having important consequences on the therapeutic interventional strategies, prognosis, and life-expectancy of patients with CKD.

Material and Method: Serum samples of 29 CKD patients (in stages II-IV, not undergoing dialysis) and 10 normal controls were analysed to simultaneously measure the level of 8 biomarkers (IL-6, IL-1β, TNFα, OPG-osteoprotegerin, OC-osteocalcin, OPN-osteopontin, PTH, FGF-23 – Milliplex MAP Human Bone Magnetic Bead Panel) using xMAP technology. Multiplexed data acquisition was performed on Luminex 200 platform using xPONENT 3.1 version.

Results: Mineral metabolism candidate biomarkers and molecules that actively regulate VC process (OPG, OC, OPN, PTH and FGF-23 respectively) showed an increased circulating level compared with control – between 1.66 and 12.37 fold higher, $p < 0.05$. The pro-inflammatory cytokines level (TNFα, IL-6) was also increased in CKD patients compared with control (1.96 and 7.03 fold higher, $p < 0.05$), with the mean values of 2.73 pg/mL and 6.96 pg/mL respectively in the CKD group, while for IL-1β no trend was visible so far. It has also been observed a positive correlation between the circulating biomarkers level and the stages inside the CKD group.

Conclusion: Detecting VC through its severe clinical manifestations may turn to be too late for any therapy to halt its progression or to reverse it. Thus, it would be particularly useful to develop a specific biomarker panel for identification of early phases of VC and for scoring its severity in CKD patients, which would allow developing further strategies aiming to control and even reversing some of the processes.

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Keywords: chronic kidney disease, circulating biomarkers.

SUN-121
Circulating endothelial microparticles in patients with chronic hepatitis C infection

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Aim: Endothelial microparticles (EMPs) can be involved in inflammatory process, blood coagulation, and regulation of vascular function. Inflammation and endothelial dysfunction have been reported in patients with chronic hepatitis C (CHC) infection, but their influence on circulating EMPs levels and diabetes prevalence remains unknown.

Methods: Seventy-four CHC patients, 30 with type 2 diabetes, and 40 healthy controls were enrolled in the study. Circulating levels of EMPs, ischemia-modified albumin (IMA), pro-inflammatory cytokines (interleukin-6, and tumor necrosis factor α), and high-sensitivity C-reactive protein (hsCRP) were assessed.

Results: Compared with the controls, the CHC patients with diabetes showed a significant increase in plasma concentrations of circulating of EMPs, IMA, tumor necrosis factor α and hsCRP ($P < 0.001$). The values of EMPs and IMA were more elevated in patients with diabetes than without diabetes (both $P < 0.01$). The positive relationships were found between tumor necrosis factor α and EMPs levels ($P < 0.01$) and the presence of diabetes ($P < 0.001$). Significant positive correlations between IMA and hsCRP levels and between EMPs and hsCRP levels were found in all patients with CHC infection.

Conclusion: We documented that significant elevation in plasma EMPs levels is associated with diabetes prevalence and increased inflammatory response reflected in tumor necrosis factor α levels in patients with chronic hepatitis C infection.

Keywords: diabetes mellitus, endothelial microparticles, HCV infection.

SUN-122
Clinical implication of afamin and its SNP rs4694619 G>C as a novel diagnostic marker for breast cancer

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Comparative proteomics identified the vitamin E binding plasma protein, afamin, as a potential novel tumor marker. We aimed to identify the diagnostic utility of afamin in detection of breast cancer. Moreover, genome-wide association studies of breast cancer revealed that single nucleotide polymorphisms (SNPs) in some genes with novel association to disease susceptibility. Thus we attempt to study SNP in afamin gene (SNPrs4694619). Plasma concentrations of afamin using ELISA assay was measured in 240 breast cancer patients, 80 benign cases, and 60 controls and compared the results with conventional breast tumor markers; CA15.3, CEA, and angiogenesis; VEGF and NO. Genotyping of afamin rs4694619 G> C polymorphism was identified using poly-

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merase chain reaction (PCR). Afamin median concentrations were superior in healthy controls followed by benign cases and breast cancer patients (136, 105, 26 μg/mL, respectively, at $P < 0.001$). Receiver operating characteristic curve was used for differentiating malignant- from non-malignant breast patients, revealed 98.6% specificity, 98.3% sensitivity for afamin versus specificities at 91.7% and 98.3%, and sensitivities at 87.5% and 96.7% for CEA and CA 15.3, respectively. Concordance between investigated markers was statistically significant. Hetero-mutant SNP rs4694619 was significantly expressed in breast cancer patients as compared to either homo-wild or mutant SNP.

Conclusion: Afamin added independent diagnostic information to CEA and CA 15.3 for differentiating breast cancer from benign and healthy samples. Afamin and its SNPrs4694619 might have the potential to become valuable biomarker for diagnosis of breast cancer.

Keywords: Afamin, breast cancer, polymorphism.

SUN-124

Comparative study of the biochemical and biological properties of *Bothrops jararaca* snake venoms milked and stored from 1963 up to 2008

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Introduction: Bothropic envenomation is characterized by proteolytic, coagulant and hemorrhagic activities. Variations in the snake venom composition may cause an impact on primary venom research and antivenom production. Considering the potential applications of snake venom research and antivenom production, it is of huge importance that venom samples are stored correctly after collection. Proper storage may limit the need to collect additional samples from wild snakes. Additionally, as some snake species are infrequently encountered, it might be impossible to collect additional samples in the future. Studies indicate that, in order to preserve the biologic and enzymatic activities, venoms may be lyophilized and frozen. However, the effect of long-term storage conditions is not fully elucidated.

Fig. 1. *Bothrops jararaca* snake.

Objectives: The present study aims to investigate the longevity of the biochemical and biological activities present in *B. jararaca* venom.

Materials and Methods: Protein profile of *B. jararaca* venom from 1963, 1973, 1977–88, 1997 and 2008 was analyzed by HPLC (C18 column – ACE). The minimal coagulant dose (MCD) was determined upon human plasma and bovine fibrinogen. The proteolytic activity was evaluated using casein and gelatin as substrates. The immunogenicity was assessed by the immunization of Balb/C mice using *B. jararaca* venom and Al_2OH as adjuvant. Serum titers was determined by ELISA assay.

Results and Discussion: Venoms analyzed showed similar HPLC profile, with some differences in the relative abundance of molecules. The MCD-plasma is quite equivalent among the venoms, while the MDC-fibrinogen increases with the time of storage. The proteolytic profile was also similar, but the proteolytic active varies among the venoms. The same was observed for the venom immunogenicity, which differs among the venoms.

Conclusion: The results suggest that some venom activities may be affected by the period and conditions of storage. For further elucidation of this study, other experiments related to the biological activities will be performed.

Keywords: *Bothrops jararaca*, longevity, snake venom proteins.

SUN-125

Comparison of 'tim' gene of *Giardia lamblia* in laboratory animals and human and the importance of cross transmission probability in Iran

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Background: Variably divided into two or three genotypes by different investigators, and each group can be divided into subgroups. *Giardia* has the ability to infect many mammals including the dog, cat, deer mouse, ground squirrel, chinchilla, swine, rabbit, pocket mouse, ox, guinea pig, and humans *Giardia lamblia* (also *Giardia duodenalis*, *G. intestinalis*) isolates have been.

Objective: The aim of the present study was to biotype groups of *G. lamblia* in laboratory animals which were used in research and the possibility of cross transmission between human and these animals and the importance of this transmission in research which was performed for the first time in Iran .Comparison of triose phosphate isomerase (tpi) sequences of these genotypes by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) was studied to determine *G. lamblia* genotype.

Methods: In this study 4 sets of primers were used in which 2 sets were designed by other investigator and 2 sets were designed by us to confirm the results of the first two primers and also to differentiate the subgroups.

Results: Two sets of primers were designed to differentiate between *Giardia* isolates. The PM924 primer set were used to differentiate between AC#L0210 (WB) and AC#U57897 (JH) isolates, and PM290 primer set were used to differentiate between C#AF069559, AC#AF069558. Among Giardia isolates, 20% and 5% of PCR-RFLP of rabbit and mouse was amplified with primer PM290 respectively.

Conclusion: There is an evidence that suggests the direct transmission from companion animals to human does occur. Zoonosis is controversial regarding *Giardia*, but most researchers believe that its zoonotic potential merits adequate precaution when working with feces of animals that may be infected.

Keywords: zoonose, *Giardia lamblia*, PCR- RFLP.

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SUN-126
Comparison of hERG channel blocking effects of anti-arrhythmic mexiletine and its metabolite m-hydroxymexiletine (MHM)

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Mexiletine, 1-(2,6-dimethylphenoxy)-2-propanamine, is a class IB antiarrhythmic drug. In addition to well-known clinical application in treating ventricular arrhythmias, recent studies have suggested the use of mexiletine for the treatment of neuropathic pain and myotonia. However mexiletine was reported to cause a number of adverse effects such as nausea, hypotension, sinus bradycardia, paresthesia, atrioventricular heart block, ventricular arrhythmias.

Recently it has been demonstrated [1] that a minor metabolite of mexiletine, *m*-hydroxymexiletine (MHM), has twice the blocking activity of mexiletine on cardiac sodium ion channels – which may account for its increased antiarrhythmic and vasorelaxant activities on isolated cardiac tissues.

Since the cardiotoxicity of drugs is mainly due to blockade of K^+ channel currents in the human heart, in the present study we have compared the effects of mexiletine and MHM on the hERG (the human Ether-à-go-go-Related Gene) K^+ channel stably transfected into mammalian cells, using the patch clamp technique.

Our data showed that MHM and mexiletine block hERG tail currents, elicited on repolarization from positive voltage to -50 mV, with IC_{50} values equal to 2.33 ± 0.13 and 0.28 ± 0.02 μM, respectively. Moreover both compounds induced a shift of voltage dependence of steady-state activation, that was more pronounced for mexiletine. Finally the block of current induced by MHM and mexiletine required preferentially channel activation, suggesting that both compounds have a similar mode-of-action, but a different hERG binding site affinity.

In summary, our data suggest that MHM might be used in substitution of mexiletine ('metabolite switch'), because it might have similar therapeutic properties but fewer cardiotoxic effects.

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Reference

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Keywords: cardiotoxicity, hERG channel.

SUN-128
Comparison of the effects of quercetin derivatives on the formation of advanced glycation endproducts in vitro

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Hyperglycaemia causes increased protein glycation and the formation of advanced glycation endproducts (AGEs). Accumulation of AGEs in body tissue are believed to be responsible for long-term complications of diabetes and aging. The major AGE in vivo is carboxymethyllysine (CML), which is not a crosslink but formed by oxidative breakdown of Amadori products and its level increases two-fold in the skin of diabetic patients. As such, inhibition of the formation of AGEs represents a potential therapeutic target for the prevention and treatment of diabetic complications. Various pharmacological compounds have been evaluated for their antiglycative properties. However, natural inhibitors have been proven relatively safer for human consumption when compared with synthetic compounds.

In this study, the inhibitory effects of quercetin, isoquecitrin, 3-O-galloylquercetin, and 7-O-galloylquercetin on the formation of advanced glycation endproducts (AGEs) were compared. Besides polyacrylamide gel electrophoresis, inhibitory effects were studied using fluorometric and immunochemical detection of AGEs (non-competitive ELISA) in bovine serum albumin (BSA)/glucose, BSA/methylglyoxal and lysozyme/methylglyoxal systems. From the tested compounds, 7-O-galloylquercetin showed the most promising results.

The effect of quercetin derivatives on the formation of AGEs suggests their possible beneficial roles in the prevention of glycation-associated diseases. This effect will be further verified in vivo. Equally, the mechanism of action of quercetin derivatives will be studied.

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Keywords: 3-O-galloylquercetin, 7-O-galloylquercetin, isoquercitrin.

SUN-129
Computational association of autoimmune diseases: a case study

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Microarray technologies have enabled rapid and efficient expression profiling of thousands of genes simultaneously. Studies on disease datasets have already revealed genes that could potentially be implicated in the pathogenesis of that disease thus opening the path to accurate diagnosis and potential biomarkers. Autoimmune diseases have a complex genetic basis with multiple genes contributing to disease risk, each with generally modest effects independently. There is enough evidence indicating that common genes underlie multiple autoimmune diseases. Previous studies point to a greater frequency of autoimmune diseases among patients with psoriasis than in the general population and many inflammatory autoimmune diseases are a result of derangements in multiple cytokine pathways. This study examined the association between psoriasis and rheumatoid arthritis, both of which are declared inflammatory autoimmune diseases.

A comparison of gene expression data associated with psoriasis and rheumatoid arthritis (RA) from four independent studies, obtained from Gene Expression Omnibus has been performed. Each dataset was statistically analyzed in order to identify differentially expressed genes (DEGs). Proteins encoded by DEGs were determined and integrated with protein-protein interaction data for further analyses and hub proteins were identified. Enrichment analyses were performed to map the interconnectivities between diseases and biological pathways.

Comparative analyses indicated that psoriasis and rheumatoid arthritis have 20 common DEGs. 12 of these DEGS have previously been linked to RA and 9 have been linked to psoriasis. Related pathways of these DEGs are: chemokine signalling pathways and Cytokine-cytokine receptor interaction. Main hubs for the PPI network are STAT1, CEBPD, MMP1 and SERPINA1.

This study provides additional insight into the molecular mechanism of autoimmune diseases: psoriasis and rheumatoid arthritis. Results indicate that psoriasis has a strong association with rheumatoid arthritis thus suggesting a common genetic cause between

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them. Further evaluation of other autoimmune diseases may lead to a common mechanism between these diseases.

Keywords: autoimmune diseases, network analysis.

SUN-130

Crystal structure of human aminopeptidase ERAP2 in the absence of catalytic Zn(II) atom

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The human adaptive immune response recognizes diseased cells and eradicates them through the action of cytotoxic T-lymphocytes (CTLs). CTLs recognize small peptide fragments, arising from pathogen-derived proteins, bound onto molecules of the Major Histocompatibility Complex (MHC) Class I on the cell surface. These antigenic peptides are produced by complex intracellular proteolytic cascades. Aberrant antigenic peptide generation can contribute to pathogen evasion and autoimmunity, necessitating tight control of the antigen generation process.

Antigenic epitopes are initially degraded by the proteasome and immunoproteasome while the final processing step occurs within the endoplasmic reticulum (ER) by at least two highly homologous zinc aminopeptidases, namely ERAP1 and ERAP2 (~50% sequence identity). The two proteins are suggested to have a complementary role in the trimming of the large pool of antigens in the ER.

The crystal structures of ERAP1 and ERAP2 contributed to our understanding on how antigenic peptide precursors are bound and processed. ERAP1 has been crystallized in two conformations; the 'open' conformation is hypothesized to be inactive but to facilitate initial substrate capture while the 'closed' conformation is enzymatically active. The conversion inactive to active was suggested to center around the reorientation of a key Tyrosine catalytic residue. Structural analysis of ERAP2 showed a similar domain topology and highly conserved active site as ERAP1 and provided structural insight on the different substrate specificities between the two ER aminopeptidases. ERAP2, however, has only been crystallized in the 'closed', presumably active conformation.

We report here the X-ray crystal structure of apo-ERAP2, lacking the active site Zn(II) ion at 3.02 Å in a conformation resembling the 'closed' ERAP1. Overall, the structure is highly similar to the crystal structures of ERAP2 in complex with the product of a reaction and ERAP2 in complex with a transition-state analogue. However, while key residues comprising the zinc-binding motif remain unaffected, important residues for the enzymatic activity, stabilization of the tetrahedral intermediate and binding to the inhibitor are perturbed. Importantly, the key catalytic Tyr455 is shifted away from the active site, in a configuration resembling the ERAP1 'open' conformation. This finding suggests that the orientation of the catalytic site Tyrosine correlates to the presence of a substrate or transition-state analogue rather than to the overall conformational state of the proteins as was originally hypothesized for ERAP1.

Keywords: adaptive immunity, aminopeptidase, X-ray crystallography.

SUN-132

Cytotoxic effects of cobalt chloride and ciprofloxacin in C6 glioma and vero cells: a preliminary study

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The objective of this study was to determine possible time- and dose-dependent cytotoxic effects of cobalt chloride ($CoCl_2$) and a fluoroquinolone antibiotic, ciprofloxacin (CPFX) in C6 glioma and vero cells. The C6 glioma cells were exposed to various concentrations of $CoCl_2$ (50–500 μM) and CPFX (10–150 mg/l) for 24, 48 and 72 h. However, as indicated in parenthesis, the concentrations of $CoCl_2$ (0.5–1000 μM) and CPFX (0.5–300 mg/l) and incubation times (24, 48, 72 and 96 h) were different with Vero cells. Cytotoxic effects of these substances were determined by MTT and/or rezasurin assays. Possible protective effects of vitamin E, coenzyme Q_{10}, and/or zinc chloride were also tested in vero cells. Our results showed that with MTT assay, in the presence of $CoCl_2$, a gradual decrease in cell survival was noted at concentrations 200 μM or higher in incubation periods of 24, 48, 72, and 96 h in vero cells. Similar results were obtained with $CoCl_2$-treated C6 glioma cells. With resazurin assay, $CoCl_2$-induced cytotoxicity was found similar to the results of MTT assay, particularly at high concentrations and long incubation periods. Pretreatment of vero cells with $ZnCl_2$ for 4 or 24 h provided significant protection against $CoCl_2$ induced cytotoxicity when measured with MTT assay. However, vitamin E or coenzyme Q_{10} was not protective. For CPFX-treated cells, a significant decline was noted in viability ≥50 mg/l of the drug at 24, 48 and 72 h for both cell types. With extended incubation period, decline in cell survival was more significant. Vitamin E or coenzyme Q_{10} pretreatment of cells for 4 h caused complete protection against CPFX-induced cytotoxicity. These preliminary results suggest that oxidative-stress might be involved in the cytotoxicity mechanism of $CoCl_2$ and CPFX in these systems.

Keywords: ciprofloxacin, Cobalt chloride.

SUN-133

DCC, MLH1, GSTT1, GSTM1, and TP53 genes polymorphisms and risk for development of colorectal cancer in population from Kazakhstan

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Association analysis between specific gene polymorphism and disease development is the leading focus in molecular epidemiology and one of the promising trends in predictive medcine.

Colorectal cancer continues to be one of the most common fatal types of cancer. The colorectal cancer (colon and rectal cancer) morbidity and significant rejuvenation have been considerable increased worldwide in recent years. Among Eurasian countries, Kazakhstan is in the seventh place regarding to the colorectal cancer incidence. There are many familial syndromes which predispose to colorectal cancer development, the percentage of patients with cancer burdened histories is 20–30%. But the majority of colorectal cancers cases are the sporadic forms. Colorectal carcinogenesis is characterized by the successive accumulation of mutations in genes controlling epithelial cell growth

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and differentiation leading to genomic instability whereby widespread loss of DNA integrity is perpetuated. In most familial cases there is a spectrum of expected mutations of key genes involved in disease pathogenesis. In sporadic cases there is no identifiable inherited gene involved, but the cancers developed as a result of accumulation of mutations in genes responsible for interaction with environmental factors.

Here we present the results of case-control study of 494 residents from Kazakhstan to reveal an association of the DCC G32008376A, MLH1 -93G/A, TP53 Arg72Pro single nucleotide polymorphisms, and GSTT1, GSTM1 genes deletion polymorphism with colorectal cancer risk. The blood samples were collected from the patient of cancer clinics of Almaty and Semey cities. Detailed questionnaires and informed consents were filled prior collection of samples. The clinical diagnosis of cancer patients was verified by the cytological or histological methods using biopsy and post-surgery materials. 16 patients (6.13%) with colorectal cancer cases in family history were excluded from the case-control analysis. The control groups of healthy individuals were selected in accordance to the ethnic and age data of cancer patients. 245 healthy persons were included to the control cohort and case cohort consists from 249 patients with sporadic colorectal cancer.

We found out the significant associations of increased risk of colorectal cancer and fallowing genotypes: DCC (32008376 G/G and G/A versus A/A – OR = 3.45, p = 0.0002), MLH1 (-93G/G, OR = 1.45, p = 0.04), TP53 homozygous (Pro72Pro, OR = 3.80, $p < 0.0001$), GSTT1 (deletions and heterozygous versus normal homozygous – OR = 1.43, p = 0.05) and GSTM1 deletions (OR = 1.83, p = 0.001).

Keywords: case-control study, colorectal cancer, gene polymorphism.

SUN-134

Decreased expression of heme oxygenase-1 as a putative risk factor linking redox imbalance and inflammation in depression. The role of HMGB1 signaling in progression of inflammation

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Objectives: Inflammation and oxidative stress are both related to the pathology of depression. The aim of this study was to examine selected parameters of redox homeostasis and inflammation in depressed patients and healthy controls and determine their association with the risk of depression. Furthermore, we assessed HMGB1 protein as a plausible signal for progressing inflammation.

Methods: The blood was collected from patients with depression (Group I, N = 18) and from healthy controls (Group II, N = 20). Antioxidant enzyme activities (gluatathione peroxidase, glutathione reductase, superoxide dismutase), reduced gluatathione, hydrogen peroxide and malondialdehyde were measured spectrophotometrically, heme oxygenase (HO-1), IL-6, IFN-gamma and HMGB1 were assayed with ELISA technique.

Results: In this study we observed significantly decreased activity of glutathione peroxidase, significantly increased activity of GR, significantly decreased activity of SOD-1, significantly decreased concentration of HO-1, significantly increased concentration of H_2O_2, significantly increased concentration of MDA in the group of depressed patients when compared with healthy controls. HO-1 was significantly correlated with the risk of depression such as decreased concentration of the enzyme increased the risk for depression. Moreover, decreasing activity of heme oxygenase was accompanied by increased severity of depression. In depressed patients IL-6 and IFN-gamma levels were increased when compared with healthy controls. HMGB1 remained in relation with redox potential and immune status of the blood.

Conclusions: Our observation of decreased activity of HO-1 in depression suggest significant role of this enzyme in maintaining the brain functions under the conditions of oxidative stress and inflammation. Immunomodulatory role of HMGB1 might be related to the change of its activity caused by redox imbalance in depressed patients.

Reference

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Keywords: depression, inflammation, Oxidative stress.

SUN-135

Dehydroepiandrosterone has strong antifibrotic effects and is decreased in idiopathic pulmonary fibrosis

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Introduction: Idiopathic pulmonary fibrosis (IPF) is a progressive and lethal disease of unknown etiology occurring in individuals older than 50 years old and reaching its peak at 60–65 years. The disease is characterized by epithelial cell injury/activation, expansion of the myofibroblast population and aberrant lung remodeling. Dehydroepiandrosterone (DHEA), a steroid pro-hormone, physiologically decreases with age, but an exaggerated decline has been associated with some chronic-degenerative diseases such as, atherosclerosis and diabetes.

Methods and Results: We quantified the plasma levels of DHEA and its sulfated form (DHEA-S) in 137 IPF patients and 58 controls and examined the effects of DHEA on human lung fibroblasts in vitro. Plasma DHEA/DHEA-S was significantly decreased in male IPF patients (median (range) DHEA: 4.4 (0.2–29.2) versus 6.7 (2.1–15.2) ng/mL, $p < 0.01$; DHEA-S: 47 (15.0–211) versus 85.2 (37.6–247.0) mg/dL, $p < 0.001$), while in females only DHEA-S was significantly decreased (32.6 (15.0–303.0) versus 68.3 (16.4–171) mg/dL, $p < 0.001$). DHEA caused a decrease in fibroblast growth rate (35% of decrease compared with controls), this finding was partially due to the induction (two-fold over the control) of fibroblast apoptosis as measured by Annexin V, probably through the intrinsic pathway with activation of caspase-9. This effect was accompanied by upregulation of several pro-apoptotic proteins (Bax and cyclin-dependent kinase-inhibitor CDNK1A) and downregulation of anti-apoptotic proteins, such as cellular inhibitor of apoptosis c-IAP1 and c-IAP2. DHEA also caused a significant decrease of transforming growth factorb1-induced collagen production, fibroblast to myofibroblast differentiation (measured by alpha-smooth muscle actin expression) and inhibited platelet-derived growth factor-induced fibroblast migration (measured in Boyden chambers covered by collagen).

Conclusions: These findings demonstrate a disproportionate decrease of DHEA/DHEA-S in IPF patients and indicate that this molecule has multiple antifibrotic properties.

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Keywords: Dehydroepiandrosterone, Fibroblasts, Idiopathic Lung Fibrosis.

SUN-136

Der f 2 directly binds to toll-like receptor4 and triggers phospholipase D1 activation leading to IL-13 production

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Background: House dust mites (HDMs) are the most important source of indoor aeroallergens that contribute to the rising incidence of allergic diseases such as allergic asthma. The major HDM, Der f 2, induces inflammatory cytokine expression. Little is known about the signaling pathway involved.
Objective: We wanted to define the Der f 2 signaling pathway from its receptor to the transcription factor responsible for IL-13 expression and production.
Methods: Human bronchial epithelial cells were stimulated with Der f 2. The release and gene expression of IL-13 were measured by means of ELISA and RT-PCR, respectively. We investigated the ability of Der f 2 in the HDM mouse model of allergic airway inflammation.
Results: Here we show that Der f 2 binds to TLR4 and induces IL-13 expression and production. Activation of TLR4 by Der f 2 requires the recruitment and activation of Syk, which leads to phosphorylation of PLCγ and membrane translocation of PKCα. p38 MAPK is then activated by PKCα and stimulates PLD1 activity by phosphorylating the Thr147 residue of PLD1. PLD1 activation enhanced binding of ROCK1 to ATF-2 and leads to increased expression of IL-13. In the airway inflammation mouse model, IL-13 production significantly increased with treatment of Der f 2.
Keywords: Der f 2, TLR4, IL-13.

SUN-137

Deregulation of the PDK1/TACE α-secretase signaling axis at the cross-road of prion and Alzheimer's diseases

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Prion and Alzheimer's (AD) diseases are a group of pathologies characterized by separate etiologies with distinct pathophysiological features. Each disease has its own clinical manifestations but some common pathogenic cascades might occur and their identification would represent a biomedical challenge for therapies. The precise mechanisms by which PrPSc in prion diseases and the amyloid Ab peptides in AD exert their toxicity however remain unknown. By combining in vitro and in vivo approaches, we showed that PrPSc or Ab peptides trigger the overactivation of the 3-phosphoinositide-dependent kinase-1 (PDK1) in prion-infected or AD neurons, respectively. PDK1 then promotes the phosphorylation and displacement of TNFa Converting Enzyme (TACE) a-secretase from the plasma membrane to caveolin-1-enriched microvesicles, leading to TACE internalization. TACE thus loses its cell surface neuroprotective function. PrPSc- or Ab-induced TACE internalization indeed diverts TACE activity away from its substrates (i) the normal cellular prion protein (PrPC),

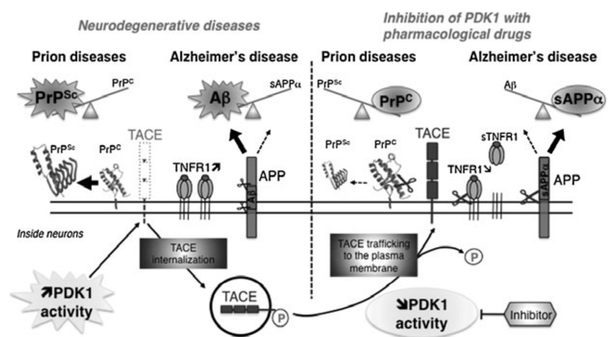

Fig. 1. In prion and Alzheimer's diseases, PDK1 triggers the internalization of TACE α-secretase in neurons. TACE dysregulation at the surface of neurons contributes to neurodegeneration by increasing the conversion of PrP^{c} into pathogenic prions PrP^{Sc} and the formation of neurotoxic amyloid Aβ peptides and reducing shedding of TNFα receptors (TNFR1). Inhibition of PDK1 promotes localization of TACE to the plasma membrane, restores TACE-dependent α-secretase activity and neuroprotective cleavage of APP, PrP^{c} and TNFR1, and attenuates PrP^{Sc}- and Aβ-induced neurotoxicity.

which amplifies the replication of PrPSc in prion diseases, (ii) the amyloid precursor protein (APP), which favors the production of Ab peptides in AD, and (iii) TNFa receptors (TNFR), which accumulate at the plasma membrane and render diseased neurons highly vulnerable to TNFa toxicity. Pharmacological inhibition of PDK1 is sufficient to target TACE back to the plasma membrane of diseased neurons, where it recovers its protective activity by triggering the cleavage of PrPC, APP, and TNFR, thus lowering the amount of amyloid proteins and desensitizing diseased neurons from TNFa toxicity. Inhibition of PDK1 in prion-infected mice increases the lifespan and counteracts motor deficits. In three transgenic mouse models of AD, PDK1 inhibition reduces AD pathology and memory impairment. We thus uncovered that deregulation of PDK1-dependent TACE activity is a central mechanistic event in neurodegenerative pathways involved in both prion diseases and AD. We also demonstrate that rescuing TACE activity at the plasma membrane upon PDK1 inhibition is a valuable entry point to fight these two neurodegenerative diseases. Importantly, the therapeutic relevance of targeting PDK1 in AD is supported by a rise in PDK1 activity and reduced TACE activity in the brain of AD subjects.
Keywords: neurodegenerative diseases, signaling, Therapeutic target.

SUN-138

Development of a new microchip based ELISA platform for detection of NGAL: a biomarker of contrast-induced acute kidney injury

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Introduction: Contrast-induced acute kidney injury (AKI) presents a serious complication during the medical use of iodinated contrast media. At the moment renal disfunction is detected by the measurement of serum creatinine that is not a sufficiently sensitive marker for early detection of AKI. Neutrophil gelatinase-associated lipocalin (NGAL), a member of the lipocalin protein family emerged as early biomarker of AKI.

Currently, NGAL published studies have been performed mainly with commercial research-grade ELISA assays. These assays are accurate, but still not practical for clinical purposes

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because they are time demanding. For the determination of NGAL, we present here a combined microfluidic Flow Injection Analysis-Thermal Lens Microscopy (μFIA-TLM) as an alternative detection tool.

Materials and Methods: Blood plasma samples were collected from four patients admitted to General Hospital Dr. Franca Derganca (Šempeter pri Novi Gorici, Slovenia) for percutaneous coronary intervention (PCI). All patients received low-osmolar contrast medium (Iomeron-350). Plasma samples were collected before and up to 12 hours after PCI. For the control group were used six individuals with normal serum creatinine values before PCI.

We used commercial ELISA assay (BioPorto, Gentofte, Denmark) to measure NGAL levels in serum and plasma. The final reaction product from ELISA of each sample was also measured by the μFIA-TLM system on microchip to demonstrate possible advantages of TLM detection.The μFIA served only as a device for the delivery of final ELISA product to the TLM detector. In addition, an 'in-house' ELISA assay for NGAL detection was developed and validated in our laboratory. With this method we measured samples from six healthy individuals and performed a comparative analysis of obtained results with different experimental approaches to assess their capability and advantages/disadvantages.

Results: The presence of NGAL in blood samples was confirmed by SDS-PAGE and Western blot. The μFIA-TLM provided seven fold lower limits of detection (up to 1.5 $pgmL^{-1}$) compared to conventional ELISA limit values of 10 $pgmL^{-1}$. These data suggest that TLM could serve as a new, rapid and highly sensitive method for NGAL determination. It's use could be proposed not only for research but also for medical purposes and NGAL detection in real samples.

Conclusion: Development of 'in house' ELISA allow us to design a new lab-on-a-chip ELISA platform based on TLM detection of NGAL in biological samples enabling bed-side tests needed for example during coronarography.

Acknowledgements: TRANS2CARE project financed by European Regional Development Fund and Slovenian Research Agency through young investigator grant to T. R.

Keywords: ELISA, NGAL, TLM.

SUN-139
Development of an anticancer agent targeting polo-box domain of polo-like kinase 1

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Polo-like kinase 1 (Plk1) is a regulator of cell cycle progression during mitosis; it is overexpressed in many different tumors and has been implicated as a potential antimitotic target. Plks are characterized by the presence of a highly conserved C-terminal polo-box domain (PBD) that is involved in regulating kinase activity. The phosphopeptide Pro-Leu-His-Ser-p-Thr (PLHSpT) is a potent selective inhibitor of the PBD of human plk1 that acts by inducing mitotic arrest and apoptotic cell death in cancer cells. We synthesized cRGDyK-S-S-PLHSpT to exploit the drug delivery. In HeLa cells, the uptake of cRGDyK-S-S-PLHSpT was significantly increased, it localized in the nuclei and surroundings. We found that cRGDyK-S-S-PLHSpT inhibited cell survival in a dose-dependent manner, which was shown to significantly inhibit plk1 kinase activity in human HeLa and U87MG tumor cells. The cRGDyK-S-S-PLHSpT treated cells had multipolar spindles and misaligned chromosomes. Cell proliferation was also inhibited by cRGDyK-S-S-PLHSpT due to the induction of apoptosis. In the xenograft models mice treated with cRGDyK-S-S-PLHSpT (4 mg/kg body weight), tumor growth was significantly reduced compared with untreated and PLHSpT-treated mice. Our results demonstrated that the new peptide, cRGDyK-S-S-PLHSpT, is promising as an anticancer agent. We expect that our contribution will provide new insights into the design of novel plk1 peptide inhibitors.

Keywords: Anticancer, inhibitor, kinase inhibitor.

SUN-140
Development of LC-MS method for quantification of haemagglutinin in influenza vaccine

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Influenza is a virus that infects 15% of the world population, causing 500 000 deaths annually. Haemagglutinin (HA) is a major antigen of this virus, and the potency of influenza vaccine is based on the content of HA. Although Single Radial Immunodiffusion (SRID) is a very specific and unique method that can be used to measure the HA content in influenza vaccine visually, it needs a long time and is very labor-intensive. Also, this method can be a hindrance to develop influenza vaccine rapidly because it takes several months to prepare standard antigen and antibody used in SRID. Against this backdrop, method using Liquid Chromatography-Isotope Dilution Mass Spectrometry (LC-IDMS) was developed to measure the HA content in influenza vaccines.To begin with, we performed a 10 year study since 2000 on the amino acid sequence of influenza virus to identify the least mutated parts of the sequence, and selected a target peptide in vaccines treated with trypsin and tested by Q-TOF MS method. Isotope-labeled target peptides were used as a internal standard to correct the variations of test results caused by experimental errors. For optimization of assay conditions, a series of preliminary tests were performed to determine appropriate LC-MS parameters as well as trypsin digestion conditions. To show the validity of this method, the linearity, accuracy, precision, limit of detection and limit of quantification were measured. It showed that RSD values measured over 3 days ranged between 3.0 ~ 12.7% and the r^2 value (linearity) was above 0.99, proving the validity of the assay conditions. The established testing method includes determining the content of hemagglutinin in 3 lots of H5N1 (A/Vietnam/1194/2004) vaccines produced in 2012 preparing for a possible outbreak and 10 lots of H1N1 (A/California/07/2009) vaccine bulk produced in 2013. Not only the results showed a high correlation with those of SRID, but this new method has been also proved to be more rapid and precise than SRID. In particular, it can be useful in addressing a potential influenza pandemic, as it makes it possible to immediately shift the target peptide to measure hemagglutinin through assaying the amino acid sequence in case where significant antigen mutations occur.

Therefore, in case of emergency such as a flu outbreak, more prompt response in terms of vaccine manufacturing and conducting clinical trials will be possible without having to be on a long waiting list for reference standards to be distributed by international organizations like the WHO.

We expect that this new testing method will contribute to enhancing public health by improving the efficiency of vaccine manufacturing process as well as building up trust on test results based on extensive monitoring.

Keywords: hemagglutinine, influenza, LC-IDMS.

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SUN-141
Development of nuclear factor kappa B inhibitors: synthesis and evaluation of lantadene congeners as anticancer agents

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The pentacyclic triterpenoids reduced lantadene A and B analogs were synthesized and evaluated in vitro for their NF-κB and IKKβ inhibitory potencies and cytotoxicity against A549 lung cancer cells. The lead analog showed sub-micromolar activity against TNF-α induced activation of NF-κB and exhibited inhibition of IKKβ in a single-digit micromolar dose. The physicochemical evaluation demonstrated that the lead analog was stable in the simulated gastric fluid of pH 2, while hydrolyzed at a relatively higher rate in the human blood plasma to release the active parent moieties. At the same time, molecular docking analysis showed that lead compound was hydrogen bonded with the Arg-31 and Gln-110 residues of the IKKβ. The preliminary results showed that the lead molecule can be developed as potent anticancer agents.

Keywords: Lantadene, nuclear factor kappa B.

SUN-142
Development of potent pseudopeptide phosphinic inhibitors of antigen-trimming aminopeptidases that can enhance cytotoxic t-cell responses against cancer cells

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ER aminopeptidase 1 (ERAP1), ER aminopeptidase 2 (ERAP2) and Insulin Regulated aminopeptidase (IRAP) are three homologous enzymes that play critical roles in the generation of antigenic peptides. These aminopeptidases excise amino acids from N-terminally extended precursors of antigenic peptides in order to generate the correct length epitopes for binding onto MHC class I molecules. Loss of ERAPs function substantially alters the repertoire of peptides presented by MHC class I molecules, affecting recognition by $CD8^+$ T cells. In addition, ERAP1 has been recently shown to be involved in the regulation of inflammatory innate immune responses. Genome-wide association studies have identified common variants of ERAP1 and ERAP2 to be associated with the predisposition to several human diseases, ranging from viral infections to autoimmunity and cancer. The important roles of these enzymes in regulating both adaptive and innate immune responses suggest that inhibition of their activity may constitute an innovative therapeutic approach for cancer immunotherapy or the regulation of inflammatory responses.

In this study we followed a rational-design approach and developed a series of phosphinic pseudopeptide transition state analogs that can inhibit this family of enzymes with nM affinity. Kinetic as well as X-ray crystallographic analysis of one such inhibitor in complex with ERAP2 validated our design, revealing a canonical mode of binding in the active site of the enzyme, and highlighted the importance of the S2' pocket for achieving inhibitor potency. Also these compounds did not inhibit equally all variants of ERAP1/ERAP2 and as a result inhibitor potency is variant dependent. Antigen processing and presentation assays in HeLa and murine colon carcinoma (CT26) cells showed that these inhibitors can induce increased cell-surface antigen presentation of transfected and endogenous antigens and enhance specific cytotoxic T-cell responses. Finally, pretreatment of mouse macrophages with the best inhibitor of ERAP1 significantly ablated macrophage phagocytosis activity in a dose-dependent manner suggesting a possible application of these compounds as anti-inflammatory molecules. This class of inhibitors constitutes a promising tool for controlling the cellular adaptive and innate immune response in humans.

Keywords: Autoimmunity, Cancer Immunotherapy, Inhibitors.

SUN-143
Development of sustained release 'nanopolypill' for hypertension – an experimental study

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Blood pressure control is the key element in any cardiovascular prevention strategy. The chronic nature of the therapy is responsible for problems like adherence to the treatment leading to sub-optimal BP control and several cardiovascular complications.The use of a fixed-dose combination therapy, targeting several risk factors, in the form of a 'polypill', was first proposed by Wald and Law as a concept in 2003. An extension of the concept of polypill would be a sustained release polypill.The present study was thus planned to formulate, characterize and evaluate the pharmacokinetics of a novel 'nanopolypill' comprising three commonly prescribed anti-hypertensive drugs, hydrochlorothiazide (a diuretic), candesartan (ARB) and amlodipine (a calcium channel blocker).The candidate drugs were loaded inside Poly (DL-lactide-co-gycolide) (PLGA) nanoparticles. The formulations were evaluated for their size, morphology, drug loading and in vitro release individually. Single dose pharmacokinetic profiles of the nanoformulations alone and in combination, as a nanopolypill, were evaluated in Wistar rats.The candidate drugs encapsulated inside PLGA showed entrapment efficiencies ranging from 30%, 33.5% and 32% for hydrochlorothiazide, candesartan and amlodipine respectively. The nanoparticles ranged in size from 110 to 180 nm. In vitro release profile of the nanoformulations showed 100% release by day 6 in the physiological pH 7.4 set up with PBS (phosphate buffer saline) and by day 4–5 in the intestinal pH 1.2 and 8.0 set up SGF (simulated gastric fluid) and SIF (simulated intestinal

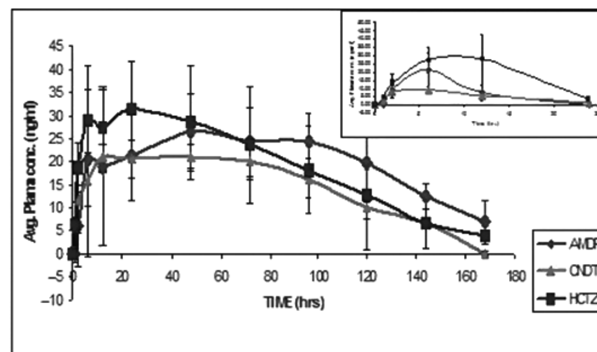

Fig. 1. Plasma concentration plot of drugs combined in the Hypertension 'Nanopolypill'; Inset – Free drugs. C_{max} – ng/ml; AUC – ng hr/L; *Free versus NP significant at $p < 0.05$ (n = 5).

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fluid) respectively. In pharmacokinetic analysis, a sustained-release for 6 days and significant increase in the mean residence time (MRT), as compared to the respective free drugs was noted [MRT of amlodipine, hydrochlorothiazide and candesartan changed from 8.9 to 80.59 hours, 11 to 69.20 hours and 9 to 101.49 hours respectively].We have shown for the first time that encapsulating amlodipine, hydrochlorothiazide and candesartan into a single nanoformulation, to get the 'nanopolypill' is a feasible strategy which has a potential of decreasing pill burden.

Keywords: Hypertension, Nanoparticles, Pharmacokinetics.

SUN-144
Diabetes-induced liver injury associated with inflammatory pathway activation: effects of chronic vitamin D3 administration

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Background and Aims: Diabetes is known to be characterized with the accumulation of pro-inflammatory factors in various tissues. It is known that nuclear factor kappaB (NF-kB) is a key regulator of the inflammatory process and its activation leads to the increased expression of inducible nitric oxide synthase (iNOS) and VEGF involved in inflammation. Vitamin D_3 (D_3) is currently recognized as a potent immunomodulator affecting various inflammatory and autoimmune diseases. However, the precise mechanisms of vitamin D_3 influence on immune homeostasis has not been clearly defined. We therefore investigated the role of D_3 in regulation of pro-inflammatory factors (NF-kb/p65, iNOS, VEGF) in liver of diabetic mice.

Materials and Methods: Type 1 diabetes was induced in male C57BL/J6 mice (weighing 25.0 ± 1.5 g) by i.p. injection of multiple low dose streptozotocin (40 mg/kg b.w.). Control and STZ-diabetic mice were treated with or without vitamin D_3 (15 IU/mouse per os, for 8 weeks). Serum 25-hydroxyvitamin D_3 ($25OHD_3$) was assessed by ELISA. The levels of cytosol and nuclear pNF-kB/p65 (pRelA), iNOS and VEGF were *measured* by Western-blot analysis. NO production was detected by DAF-DA (4,5-diamino-fluorescein diacetate) using flow cytometry.

Results: Serum level of $25OHD_3$, the main circulating metabolite of D_3, was shown to be reduced to 23.8 ± 1.9 in diabetes versus 39.7 ± 2.9 nmol/l in control, indicative of diabetes-induced D_3 deficiency ($p < 0.05$). These changes were accompanied by 1.51- and 2.04-fold overexpression of cytosolic and nuclear pRelA respectively. Diabetes was also associated with 1.32-fold increase in iNOS expression. It is worth noting that changes in iNOS expression most likely result in currently established 1.62-fold elevation of NO production in liver related to diabetes. In addition, 2.31-fold increase in VEGF was observed in hepatic tissue of diabetic mice. This finding taken together with previous data can indicate possible intensification of inflammatory processes. Full restoration of $25OHD_3$ content was achieved by D_3 treatment. Vitamin D_3 also caused partial normalization of pNF-kB/p65, iNOS and VEGF levels and NO production in liver.

Conclusions: The study confirmed that diabetes-induced liver abnormalities are associated with upregulation of inflammatory markers expression (NF-kB/p65, iNOS and VEGF) that correlated with insufficient vitamin D_3 availability. Our data suggest a potential efficacy of vitamin D_3 to counter hepatic inflammation associated with diabetes.

Keywords: Type 1 diabetes, vitamin D3, liver, proinflammatory factors, NF-kB-mediated pathway.

SUN-145
Diagnostic potential of new methods to detect stenosis of aortic heart valve

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Aortic stenosis in heart valve is associated with the narrowing of the aortic heart valve and remodelation of the extracellular matrix. Emilin-1 is protein of extracellular matrix which is expressed in the endocardium, endothelial cells and smooth muscle cells. Emilin-1 can influence values of blood pressure, structure of vessels wall and it can cause the formation of cardiovascular diseases. A goal of our study is to detect the pathological changes in a blood of patients with heart valve stenosis by using determination of gene expression of Emilin-1 and perform fluorescence analysis together with detection of the molecular structural changes of blood proteins.

RNA was isolated from peripheral blood of patients with stenosis of aortic heart valve (10) and the control group of healthy 35 peoples. The reverse transcription from mRNA to cDNA was realized. The gene Emilin-1 and housekeeping gene β-actin (for the standardization) were amplificated by real-time PCR. Autofluorescence of the blood serum/plasma samples was analysed by synchronous fluorescence fingerprint on Luminescence Spectrophotometer Perkin-Elmer LS 55. The structure of blood serum/plasma was also analysed by atomic force microscope (ICON, Bruker, USA).

Expression of Emilin-1 gene at mRNA level was significantly increased (about 25.07 ± 0.05%) in comparison with healthy subjects evaluated by statistical analysis. Changes in nanostructure of blood was detected in the patients in comparison with healthy subjects. The autofluorescence of proteins was significantly increased in the serum ($p < 0.001$) but significantly decreased ($p < 0.001$) in the plasma and tissue of patients with stenosis of aortic heart valve in comparison with healthy subjects.

This pilot study uses the combination of several experimental techniques like RT-PCR, fluorescence analysis and atomic force microscopy which could be a prospective new screening procedure for the detection of aortic stenosis in heart valve. The formation of Emilin-1 will be correlated with the determination of transforming growth factor b (TGF-b). Emilin-1 influences the biosynthesis of transforming growth factor β_1 (TGF-β_1), whose deregulation results in the systemic hypertension accompanied with the structural changes and narrowing of the walls of arteries. These results can lead to the development of new and more sophisticated methods for detection of cardiovascular diseases.

This study was supported by VEGA 1/0115/14 and MediPark Košice, ITMS:26220220185, Operational Programme Research and Development (OP VaV-2012/2.2/08-RO).

Keywords: cardiovascular disease, Emilin, fluorescence.

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SUN-146
Different sized silver-citrate nanoparticles kill cancer cells through the (re)activation of the p53 dependent apoptotic pathway

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Silver nanoparticles (AgNPs) are the most broadly used nanomaterials in industry and medicine due to their unique physical, chemical properties and their outstanding antibacterial and antifungal features. Recent studies suggest that AgNPs induce cellular stress in several mammalian cell types thus their cytotoxic properties could be exploited to kill cancer cells. AgNPs seem to be very promising novel chemotherapeutic agents however the exact molecular mechanisms behind their activity are still unclear. AgNPs kill cancer cells through a Trojan horse-like mechanism: after the cellular uptake, nanoparticles probably release a vast quantity of highly toxic silver ions within the cell, which eventually results in apoptosis mediated cell death. Although several studies describe the cytotoxic effect of AgNPs, it is still unclear how the size, the shape, the coating and the functionalizing groups might influence their anti-cancer activity.

In this study we used the biocompatible sodium-citrate as a reducing agent to synthesize different sized (~4 and 35 nm) quasi-spherical citrate coated AgNPs. We found that both 4 nm and 35 nm sized AgNPs kill osteosarcomic U2Os cells with the same extent in a concentration dependent manner. However, cleaved caspase 3 staining verified apoptosis during AgNP mediated cell death, in either 4 or 35 nm silver particle treated cancer cells, 4 nm sized AgNPs induces caspase 3 activation with a higher degree than 35 nm sized nanoparticles. Using transmission and scanning electron microscopy we observed aggregated nanoparticles both on the surface and inside the cells where the aggregated AgNPs were largely localized in membrane coated vesicles which suggest a receptor mediated uptake mechanism of AgNPs. During both sized AgNP treatments we detected degraded membrane structures and increased levels of reactive oxygen species suggesting the induction of the mitochondrial apoptosis pathway. Using Western blot analysis and reporter assay experiments we revealed that the p53 mediated apoptotic pathway is in fact involved in Ag-citrate NP triggered cell death.

Our results show that cancer cells are able to uptake both 4 nm and 35 nm spherical shaped silver-citrate nanoparticles which induce cancer cell death by apoptosis via the activation of p53 pathway. On a broad scale, AgNPs represent a feasible platform for the rational design of therapeutically useful anticancer agents so long as their actions are fine-tuned with bioactive functionalizing molecules.

Keywords: Cancer therapy, Cell death, nanoparticles.

SUN-147
Differential expression of ID gene family members in eutopic and ectopic endometrial tissue may be considered as probable casing factor of endometriosis

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Inhibitors of DNA binding (*ID*) transcription factor are helix-loop-helix regulatory proteins that induce proliferation of cells during development.

Endometriosis is the gynecologic disorder characterized by the presence of the endometrial tissue outside of the uterus and causes 30–40% of infertility.

Due to the characteristic of the endometriosis and the regulatory role of *ID* genes, it seems that this gene family may have a role in occurrence of endometriosis as well as its infertility cause. The aim of this study is to determine whether expression of *ID* genes alter in endometriosis.

For this respect, eutopic and ectopic endometrium were obtained from 26 laparoscopically confirmed endometriosis patients. Also, endometrial tissues were collected from 18 healthy fertile women, between 20 and 45 years old, undergoing tubal ligation surgery. The Expression levels of *ID* genes were detected by real-time PCR technique, using designed primer. Ethical approval and informed patient consent was gained for the use of tissue samples.

Our results revealed that mRNA expression of all members of *ID* gene family were significantly lower in eutopic and ectopic samples during the secretory phase, compared with control group. Also in proliferative phase, *ID*1 and *ID*4 showed lower expression in comparison to control group; while, *ID*2 and *ID*3 pointed higher expression levels. On the other hand, *ID*2 showed more expression level in eutopic and ectopic samples and *ID*3 showed sizeable expression in ectopic phase.

The present findings suggest for the first time that deregulation of *ID* gene family members – especially *ID*2 and *ID*3 – can be listed as potential molecular markers of endometriosis and maybe contribute to the infertility that caused by this disorder.

Keywords: Endometriosis, ID gene family, Infertility.

SUN-148
DJ-1 protein and apolipoprotein A are targets for LVVYPW in the brain of streptozotocin-induced diabetic rats

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Earlier we have shown that hemorphins (LVVYPW, hemorphin-7 and LVV-hemorphin-7), demonstrating a wide spectrum of biological activity, are involved in pathophysiology of severe diseases (cancer, diabetes, stress) and act as homeostatic agents by modulation of protein phosphatase calcineurin activity, by affecting different receptors function and by regulation of hypothalamo-pituitary-adrenocortical axis activity. Very recently, by using the isotope-coded protein label (ICPL) quantitative proteomic technology (Lottspeich F. et al., 2005; Kellermann J. et al., 2012), the effect of in vivo treatment of diabetic rats by LVVYPW has been studied. Brain proteins from 3 rats (control

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rats, diabetic rats and diabetic rats, treated by LVVYPW) were labelled with the ^{12}C, ^{13}C and deuterium version of ICPL-label respectively. Then these three samples were mixed together and Triplex-ICPL aliquots were cleaved by combination of trypsin and endoproteinase GluC and analysed directly using LC-ESI mass spectrometry. In the experiments was used OFFGEL electrophoresis as well. Among the proteins differently regulated by LVVYPW in diabetic rat brain were identified DJ-1 protein and apolipoprotein E as well. It should be noted that differently regulated proteins were validated by immunological methods as well. The expression levels of both DJ-1 and apolipoprotein E (ApolipoE) were significantly down regulated in the brain of diabetic rats, treated by LVVYPW: 4.8 fold for DJ-1 and 5.6 fold for A-polipoE respectively. It is well known that DJ-1 and ApolipoE are involved in pathophysiology of neurodegenerative diseases (Parkinson's disease (PD) and Alzheimers disease (AD). ApolipoE is genetic risk factor for sporadic AD (Rebeck G., et al., 1993). It should be noted that the implication of DJ-1 with tumor progression was also demonstrated (Tillman J.E. et al., 2007). PD is a progressive neurodegenerative movement disorder characterized by selective loss of nigrostriatal dopaminergic neurons. By using in vivo microdialysis we have shown that intracerebroventricular administration of LVV-hemorphin-7 induces moderate, but statistically significant elevation of extracellular dopamine (DA) concentrations in the striatum of freely moving rats. As mentioned above hemorphins modulate calcineurin activity in the brain and immune system (Barkhudaryan N. et al., 1991; 2002), and calcineurin in turn participates in DA release (Day M. et al., 2002). Data obtained once again confirm that pleiotropic nature of hemorphins allows them to act as homeostatic agents by implication of different signalling pathways.

Keywords: diabetes, hemorphin, quantitative proteomics.

SUN-149
Dual oxidase 2 in lung epithelia is essential for hyperoxia-induced acute lung injury in mice

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Acute lung injury (ALI) induced by excessive hyperoxia has been employed as a model of oxidative stress imitating acute respiratory distress syndrome. Under hyperoxic conditions, overloading quantities of reactive oxygen species (ROS) are generated in both lung epithelial and endothelial cells, leading to ALI. Some NADPH oxidase (NOX) family enzymes are responsible for hyperoxia-induced ROS generation in lung epithelial and endothelial cells. However, the molecular mechanisms of ROS production in type II alveolar epithelial cells (AECs) and ALI induced by hyperoxia are poorly understood. In this study, we show that dual oxidase 2 (DUOX2) is a key NOX enzyme which effects hyperoxia-induced ROS production, particularly in type II AECs, leading to lung injury. In DUOX2 mutant mice ($DUOX2^{thyd/thyd}$) or mice in which DUOX2 expression is silenced in lung epithelia, hyperoxia-induced ALI was significantly lower than in wild-type (WT) mice. DUOX2 was mainly expressed in type II AECs, but not endothelial cells, and hyperoxia-induced ROS production was markedly reduced in primary type II AECs isolated from $DUOX2^{thyd/thyd}$ mice. Furthermore, DUOX2-generated ROS are responsible for caspase-mediated cell death, inducing ERK and JNK phophorylation in type II AECs. To date, no role for DUOX2 has been defined in hyperoxia-mediated ALI despite it being a NOX homologue and major ROS source in lung epithelium. Here, we present the novel finding that DUOX2-generated ROS induce alveolar epithelial cell death, leading to hyperoxia-induced lung injury. This work was supported by the Brain Korea 21 PLUS Project for Medical Science, Yonsei University

Keywords: hyperoxia, reactive oxigen species.

SUN-150
Effect of arazyme on the lipopolysaccharide-induced inflammatory response

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Arazyme is a novel extracellular metalloprotease secreted by *Aranicola proteolyticus*. Endothelial cells are involved in the pathogenesis of various inflammatory diseases, induce uncontrolled cell viability, and express various inflammatory mediators, including cytokines, chemokines, adhesion molecules, and reactive oxygen species (ROS). In this study, human umbilical vein endothelilal cells (HUVECs) were used to investigate the anti-inflammatory effects of arazyme after lipopolysaccharide (LPS) stimulation. Apoptosis of HUVECs due to LPS was inhibited by arazyme. In various inflammatory responses induced by LPS, arazyme inhibited the secretion of the monocyte chemoattractant protein-1 and interleukin-6 and expression of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1. Arazyme also suppressed ROS production in HUVECs. The action of arazyme was not associated with NF-κB activity in HUVECs. These results indicate that arazyme has anti-inflammatory properties in inflamed endothelial cells and may be useful as a therapeutic agent for inflammatory diseases associated with endothelial cells.

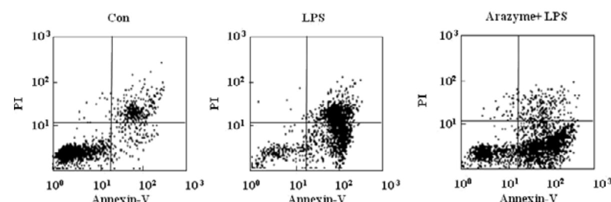

Fig. 1.

Keywords: arazyme, endothelial cells, anti-inflammatory effect.

SUN-152
Effect of cytokines on human primary hepatic stellate cell

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The liver is known as one of the regenerative organ. It is composed of parenchymal and non-parenchymal cells. The hepatic stellate cell (HSC), one of the non-parenchymal cells, is a key cellular element involved in wound healing by responding to various kinds of cytokines such as TGF-β and IL-6 in liver injured by viral, chemical and alcohol so on. However, as a consequence of

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excess inflammation, it develops hepatic fibrosis. We investigated response of primary human HSC (pHSC) and cell line LX-2 to various cytokines. pHSC was prepared from a piece of liver which was resected surgically from patients with metastatic liver cancer or with hepatocellular carcinoma. Expression of liver fibrosis marker genes, Matrix metalloprotease 1 (MMP1) and alpha-smooth muscle actin (α-SMA), were examined in pHSC and LX2 following exposure of cytokines. The each cytokine had some effects on MMP1 and α-SMA expression and, unexpectedly, a simultaneous treatment with cytokines had greater effects on their expression. The simultaneous treatment with cytokines synergistically induced MMP1 gene expression and reduced α-SMA in pHSC, suggesting these cytokines have anti fibrosis activity. Next, the signaling pathway for the synergistic effect was examined by using inhibitors for signaling protein-kinases. As a results, when Mitogen-activated protein kinase (MAPK) pathway, p38 and ERK1/2, were inhibited, the synergistic effect of the cytokines was decreased. From these results, it was suggested that the simultaneous treatment with the cytokines show the anti-fibrotic activity through MAPK pathway in pHSC.

Keywords: liver cirrhosis, cytokine, hepatic stellate cell.

SUN-153

Effect of melatonin on serum glucose, insulin and TNF-alpha levels in rats with impaired glucose tolerance

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Impaired glucose tolerance is a pre-diabetic state of hyperglycemia associated with insulin resistance that may precede type 2 diabetes mellitus by many years. Tumor necrosis factor-a (TNF-α) over-secretion has already been implied in the pathogenesis of insulin resistance and diabetic complications. Melatonin is a circulating hormone that is mainly released from the pineal gland. It is best known as a regulator of seasonal and circadian rhythms, its levels being high during the night and low during the day.

Objective: The aim of the present work is to clarify whether treatment with melatonin has beneficial effect on serum glucose, insulin and TNF-α levels in rats with impaired glucose tolerance.

Methods: Twenty-eight 10-week-old male Wistar rats were divided into four groups, A (control), B (control receiving melatonin), C (pre-diabetic) and D (pre-diabetic receiving melatonin). Noninsulin dependent diabetes mellitus was induced by intraperitoneal streptozotocin injection following nicotinamide injection (groups C and D). All groups received standard diet for 4 weeks. After 4 weeks, when the model was fully developed, oral therapy with melatonin (2 mg/kg body weight) was introduced to groups B and D. Treatment with melatonin lasted for 2 weeks.

Results: Fasting serum glucose levels were significantly higher in group C when compared to the group A (group A, 4.81 ± 0.18 mM versus group C, 6.51 ± 0.32 mM; $p < 0.001$). Melatonin significantly reduced fasting serum glucose levels in diabetic group, but had little effect on fasting glucose levels in healthy rats (group D, 5.92 ± 0.28 mM versus group C, $p < 0.01$; group B 4.58 ± 0.39 versus group A, NS). Furthermore, melatonin significantly reduced serum TNF-α in pre-diabetic animals when compared to untreated group (group A, 11.27 ± 0.48 pg/ml versus group C, 13.21 ± 0.41 pg/ml, $p < 0.001$; group D, 11.34 ± 0.28 pg/ml versus group C, $p < 0.001$; group B, 10.95 ± 0.27 pg/ml versus group A, NS). No significant difference in insulin levels between pre-diabetic and healthy rats was found (group C, 1.09 ± 0.09 ng/ml versus group A, 1.24 ± 0.10, NS). However, insulin was significantly lower in groups treated with melatonin (group D, 0.69 ± 0.05 ng/ml versus group C,$p < 0.001$;group B, 0.98 ± 0.05 versus group A, $p < 0.01$).

Conclusion: Early application of melatonin may postpone the onset of diabetes type 2 by reducing serum glucose levels and prevent its complications due to TNF-α production and/or activity suppression. Furthermore, melatonin might play important role in regulating insulin secretion, linked to the fact that insulin levels are also adapted to day/night changes.

Keywords: diabetes mellitus, Melatonin.

SUN-154

Effect of NAC and curcumin on the increase of MMP-3 induced by oxidative stress in intestinal myofibroblasts

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Matrix metalloproteinases (MMPs), proteins involved in the metabolism of extracellular matrix (ECM), are secreted as latent enzymes, activated extracellularly after the proteolytic cleavage. The rapid and excessive production of pro-inflammatory cytokines, such as the tumour necrosis factor (TNF)α, and the up-regulation of transcripts of matrix metalloproteinases (MMPs) play a crucial role in the ulcerations present in the gut of Crohn's Disease (CD) patients [1]. A pivotal role is attributed to increased MMP-3 (stromelysin-1) in fistulae formation in intestine of CD patients [2]. MMP-3 degrades a wide range of ECM components and is expressed by fibroblastic cells [2]. The oxidative stress is involved in the maintenance of chronic inflammation in CD [3] and plays an important role in the regulation of MMPs activity and expression [4]. Therefore, the aim of this study was to investigate the relationship among oxidative stress, activity of antioxidants and MMP-3 production in a myofibroblasts cell line derived from human colonic mucosa, CCD-18Co (Co) stimulated or not by TNFα. Oxidative state was modulated by buthionine sulfoximine, an inhibitor of glutathione (GSH) synthesis, N-acetylcysteine (NAC), GSH precursor, and curcumin, antioxidant with anti-inflammatory properties. An up-regulation of MMP-3 due to increased oxidative state was found in 18Co treated with BSO. Stimulation by tumor necrosis factor (TNF)α increased further MMP-3 levels. NAC and curcumin treatments removed BSO induced oxidative state and up-regulation of MMP-3. However, these antioxidants normalized MMP-3 levels mainly in TNFα stimulated cells. Moreover, the effects of NAC and curcumin on MMP-3 were not closely related to the change of redox state measured in these conditions. For this, we hypothesize a possible direct action of NAC and curcumin on transcriptional factors involved on MMP-3 production. Our data suggest that, in condition of oxidative stress, the antioxidants, by reducing and/or restoring to normal values the altered levels of MMP-3 may have a therapeutic use for the prevention and treatment of fistulae in intestine of CD patients.

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Keywords: antioxidant activity, Crohn's Disease, myofibroblasts.

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SUN-155

Effect of obesity and tobacco smoke exposure on adipokines, cytokines and matrix metalloproteinases in a rat model

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Obesity is characterized by hypertrophy of adipose tissue, while chronic obstructive pulmonary disease-(COPD) for induce lung damage, mainly emphysema. Both diseases are associated with systemic low-grade inflammation. There are not animal models combining obesity and COPD, therefore these diseases were induced simultaneously in rats to analyze their effects on expression of some inflammatory mediators and enzymes involved in lung tissue remodeling. Obesity was induced with sucrose (30%) for four months plus/minus tobacco smoke exposure (20 cigarettes/day, 5 days/wk) last two months. Body weight, abdominal fat, dyslipidemia, glucose tolerance test-(GTT) and histology were evaluated; inflammatory mediators were analyzed with qPCR and ELISA, while MMP-2, MMP-9, MMP-12, TIMP-1 and TIMP-2 by qRT-PCR; additionally, MMP-2 and MMP-9 by gelatin zymography. Rats with sucrose diet enlarged: body weight, abdominal fat, triglycerides, GTT, plasma levels of insulin, adiponectin, leptin, resistin, IL-6, IL-1β, TNF-α and IFN-γ, upregulating lung IL-6, IL-1β, TNF-α and IFN-γ, and showing hyperplastic bronchial and alveolar epithelium. In animals exposed to sucrose and tobacco smoke were reduced: body weight, abdominal fat, plasma levels of leptin, resistin, IL-1β, IFN-γ, reducing inflammation but exhibiting similar emphysematous lesions than tobacco smoke exposure. Expression of gelatinases and MMP-12 augmented in rats exposed to tobacco smoke alone or combined with sucrose; zymography showed prominent activity of MMP-2 and MMP-9 in all experimental groups. Results suggest that simultaneous exposure to sucrose and tobacco smoke diminish inflammation, but produce similar emphysematous lesions than observed with tobacco smoke exposure, evidencing that obesity did not confer any protector effect for lung damage.
Keywords: COPD, matrix metalloproteinases, Obesity.

SUN-156

Effect of therapeutic low-intensity pulsed ultrasound (LIPUS) after the treatment of rat extraction socket

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Objective: Low-intensity pulsed ultrasound (LIPUS) has been widely accepted as a potent therapeutic tool to accelerate fracture healing. The effect of LIPUS on wound healing in the oral environment, however, has not been well explored. This study aimed to characterize the effect of LIPUS on tooth extraction sockets in aged rats.
Methods: Right maxillary first molars were removed from retired female breeder rats in 2 groups ($n = 5$), control and LIPUS. LIPUS was applied extrabuccally to the socket every 24 hrs starting one day after the extraction. The blood flow rate was measured by laser-Doppler flowmetry at the extraction socket, the dorsum of the tail base, and the dorsum of foot prior to and 20 min after the 20-min LIPUS treatment. We also evaluated cytokine mRNA levels in the cells at the remote sites.
Results and Discussion: On days 3 and 7 after extraction, the blood flow rate in the socket dropped significantly 20 min after LIPUS treatment. On day 3, the rate also dropped in the tail, which is remote from the wound/exposure site. The baseline flow rate in the tail in the LIPUS group was significantly higher than in the vehicle group on day 3. In the socket measurements, the LIPUS group showed a trend to higher baseline values that was not statistically significant. Cytokine messages at the remote sites also showed some changes as a result of LIPUS treatment. Mechanical stimulation at wound sites may have effects far away from the healing tissue through as-yet-unknown mechanisms.
Keywords: socket healing, laser-Doppler flowmetry, low-intensity pulsed ultrasound.

SUN-157

Effects of beta-hydroxybutyrate on brain vascular permeability in rats with traumatic brain injury

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Traumatic brain injury (TBI) causes various pathological conditions including increased blood-brain barrier (BBB) permeability and cerebral edema. This study investigates the effect of beta-hydroxybutyrate (B-OHB) treatment on the functional and structural BBB characteristics during TBI induced by lateral fluid percussion. Adult female rats were allocated into the following experimental groups: 1-Control, 2-Sham, 3-B-OHB, 4-TBI and 5-TBI + B-OHB. Evans blue (EB) and horseradish peroxidase (HRP) traces were used as determinants of BBB permeability. The intensity of immunostaining for occludin, aquaporin-4 (AQP-4), glial fibrillary acidic protein (GFAP) and c-fos were evaluated and gene expression levels of occludin AQP-4, c-fos, glucose transporter-1 (GLUT-1) and nuclear factor kappa-B (NF-κB) were analyzed. Lipid peroxidation and glutathione (GSH) levels were measured. Occludin and GFAP immunostaining intensities did not differ between experimental groups, while AQP-4 and c-fos immunostaining intensities were higher in TBI. MDA levels increased and GSH levels decreased in TBI ($p < 0.05$). c-fos, GLUT-1 and NF-κB expressions were increased in TBI and TBI+B-OBH ($p < 0.01$). EB dye extravasation into brain and HRP reaction products increased in TBI, while B-OHB administration caused alleviation of these findings. B-OHB treatment in TBI caused a reduction in c-fos immunostaining intensity accompanied by decreases in c-fos and NF-κB expressions and increase in GLUT-1 expression ($p < 0.01$). The results of this study indicate that transcellular pathway is primarily responsible for the increased BBB permeability in TBI and that B-OHB treatment may, at least partly, provide a protection of BBB integrity mainly by reducing the effects of free radicals and increasing GLUT-1 production.
Keywords: Beta-hydroxybutyrate, blood-brain barrier, traumatic brain injury.

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SUN-158

Effects of feeding *Ricinus communis* seed meal on liver enzymes makers of albino rats

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The effect of feeding a *Ricinus communis* seed meal on some liver enzymes was evaluated in albino Wister rats. Sixteen (16) male albino rats were randomly divided into 4groups of 4 rats each. Raw *Ricinus communis* seeds were incorporated into normal rat diet at 5%, 10% and 20% inclusion levels and fed to rats in groups 2, 3 and 4 respectively, while rats in group 1 (control) were fed normal rat chow. The study lasted for 28 days, after which the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP), serum bilirubin (conjugated and total) were determined using standard methods. The mean values for ALT and ALP, and those for conjugated bilirubin significantly decreased relative to the control. The mean AST and total bilirubin values significantly increased relative to the control group. The results of the experiment suggest that *Ricinus communis* meal may possess some hepatoprotective property.

Keywords: diet, enzyme activity, liver.

SUN-159

Effects of indole α-lipoic acid derivatives on nitric oxide production in LPS/IFN-γ activated RAW 264.7 macrophages

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In this study, nitric oxide inhibitory effects of indole α-lipoic acid derivatives in LPS/IFN-γ activated RAW 264.7 macrophages were investigated. Antioxidant effects of these derivatives were reported before and potential anti-inflammtory activities were examined in this study. Cell proliferation, nitric oxide levels and iNOS protein expression were examined with MTT test, griess assay and western blot respectively. Our results showed that, all of the α-lipoic acid derivatives showed significant inhibitory effects on NO production and iNOS protein levels dose dependently ($p < 0.05$). Compound III-5a was found to be the most potent inhibitory compound which showed %45 inhibition in NO levels at 2.73 μM concentration. In conclusion, these lipoic acid derivatives may have potential for the treatment of inflammatory conditions related with high NO production.

Keywords: lipoic acid, macrophage, nitric oxide.

SUN-160

Effects of salicylic acid derivatives on reactive oxygen and nitrogen species and the redox state of mitochondria

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Salicylic acid (SA) and its metabolites, frequently found in fruit and vegetables, constitute a natural source of salicylates. SA itself and its salts are commonly used as drugs with vasodilatatory or anti-inflammatory activities. Their low solubility and possible side effects in the gastrointestinal tract force the requirement to find potential replacements. We therefore compared selected properties of SA and 4 other derivatives in vitro.

The ability of gentisic acid (GA) and hypogallic acid (HGA) to scavenge hydroxyl radical ($HO^{\cdot}$) increased with increasing concentration, exceeding 50% at the highest test concentration of 100 $\mu g.ml^{-1}$. However, the highest inhibition capacity, (up to 35%) in the case of protocatechuic acid (PCA) was recorded only at a concentration of 50 $\mu g.ml^{-1}$. SA and 2,4-dihydrobenzoic acid (2,4-DHBA) showed a similar trend, and their scavenging ability was only about 10%. The ability to convert NO to nitrites showed an opposing trend, decreasing from about 20 to 2%, with increasing SA concentration. Maximum values in PCA, 2,4-DHBA, HGA reached up to 27%. However, GA reached 51% efficiency. We therefore investigated the effect of derivatives 50 $\mu g.ml^{-1}$ in rat liver mitochondria as the location of 90% of total cell energy production, leading to the production of superoxide radicals ($O_2^{\cdot -}$) and precursors of other reactive particles. Activities of superoxide dismutase (SOD) were significantly increased, even with SA at $p < 0.001$, and HGA, 2,4-DHBA at $p < 0.01$. However, glutathione peroxidase activity (GPx) in SA did not change significantly and had a significant decreasing trend in comparison with other derivatives. In neither case were there significant changes in the activities of GR nor in reduced glutathione levels, except for increased levels in SA and 2,4-DHBA.

It was shown that acetate, salicylate and benzoate form Cu-complexes exhibited excellent SOD-mimicking activity. It is therefore possible that more active complexes can be formed, especially by 2,4-DHBA and HGA. According to the significantly lower GPx activity, a number of peroxides formed could cause substrate-inhibition, but could also be converted into $HO^{\cdot}$. Despite the potent ability of derivatives to scavenge $HO^{\cdot}$, especially GA and HGA, there is no implied offset of oxidative stress conditions. In this case, the relatively high ability of PCA, 2,4-DHBA, HGA and GA to convert NO to nitrites can be important. At the same time, a high concentration of peroxides and nitrites allows the catalase of their conversion to harmless nitrates. However, we would like to examine this more closely, as 2,4-DHBA seems to be a more effective derivative than GA, PCA or the HGA.

This study was supported by VEGA 1/1236/12.
Keywords: Reactive Nitrogen Species, Reactive Oxygen Species, Salicylates.

SUN-161

Efficient therapeutic usage of pioglitazone to reduce the progress of neurodegenerative conditions in oligodendrocyte progenitor cells

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One of the world wide neuropathologic diseases is Multiple sclerosis (MS) which is considered by inflammation of the central nervous system (CNS) and demyelination. Neurons and oligodendrocytes, which are CNS cells cooperated in MS. Lipopolysaccharide led to NO production and extensive oligodendrocyte and cells death and IFN gamma induce this death. We have focused on the therapeutic role of Peroxisome proliferator-activated receptor gamma (PPARγ) which is a nuclear receptor that response to inflammation. Recently demonstrated that PPAR gamma ligands can effects on viability of neurons and oligodendrocytes during inflammation. Thus as a step to obtain OPC cells, human embryonic stem cells were treated with specific factors and media to obtain OPC cells after 40 days. These cells

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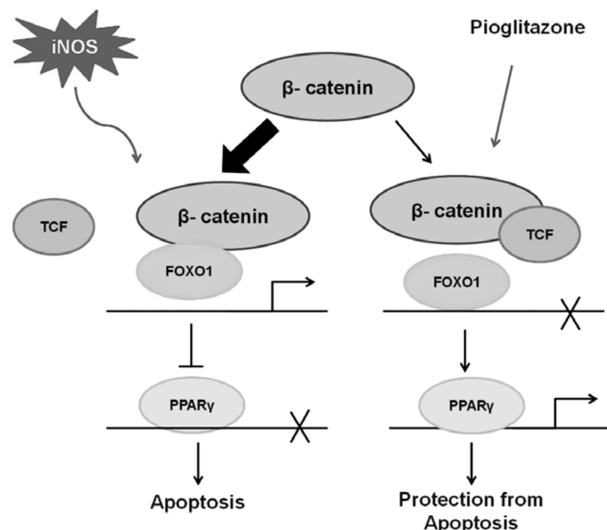

Fig. 1.

were characterized in terms of cellular and molecular analyses. The obtained OPCs were pretreated by one of the potent PPARγ agonists, Pioglitazone, at different concentrations. Then the pretreated cells were used for inflammation induction. Interestingly, pioglitazone reversed the inflammation conditions and enhanced OPC cells viability. Data showed that pioglitazone reduced NO production while did not change mRNA levels of inflammation markers. More over pioglitazone enhanced cell viability through different mechanisms including apoptosis reduction, anti-apoptotic marker increment and regulation of cell cycle markers. This study demonstrated that NO induced apoptosis through FOXO1 and degradation of β-catenin while presence of pioglitazone and CHIR limited this effect and saved hOPCs from apoptosis. Thus we suggest the therapeutic use of Pioglitazine to reduce the progress of neurodegenerative conditions.

Keywords: inflammation, oligodendrocyte, Pioglitazone.

SUN-164

EPA and DHA are able to modulate markers of insulin resistance in ageing 3T3-L1 adipocytes

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Currently, the most frequent cause of insulin resistance is obesity. Adipocytes of obese individuals secrete higher levels of proteins which are involved in development of insulin resistance. On the other hand in obesity secretion of adipokines, which increase insulin sensitivity is decreased. A properly balanced diet and bioactive substances present in food are able to improve insulin sensitivity of body tissues. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are nutrients which potentially decrease insulin resistance. EPA and DHA are able to modulate secretory functions of adipocytes. In most cases they decrease secretion of adipokines involved in development of insulin resistance, such as interleukin 6 (IL-6), and increase production of insulin sensitizers, like adiponectin. Effects of EPA and DHA on young adipocytes were investigated in several studies. However, influence of these fatty acids on old cells is still unknown. The impact of EPA and DHA on secretory functions and markers of insulin resistance in old adipocytes seems to be important because ageing is increasingly connected with fat mass accretion and insulin resistance. Also it was demonstrated in in vivo studies that the proliferation of preadipocytes and their ability to differentiate decreases with age. This observation suggests that old adipocytes could constitute a large part of the adipose tissue, especially in older individuals.

Therefore, the aim of the present study was to evaluate the influence of EPA and DHA on secretory functions and markers of insulin resistance in ageing 3T3-L1 adipocytes.

Differentiated young and old 3T3-L1 adipocytes were cultured for 48 h in the presence of 100 μmol/l EPA, or 50 μmol/l DHA complexed to albumin, whereas in control conditions only albumin was added to the medium. The concentration of adipokines (IL-6 and adiponectin) in conditioned media was measured using mouse-specific ELISA kits. The expression of IRS1 and GLUT4 genes in adipocytes was determined by real – time PCR.

Both investigated fatty acids increased the secretion of adiponectin compared with the control, but only by young cells. Moreover, EPA supplementation increased IL-6 concentration in conditioned medium, while DHA exerted an opposite effect in young and old adipocytes. The expression of IRS1 and GLUT 4 in cells was decreased by EPA and increased by DHA treatment in both examined time points.

In summary, to our knowledge this research is the first showing that the influence of investigated fatty acids on adipokines secretion is age dependent, and seems to be stronger in young adipocytes then old. Furthermore, we demonstrated that EPA and DHA may exert opposite effects on expression of genes connected with insulin resistance, which could be caused by different impact on secretion of IL-6.

Keywords: adipocytes, EPA, insulin resistance.

SUN-165

ERK phosphorylation is suppressed by H_2O_2 in human umbilical vein endothelial cells

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The mitogen-activated protein kinases (MAPK) are key signaling transducers involved in various cellular events such as cell growth, proliferation and differentiation. MAPK signaling pathways are usually activated by a variety of extracellular stimulants including reactive oxygen species. Previous studies have reported that extracellular H_2O_2 leads to phosphorylation of extracellular signal-regulated kinase (ERK), one of the MAP kinases, in endothelial cells. However, in this study, we demonstrate that H_2O_2 suppressed ERK1/2 activation and phosphorylation in some concentration range especially in human umbilical vein endothelial cells (HUVEC), not in immortalized mouse aortic endothelial cells (iMAEC) and human astrocytoma CRT-MG. Physiological laminar flow abrogated H_2O_2-induced suppression of ERK1/2 phosphorylation, but oscillatory flow did not affect H_2O_2-induced ERK1/2 suppression. Decreased ERK1/2 phosphorylation level induced by H_2O_2 was inversely correlated with the level of Src Y530 phosphorylation. Blocking Src using pharmacological inhibitor PP2 abolished H_2O_2-induced phospho-ERK1/2 suppression, leading to a significant increase of ERK1/2 phosphorylation. Csk knock-down using siRNA also abrogated H_2O_2-induced suppression of ERK1/2 phosphorylation. Further study is in progress to identify the effect of H_2O_2 on the membrane translocation of Csk, which known as a kinase of Src Y530 residue, and the effect of H_2O_2-induced ERK1/2 suppression on endothelial cell permeability.

Keywords: ERK1/2, H2O2, HUVEC.

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SUN-166
Evaluation of hyperkaliemia as an indicator of necrosis grade of caustic injuries in adults

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The caustic ingestion is often accompanied by severe metabolic disorders. In general, hyperkaliemia was always correlated with the severity of the tissular necrotic process but has never been studied as an indicator of severity of mucosal lesions in the caustic ingestions.

The aim of our study was to evaluate this biochemical marker as an indicator of severity in caustic digestive necrosis, especially in extreme cases or upper gastrointestinal endoscopy can not be performed.

Materials and Methods: This is a monocentric prospective study, which included all cases of caustic ingestion from January 1st, 2006, to December 31, 2008. All the patients were studied after their hospitalization; fibroscopic examination was performed immediately and an ionogram was performed on the first day from ingestion.

Results: 314 adult patients were hospitalized for caustic ingestion. Among these, 176 were women and 136 were men; their mean age was 28.12 ± 12.00 years (15–79 years).

The ionogram was performed in 255 patients. It was pathologic in 47.5% of cases.

We noted 2% cases of hyperkalemia.it was correlated with acid ingestion in 60% of cases and alkali ingestion in 40% others. The caustic substance was (battery acid: 40%), (chlorydric acid: 40%); (caustic soda: 20%) ($p < 0.00001$).

One patient died before the endoscopic examination.

In all other cases, the lesion stage was severe (>IIb) and the burns were diffuse to the entire upper digestive tract.

Conclusion: Hyperkaliemia has long been considered as a biochemical factor of the tissular necrosis, but it must be reconsidered as an indicator of the necrosis grade of caustic injuries in adults especially in cases where the endoscopy cannot be practiced.

Keywords: caustic, hyperkaliemia, necrosis.

SUN-167
Evaluation of oxidative stress markers in type-2 diabetic rat kidney with treated Δ^9-THC

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Oxidative stress plays a major role in the pathogenesis of diabetes mellitus. Impaired redox homeostasis can lead to the damage of macromolecules and insulin resistance. Thus, oxidative stress promotes the development of complication of diabetes. Δ^9-tetrahydrocannabinol (Δ9-THC) has been shown to interfere with both the function of insulin and its release. The activation of cannabinoid receptors stimulates insulin secretion to maintain glucose homeostasis. The current study, we aimed to evaluate effects of Δ^9-THC on oxidative stress markers with respect to antioxidant status in kidney tissue of streptozotosin + nicotinamid induced type 2 diabetic rats.

The male Sprague–Dawley rats (8–10 weeks) were arranged as Control, Δ^9-THC, Diabetes, Diabetes + Δ^9-THC groups. The type-2 diabetic rats were treated with a single dose nicotinamide (85 mg/kg) 15 min before the injection of streptozotocin (65 mg/kg). Δ^9-THC was administered intraperitoneally at 3 mg/kg/day for 7 days. On day 15 after the Δ^9-THC injections, kidney tissues were removed. Protein carbonyl (PCO), lipid hydroperoxide (LHP), malondialdehyde (MDA), Cu-Zn superoxide dismutase (Cu-Zn, SOD) and thiol fractions including total thiol (T-SH), protein thiol (P-SH) and nonprotein thiol (NP-SH) parameters were measured. Statistical analysis was performed using the SPSS version 21.0 program.

A significant increase was observed in PCO, LHP and MDA parameters of diabetic rats as compared to control rats. Moreover, PCO, LHP and MDA levels of kidney were significantly reduced in diabetes treated with Δ^9-THC compared to diabetic animals. The SOD activity and T-SH level were shown a significant increase in diabetic rats treated with Δ^9-THC as compared with diabetic group. On the other hand, P-SH and NP-SH levels were non-significantly higher in the diabetes treated with Δ^9-THC than in the diabetic rats.

According to the results, the renal tissue in type 2 diabetic animals is exposed to oxidative damage. This oxidative damage may be relieved in type 2 diabetic rats treated with Δ^9-THC.

Keywords: oxidative stress, Type-2 diabetes, Δ9-THC.

SUN-168
Evaluation of scaffolds for the delivery of mesenchymal stem cells in vivo

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Mesenchymal stem cells (MSCs) have been shown to improve tissue regeneration in several preclinical and clinical trials. These cells have been used in combination with three-dimensional scaffolds as a promising approach in the field of regenerative medicine. In order to determine the most suitable scaffold for delivering the cells to dermal wounds, in this work we compare the behavior of human adipose-derived MSCs (AdMSCs) seeded on four different biomaterials that are commonly used in clinical settings. MSCs were isolated, characterized, and seeded onto scaffolds based on bovine collagen and glycosaminoglycans, fibrin, chitosan, or decellularized porcine dermis. In vitro results showed that the scaffolds strongly influence key parameters such as seeding efficiency, cellular distribution, attachment, survival, metabolic activity, and paracrine release. Chick chorioallantoic membrane assays showed that the scaffold composition also influences the angiogenic potential of AdMSCs in vivo. This work provides valuable information to improve wound healing in scaffold-based approaches, representing a step further for optimizing the use of AdMSC-seeded biomaterials in wound repair.

Keywords: biomaterials, mesenchymal stem cells (MSCs), wound healing.

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SUN-169
Experimental pathology of Cardio-pulmonary damage induced by Moroccan vipers' venoms in rabbit model

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Viper envenomations are often associated with complex local and systemic pathological events, including edema, dermonecrosis, myonecrosis and hemorrhage. In this study, the venom of the vipers *Cerastes cerastes* and *Macrovipera mauritanica* and their respective toxic fractions were tested for their ability to induce histopathological and biochemical changes after subcutaneously administration of a subléthal dose.

The histology of the studied organs revealed that both viper's venoms and their toxic fractions induce deep lesions in the tissue structures. Sections of the lung showed an enlargement of alveolar spaces, destruction of the walls lining the alveolar spaces, hemorrhage, intra-alveolar edema and inflammatory cell infiltration, with the exception of a strong hemorrhagic suffusion in the case of *Cerastes cerastes* venom and marked presence of lymphoid islets in the case of venom *Macrovipera mauritanica.* At heart level were observed cardiomyocyte injuries with fibromuscular degeneration, hemorrhagic areas, interstitial edema. The damage was more intense for fractions marked by a discrete inflammatory infiltrate.

Correlated with biochemical markers, the venom of both vipers caused a significant increase in levels of creatine phosphokinase with a concomitant decrease in lactate dehydrogenase 24 hours after envenomation.

Our results suggest that the venom of *Cerastes cerastes* and *Macrovipera mauritanica* are likely to cause severe histopathological changes in poisoned patients. Fast and appropriate management is crucial to prevent the sequelae and preserve the normal functioning of poisoned patients vital organs.

Keywords: Biochemistry blood, Histopathologic modifications, Vipers venom.

SUN-170
Expression and purification of α-1,2 mannosyltransferase for the development of antifungal drugs

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The rise of the occurence of systemic infections has been the cause of great concern worldwide. The therapeutic options currently available are limited, and another problem is the pathogens resistance to the classical antifungal agents resulting in the requirement for the development of new antifungal agents. Preliminary results obtained by our group through *in silico* analysis identified four possible target genes which are present in seven relevant human pathogenic fungi and absent in the human genome. Among these target genes we found *Kre2 or Mnt1,* a gene highly conserved in pathogenic fungi which encodes a protein of approximately 49 kDa, the α – 1,2 mannosyltransferase. It is an important protein for cell viability and virulence of the pathogen within the host. The recombinant protein KRE2 with a degree of purity is of great importance to the knowledge of the main structural and functional characteristics of this protein *Paracoccidiodes lutzii* protein. The results obtained in this study indicate the success in obtaining this protein with high purity in heterologous expression *Escherichia coli*system. However, the refinement of purification in order to increase the purity and reduce potential future degradations are necessary. Heterologous expression of the gene of Paracoccidioides kre2 lutzii in Escherichia coli was performed in this work. Purification of the recombinant protein by affinity chromatography was also carried out. The pure protein enables the execution of other steps, such as enzyme activity to confirm the activity of the recombinant protein, circular dichroism and crystallographic assays may contribute to the knowledge of the main structural and functional characteristics of this protein. To verify possible degradation, MALDI TOFF MS technique was used, and using the software Mascot Search Results was confirmed that, the bands below the expected size of KRE2 belong to the same protein. Thus, the search continues for solving this problem, using such techniques as gel filtration, or by molecular exclusion chromatography. Additionally, DLS was performed, which the main objective of the technique is to observe the behavior of protein molecules in solution. Results generated by this work represents an advancement and may contribute to the development of new antifungal agents for the treatment of important fungal infections worldwide.

Keywords: drug target, new antifungal compounds, α – 1,2 mannosyltransferase.

SUN-171
Expression of cytokine IL-1alpha following cytotoxic treatment in vitro and in vivo

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Purpose: A number of cytokines are abundant at tumor sites, and they may influence the development of the malignant process as well as the tumor response to the treatment. Interleukin-1 (IL-1) acts as a major upstream cytokine that is involved in regulation of immune and inflammatory responses. Little is known about the role of IL-1alpha in various diseases, since most studies have concentrated on the role of IL-1beta. Here, we focused our study on the IL-1alpha expression in cells and tumors following treatment with conventional chemotherapeutic drug 5-fluorouracil (5FU) and photodynamic treatment (PDT) mediated by m-tetrakis-(3-hydroxyphenyl)-chlorin (mTHPC). PDT is an attractive addition to the conventional strategies of cancer treatment due to its specific mode of action.

Methods: Human colon tumor HCT116 cell line and its subline resistant to 5FU, *i. e.* HCT116/FU, as well as Lewis lung carcinoma LLC1 cell line and C57BL/6 mice bearing this tumor, were used for the experiments. In vitro, the cells were incubated with 5-FU or the photosensitizer mTHPC. In case of mTHPC-PDT, light emitting diode array was used for illumination. In vivo, anti-mouse IL-1alpha antibodies were injected after light exposure. The effect of the treatment was evaluated by comparison of viability of treated cells versus untreated ones, growth of tumors and survival of the treated mice versus untreated ones. Gene expression on mRNA and protein levels was revealed by qPCR and ELISA.

Results: It was shown that 5FU stimulated the expression of IL-1alpha in HCT116 and HCT116/FU cells in vitro. mTHPC-PDT significantly inhibited cancer cells survival in vitro and tumor growth in vivo. mTHPC-PDT induced overexpression of IL-

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1alpha. The blockade of cytokine IL-1alpha inhibited growth of both untreated and mTHPC-PDT-treated tumors.

Conclusions: IL-1alpha expressed in response to cytotoxic treatment promotes the tumor growth. Combination of cytotoxic agents with anti-IL-1alpha immunotherapy might be an effective therapeutic strategy.

Keywords: cancer immunotherapy, Interleukin-1.

SUN-172

Flavonoids from *Triticum aestivum* inhibit adipogenesis in 3T3-L1 cells

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Obesity is the most common metabolic disease worldwide. It is characterized with an excessive storage of body fat, and is associated with many metabolic complications, including type 2 diabetes, hypertension and cardiovascular diseases. The current study aims to uncover the effects and underlying mechanisms of flavonoids; leuteolin, isoscoparin and isoorientin isolated from *Triticum aestivum* Sprout (TA) in regulation of adipogenesis. 3T3-L1 cells were treated with different concentrations (0–10 μM) of flavonoids for 8 days. Cell viability was assayed on differentiating cells treated with or without the flavonoids using CCK-8 kit. Oil Red O staining was performed to visualize the lipid accumulation in the cells. We performed quantitative polymerase chain reaction and western blotting to examine expression of transcription factors and genes involved in adipogenesis. Flavonoids suppressed lipid accumulation in 3T3-L1 cells without affecting viability up to 10 μM. The flavonoids inhibited adipocyte differentiation by downregulation of adipogenic transcription factors such as peroxisome proliferator-activated receptor-gamma (PPARγ), CAAT/enhancer binding protein-alpha (C/EBPα) and sterol regulatory element binding proteins (SREBP)-1c and subsequent attenuation of expression of adipogenesis associated genes; activating protein (aP2), fatty acid synthase (FAS), hormone-sensitive lipase (HSL) and lipoprotein lipase (LPL). Of note, the flavonoids enhanced the expression of *insig-1* and *2* which, in turn, inhibited the transcription factor (SREBP-1c) activation, eventually leading to the suppression of adipogenesis in 3T3-L1 cells. Our study reveals an anti-adipogenic effect of the flavonoids from TA and suggests that they could be potential therapeutic agents for the prevention and treatment of obesity.

Keywords: anti-adipogenesis, flavonoids, *Triticum aestivum*.

SUN-173

Functional analysis of fractalkine gene promoter in human aortic smooth muscle cells exposed to pro-inflammatory conditions

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Fractalkine (Fk) and its receptor CX3CR1 contribute effectively to the atherosclerosis process mediating the recruitment of leukocytes and promoting the interactions between monocytes/ macrophages and smooth muscle cells (SMC). Since Fk expression is significantly increased in SMC during atherogenesis, we aimed to uncover the advanced molecular mechanism of transcriptional regulation of Fk gene. To this purpose, we clone and characterized the human Fk promoter and investigated the role of various transcription factors in its regulation in human aortic SMC activated by IFNγ. *In silico* analysis of Fk promoter indicated the existence of binding sites for different inflammatory modulators such as nuclear factor (NF)-kB, activator protein (AP)-1, and signal transducers and activators of transcription (STAT)1/3. Using a luciferase reporter plasmid, we identified a 2046 bp region spanning the transcriptional start point of Fk gene, which has strong constitutive promoter activity in SMC. The effects of IFNγ on both, Fk reporter activity and endogenous transcription, were abolished by silencing the NF-kB, STAT1 and STAT3. Transient over-expression of p65/NF-kB, and STAT1/3, but not c-jun/AP-1, increased the Fk promoter activity. Chromatin immunoprecipitation demonstrated the existence of physical interactions of p65 and STAT1/STAT3 proteins with the predicted elements of the Fk promoter. Moreover, Fk-promoted monocyte chemotaxis was dependent on JAK-STAT pathway. Approaching the advanced molecular mechanisms by cloning and characterizing potential transcriptional response elements, the results identify the fractalkine regulatory mechanism in activated human SMC.

Keywords: fractalkine promoter, smooth muscle cells, interferon-gamma, STAT1/3, NF-kB.

SUN-174

Functional and structural insights into the NADPH oxidase family: new kids on the block!

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The reactive oxygen species (ROS) are known as highly reactive metabolites of oxygen that can attack proteins, lipids, and nucleic acids. This damage includes aging associated pathologies, cardiovascular disease, cancers and chronic inflammation. However, many researches seem to show that these ROS have also essential roles in normal physiological processes, such as redox regulation, cell differentiation, hormone synthesis, and probably the most documented is their role in the host defense [1]. Several ROS producing enzyme systems exist and the phagocytic NADPH oxidase (Nox) was the first identified example of a system that generates ROS not as a byproduct, but rather in a dedicated and oriented manner. Noxs are transmembrane proteins involved in the electron transfer across biological membranes. Usually the final acceptor is the molecular oxygen resulting in the production of superoxide anion that is the precursor of ROS. In addition to ROS related damages, dysregulation of Nox-dependant ROS production can induce pathological consequences related to specific physiological context (e.g., chronic granulomatous disease, hypothyroidism, cardiovascular and neurodegenerative diseases). Accordingly, the Nox family became one of the most potential drug targets, making the understanding of their function at molecular basis crucial.

In the litterature, it has always been reported that Nox proteins exist only in eukaryotes [1]. Eukaryotic membrane proteins have proven to be difficult to study due to their partially hydrophobic surfaces, flexibility and lack of stability, all the data available on Nox enzymes are obtained from putative assignements or structure-function studies.

In our project, to overcome the difficulty of working on eukaryotic membrane proteins, we used an original approach based on bioinformatic tools. Through using specific filters and a novel program, we were able to identify hundreds of prokaryotic candidates. SpNox, the prokaryotic model from *Streptococcus pneumoniae* was expressed in *E. coli* and the pure protein is obtained after several purification steps. Purified SpNox has an NADPH oxidase activity, produces superoxydes, and shares with

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eukaryotic Nox enzymes their major characteristics. Thus, through this work we are providing the first prokaryotic model for the functional and structural study of a Nox enzyme. Crystallization trials are performed and the understanding the biological and physiological function of a Nox protein in bacteria remains to investigate.

Reference

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Keywords: Biochemical characterization, Immunity, inflammation and disease, membrane protein.

SUN-175
Functional study of a novel interferon-responsive gene, GRAMD1B, identified in multiplex MS families

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Background: While the role of common genetic variants is clearly established in multiple sclerosis (MS), the heritability of the disease is still poorly explained, suggesting the existence of rare variants implicated in the susceptibility to the disease.

Objective: To identify low-frequency and rare genetic variants contributing to MS susceptibility in an Italian multiplex family.

Methods: SNP microarray genotyping and whole-genome sequencing (mean coverage: 20×) in 4 MS patients and 4 unaffected individuals belonging to an Italian multiplex family originating from a first cousin consanguinueous marriage have been performed. The Merlin software was used for the linkage analyses and SNPeff and GATK software were applied to prioritize rare variants.

Results: Filtering criteria, narrowed down the list of variants up to a rare functional variant, which determines an amino acid change, S601P, at a unexplored gene, *GRAMD1B*. The mutation is in a context that is highly conserved across species, and it segregates along the family consistent with an autosomal recessive transmission ($p = 0.02$). By performing WG expression, we found that the gene was downregulated in affected relatives ($p = 0.01$) with the exception of the only case who was IFNβ treated. We performed an IFNβ stimulation of PBMCs isolated from 20 healthy controls, showing an increase in *GRAMD1B* expression ($p < 0.001$). We also observed a significantly higher expression of GRAMD1B in the brain tissue and in immune cells, compared to other cells and tissues ($p < 0.05$) and a significant increase of GRAMD1B expression in activated rat microglial cells compared to unstimulated ones ($p < 0.05$), suggesting a possible role of this protein in the context of glia activation. Ongoing experiments are aimed to investigate: 1) the intracellular localization of GRAMD1B 2) the function of GRAMD1B particularly the modulation of its expression under glia activation and IFNβ stimulation; 3) the effect of the S601P mutation on the expression, localization and function of GRAMD1B protein as well as its possible role in MS etiology.

Conclusions: We identified a novel rare variant in GRAMD1B, an unexplored gene recently found to be associated with IgE levels. Further investigations are ongoing to explore the role of this variant in MS.

Keywords: Disease genomics, immune system, neurogenetics.

SUN-176
Gene editing by CRISPR/Cas9: a novel approach complementing ES-cell based gene targeting technologies to efficiently modify the mouse genome

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The unique possibility to introduce virtually any modification in the mouse genome has provided investigators with invaluable tools to explore specific aspects of physiology and pathology in a mammalian system. One of the main limitations of gene targeting in mouse is the technical complexity of the entire procedure: steps such as targeting vector construction, ES cell manipulation, and blastocyst injection are rather time-consuming and require extensive effort from highly experienced scientists.

Here, we discuss the use of the clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9) technology as a complementary approach to ES cell manipulation by homologous recombination, and its advantages/disadvantages over standard gene targeting methods. As an example of CRISPR/Cas9 versatility, we describe the combination of this technology with standard gene targeting approaches to modify already existing mouse models, tailoring them to new experimental needs (model refitting). Along this line, we will also present our recent data on the use of CRISPR/Cas9 to generate conditional Knock-Outs, Knock-Ins, genomic deletions, and multiplex Knock-Outs.

Keywords: CRISPR/Cas9, genome editing, Mouse models.

SUN-177
Genetic association of SNPs in MMPs type polymorphisms with COPD secondary smoking

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Introduction: Obesity is a major risk factor to respiratory disorders, with a pathophysiological link with chronic obstructive pulmonary disease (COPD). The prevalence of obesity increased in patients with mild to moderate forms of COPD (grades I and II; GOLD), but is lower in patients with severe impairment of lung function (grades III and IV, GOLD). Adipose tissue can respond to the presence of pro-inflammatory stimuli delivered by the lungs, secreting in turn inflammatory mediators including adipocytokines, cytokines and chemokines; which can regulated either positively or negatively lipid metabolism and may also exert immunomodulatory effect on the inflammation associated with COPD. Additionally MMPs act remodeling extracellular matrix in lung and airways as part of inflammation and develop of lesions in COPD, therefore the single nucleotide polymorphisms (SNPs) associated with MMPs genes could be involved in modulate pathogenesis of obesity an COPD through genetic expression.

Patients and Methods: Were evaluated 12 SNPs; 3 SNPs in each one of the genes to *MMP1*, *MMP2*, *MMP9* and *MMP12*, in 330 smokers with COPD (EP) and 658 smokers without COPD (FS) used as controls.The genetic material was obtained from the biobank samples of DNA at the HLA Laboratory of the National Institute of Respiratory Diseases (INER). Genotyping for SNPs was performed by real-time PCR (7300 Real Time PCR System). The association analysis of genotypes and alleles was done with Epi Info 7.0 software.

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Results: Allelic frequencies of the polymorphisms evaluated were in Hardy-Weinberg equilibrium. Statistically significant differences in the frequency of TT genotype of rs3918253 in *MMP9* (11.21% versus 6.83%, p = 2.50E-02, OR = 1.72, 95% CI 1.08–2.71) when compare cases versus controls, showing this SNP is a genetic factor to COPD susceptibility in Mexican population.Two SNPs were found associated in *MMP2*, the GG genotype of rs243864 (EP = 10.3% versus FS = 1.51%, p = 1.00E-10, OR = 7.44, 95% CI = 3.62–15.26) and the GG genotype of rs116643 (EP = 15.45 versus FS = 10.33, p = 2.50E-02, OR = 1.58, 95% CI = 1.07–2.34) suggesting also genetic susceptibility to COPD in the Mexican population. There was not associated with genetic susceptibility to COPD in the *MMP1* and *MMP12* gene polymorphisms.The haplotype consisting of the polymorphisms in the *MMP1* and *MMP12* genes, located in different positions of chromosome 11 were calculated. No haplotype blocks were found in high linkage disequilibrium with r2 > 80.
Conclusion: There are polymorphisms SNP-type in *MMP2* and *MMP9* genes were associated with genetic susceptibility to COPD in the Mexican population.
Keywords: COPD, MMP, polymorphisms.

SUN-178
Glutathione intake promotes longevity through the activation of SIR-2.1 and DAF-16 (FoxO) pathway in *C. elegans*

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Oxidative stress has a prominent role in lifespan regulation of the living organisms. One of the endogenous free radical scavenger systems is associated with GSH, the most abundant nonprotein thiol in mammalian cells, acting as a major reducing agent and antioxidant defence. We have recently designed a series of novel S-acyl-GSH derivatives capable to prevent amyloid oxidative stress and cholinergic dysfunction in Alzheimer disease models, upon the increase of GSH intake. In this study we show that the longevity of wild-type N2 *Caenorhabditis elegans* strain was significantly enhanced by dietary supplementation with linolenoyl-SG (lin-SG) thioester with respect to ethyl ester of GSH, linolenic acid or vitamin E. RNA interference analysis and activity inhibition assay indicate that lifespan extension was mediated by the upregulation of SIR-2.1, a NAD-dependent histone deacetylase ortholog of mammalian SIRT1. In particular, lin-SG-mediated overexpression of sir-2.1 appears to be related to the DAF-16 (FoxO) pathway. Moreover, lin-SG derivative protects N2 worms from the paralysis and oxidative stress induced by $A\beta/H_2O_2$ exposure. Overall, our findings put forward lin-SG thioester as an antioxidant supplement triggering sirtuin upregulation, thus opening new future perspectives for healthy aging or delayed onset of oxidative-related diseases.
Keywords: Glutathione, longevity, oxidative stress.

SUN-179
GM-CSF-induced CCL17 expression in monocytes/macrophages is IRF4 dependent: a pathway of potential significance in inflammation

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Clinical trials in rheumatoid arthritis (RA) targetting the cytokine, granulocyte macrophage-colony stimulating factor (GM-CSF), are showing promise although its mode of action remains largely unknown. Once responsive cell target type to GM-CSF is monocytes/macrophages. Increased macrophage numbers in the synovial fluid from RA knee joints is highly correlated with the severeity of the disease. The interferon regulatory factor (IRF) family of transcription factors is important in controlling expression of genes involved in immune functions. Their key role in controlling gene expression in monocytes/macrophages has recently become a major focus of research.

We report here that GM-CSF induced IRF4 expression, while suppressing IRF8 in primary human monocytes. We found that the chemokine CCL17 expression was induced in GM-CSF-treated human monocytes and largely dependent on IRF4 transcription factor. Significantly, a recent study reported that synovial fluid from knee joints of RA patients had elevated levels of CCL17 as compared to healthy controls. Interestingly, CCL17 gene is clustered together with CCL22 and CX3CL1 on human chromosome 16q13. The transcriptional regulation of these three chemokines by GM-CSF in human monocytes and their role in inflammation will be presented and discussed.
Keywords: inflammation, monocytes, transcription factors.

SUN-180
Granzyme B/perforin system and serpinB9: impact on inflammation and insulin resistance in coronary atherosclerosis

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Background/Objectives: Pro-apoptotic protease granzyme B (GZB) and perforin (PRF) are the major mediators of cytotoxic T lymphocytes, the dominant immune cells accumulated in advanced atherosclerotic lesions. Protease inhibitor-9 (PI-9) or serpinB9, the only known inhibitor of human GZB, protects vascular cells against GZB-induced apoptosis. This study aims to explore the role of GZB, PRF and PI-9 in atherosclerosis through assessing their gene expression in peripheral leucocytes and atherosclerotic tissues as well as their contribution to atherosclerosis-related key regulatory processes.
Method: 69 patients with atherosclerotic coronary artery disease (CAD) divided into 26 non-diabetics and 43 diabetics were compared to 15 apparently healthy controls. 24 atherosclerotic tissues were compared to normal mammary arteries. Serum insulin, hsCRP and GZB levels were estimated by ELISA while mRNA expression levels of GZB, PRF and PI-9 were quantified by Taqman RT-PCR.
Results: Serum insulin, hsCRP and GZB levels were significantly elevated in atherosclerotic patients compared to control subjects ($P < 0.001$). There is a significant increase in GZB mRNA expression and a significant reduction in PI-9 mRNA in peripheral leucocytes and atherosclerotic lesions ($P < 0.001$). PRF mRNA levels increased significantly only in atherosclerotic tissues ($P < 0.001$). Also, diabetic patients showed lower levels of PI-9 mRNA in peripheral leucocytes and atherosclerotic tissues compared to non-diabetics ($P < 0.05$). Regression analysis revealed that GZB and PI-9 have opposite significant modulating effects on inflammation and insulin resistance markers. Interestingly, PI-9 mRNA expression in peripheral leucocytes was inversely contributed to CAD severity.
Conclusions: GZB and PI-9 are proposed key modulators for inflammation and insulin resistance in atherosclerosis. Low levels of circulating PI-9 mRNA might be a biomarker of CAD severity. Induction of PI-9 could be a novel therapeutic approach to attenuate atherosclerosis progression.
Keywords: Atherosclerosis, granzyme B, protease inhibitor 9.

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SUN-181
Haemostasis disorders caused after envenomation by vipers' *Cerastes cerastes* and *Macrovipera mauritanica*

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Envenomation by viper bites is an important cause of morbidity and mortality in tropical and sub-tropical countries including Morocco. Vipers venoms are a real source of proteolytic enzymes causing clotting, bleeding, nephrotoxicity, edema, necrosis, hemorrhage, pain at the bite site and systemic changes. This study was conducted to evaluate the changes induced in hematological parameters in rabbits after 1, 3, 6 and 24 hours of subcutaneously administration of a subletlal dose of *Cerastes cerastes* and *Macrovipera mauritanica* venoms.

Our results indicated that most hematological and hemostatic parameters showed significant changes 3 and 6 hours after envenomation. The hemoglobin, hematocrit, red blood cells, platelets and the Quick time were reduced significantly 3 hours after envenomation. A very significant increase in the rate of lymphocytes, monocytes, actived thromboplastin time and fibrinogen were recorded 6 hours following envenomation. However, no significant difference was found for the mean corpuscular volume, corpuscular hemoglobin content, mean corpuscular hemoglobin concentration and the levels of polymorphonuclear neutrophils, eosinophils, basophils throughout the whole duration of the experiment.

These results suggest that severe hematological and hemostatic changes may be initiated during the early stages of poisoning leading to local and systemic hemorrhages and coagulopathies which are the main cause of death in case of vipers envenomations.

Keywords: Envenomation, Hematology blood, vipers.

SUN-182
Harnessing nanothecnology for therapy of inflammatory bowel disease

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Inflammatory bowel disease (IBD) is a chronic immune cell mediated autoimmune disorder affecting the western population with a rising incidence rate. Its etiology is estimated to result from intestinal loss of tolerance, in the presence of constant antigenic stimulus derived from gut-inhabiting bacteria. Our main goal is to develop a novel, versatile, and specific nanoparticle-based delivery strategy (tsNP) that will target the intestinal mononuclear phagocyte (iMNP) system, which is pivotal in the pathogenesis of intestinal auto-inflammation. We have identified a specific and highly expressed surface marker, Ly6C, which characterizes a pro-inflammatory subset of iMNPs. This subset is up-regulated during IBD and a major driver of the disorder, thus making it a promising therapeutic target.

The Ly6C^{high} population showed a high and rapid rate of receptor-mediated endocytosis following the Ly6C ligand binding. Thus, we have generated tsNPs targeting Ly6C in order to deliver cytotoxic doxorubicin (DOX) to this subset of pro-inflammatory iMNPs. Utilization of the tsNPs in ex-vivo studies resulted in an efficient internalization and cargo release of the tsNPs in IBD and not in steady state, enabling a selective and safe way to target the iMNP and consequently their pro-inflammatory activity. Moreover, employment of the anti-Ly6C-tsNPs in-vivo led to a specific targeting and elimination of the Ly6C^{+} iMNP population in IBD solely, leading to amelioration of the acute colitis and rescue of the mice.

These findings may contribute to a long-sought personalized medicine platform, by which therapies will be targeted to cells of choice, where they will release a customized therapeutic target, while minimizing collateral adverse effects.

Keywords: None.

SUN-183
Heterologous expression and enzymatic characterization of Trr1 as a target for antifungal drugs

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Although fungal infections contribute substantially to human morbidity and mortality, the impact of these diseases on human health is not widely appreciated. *Cryptococcus neoformans* is a basidiomycete fungus that causes cryptococcosis worldwide in both immunocompromised and healthy individuals. It affects about one million people each year and kills about 650 000. Several factors are required for intracellular survival. The polysaccharide capsule is an established virulence-associated phenotype of *Cryptococcus*. Likewise, the abilities of the fungus to regulate oxidative stress responses and produce melanin are also important for virulence. The *TRR1* gene is essential and encodes the cytoplasmic thioredoxin reductase enzyme. This protein has a role in maintaining the redox balance of the cell and forms part of a complex, which contains thioredoxin (Trx), thioredoxin reductase (Trr) and NADPH, protecting cells against oxidative and nitrosative stress. Some drugs to treat systemic and superficial fungal infections are available; however, the emergence of *C. neoformans* drug resistance strains due to its inherent 'hetero-resistance' to azoles, the side effects of amphotericin B, and the ineffectiveness of the most recent antifungal echinocandins highlight the critical need of a new generation of antifungal agents. In this study, heterologous expression, purification and enzymatic characterization of Trr1 and its substrate Trx1 of the fungal pathogen *C. neoformans* were performed. The *TRR1* and *TRX1* genes were expressed in *Escherichia coli* using recombinant strategy. The gene fragments were obtained by assembling synthetic genes and these were inserted into pET21a vector. The expressed products were purified by affinity chromatography and detected by western blot. It was possible to identify a band of 39 kDa for Trr1 and another of 12 kDa for Trx1. The enzymatic activity of the recombinant protein was also carried out. As a result, the kinetic parameters K_m and $V_{máx}$ indicated that Trr1 was able to efficiently reduce Trx1. This work shows a viable system for the production of recombinant proteins providing amount necessary to perform three-dimensional structural studies. Results generated by this work represents an advancement and may contribute to the development of new drugs with broad-spectrum activity for the treatment of fungal infections of global significance. In addition, *C. neoformans TRR1* has no human homologs and could therefore be a promising target for new antifungal drugs.

Keywords: Antifungal drugs, *Cryptococcus neoformans*, Thioredoxin reductase.

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SUN-184
Heterologous expression and purification of Ric c 1 and Ric c 1 mutant IN *Escherichia coli* and studies of defense and allergenic activities

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The 2S albumin isoforms from *Ricinus communis,* Ric c 1 and Ric c 3 are storage and defense proteins in castor seeds. Among the functions of defense, include the inhibition of α-amylase of larval insects. Ric c 1 and Ric c 3 have allergenic properties that promote health risks to farm workers and distributors of castor bean seed. Our group recently identified six epitopes responsible for triggering allergic (IgE-binding epitopes). The cross reaction between these allergens and IgE proteins, is mediated by two glutamic acid residues present in each of the six identified epitopes. We have verified, using docking analysis, that only one residue of glutamic acid of one of allergenic epitope is required for the interaction between Ric c 1 or Ric c 3 with α -amylase for enzyme inhibition. Molecular modeling studies indicated that substitution of some strategic glutamic acid by leucine residues in the IgE-epitopes do not interfere with the inhibitory activity of α-amylase, but possibly reduces the allergenicity. To validate the modeling studies this work aimed to suit the conditions for expression of allergenic proteins Ric c 1 and Ric c 1 mutated in *Escherichia coli*. Ric c 1 consists of two subunits linked by disulphide bonds whoever the recombinant protein was produced as a single protein containing the linked peptide present in its precursor. DNA extraction was performed using the DNeasy® Plant DNA kit and subjected to PCR. The PCR product was inserted into the expression vector pET 32 EK LI to transform *E. coli* (DE3). Induction of recombinant protein was performed by addition of IPTG into the culture. The recombinant proteins were purified by affinity chromatography on Ni-NTA column and by reverse phase chromatography in HPLC C_2C_{18} column. The purification was verified by SDS-PAGE, immunoblotting and checked by N-terminal partial sequence. The allergenic activity of recombinant Ric c 1 was measured by mast cell degranulation assay and inhibitory alpha amylase activity was tested against insect enzyme. These activities were then compared with those of the native component. Following successful cloning, Ric c 1 recombinant containing mutations in specific glutamic acid residues was performed. For this, synthetic gene using the GeneArt (Invitrogen) was marketed, already inserted into a cloning vector pMA. The pMA vector containing the synthetic gene was used to transform a strain XL -10 clones. Molecular studies showed that it was possible to express recombinant Ric c 1 and Ric c 1 mutant. The biological tests demonstrated the reduction of allergenicity and preservation of inhibition of α – amylase in the mutant variety.

Supported by CNPq and FAPERJ.
Keywords: Allergy, Castor Bean, Ric c 1.

SUN-185
High cholesterol diet induced endoplasmic reticulum stres related apoptotic process on cardiac myocyte failure

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The injured myocardium represents an environment in which there are various factors that promote cell apoptosis, such as oxidative stress, hypoxia and inflammatory reactions. High cholesterol raises important for cardiac dysfunciton, heart disease, heart attack, and stroke. Oxidative stress describes an imbalance between antioxidant defense and the production of reactive oxygen species (ROS), increased reactive oxygen species (ROS) production and the resulting oxidative cell stress has been shown to cell death/apoptosis, mitochondrial dysfunction, cardiac remodeling, and dysfunction. Protein kinase-like endoplasmic reticulum kinase (PERK) is an eIF2α kinase. PERK activation during ER stress correlates with autophosphorylation of its cytoplasmic kinase domain. pPERK has been implicated as a mechanism responsible for cardiomyocyte apoptosis. Process of apoptotic cell death shown that changes include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNAfragmentation. Bcl-2-family proteins such as Bcl-2, Bcl-XL, Bax, Bak, Bad and Bid are central regulators of cell life and death. Caspases are a family of cysteine proteases that play essential roles in apoptosis which are cleavage some of the final targets such as lamins ICAD/DFF45 (inhibitor of caspase activated DNase or DNA fragmentation factor 45) PARP (poly-ADP ribose polymerase).

In our experiment twenty male albino rabbits (1–2 months old) were assigned randomly to four groups. The first group of rabbits, the control rabbits, was only fed with diet. The second group was fed with diet containing 2% cholesterol, third group diet and received injections of 50 mg/kg/day of vitamin E intramuscularly and the rabbits in the fourth group were fed with diet containing 2% cholesterol and received injections of 50 mg/kg/day of vitamin E intramuscularly. After 8 weeks; MDA and vitamin E in blood was detected by HPLC. Apoptosis of heart tissue showed by DNA fragmentation. The left ventricule of hearth tissues were removed and protein levels of Perk, pPerk, Bax, pBcl2, Procaspase-Caspase-9 and Procaspase-Caspase-3 measured by immünoblotting. The results, endoplasmic reticulum stress related apoptotic process for hypercholesterolemic rabbits will be discussed.

Supported by Marmara University Research Fund SAG-A-130612-0202.
Keywords: Hypercholesterolemia, Endoplasmic stress, Apoptosis.

SUN-186
Homocysteine as a marker for inflammation in woman with polycystic ovary syndrome

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Polycystic ovary syndrome is characterized by oligo-/anovulation and hyperandrogenism including hirsutism, and infertility. There are many studies suggesting that PCOS may increase risk for several conditions including insulin resistance, type 2 diabetes, dyslipidemia, obesity, hypertension, cardiovascular risk. Based on most of the risk factor profiles, woman with PCOS would be expected to be at significantly increased risk for atherosclerotic disease. Although a definitive link between PCOS and these chronic illnesses has not been demonstrated, there is significant overlap in the clinical characteristics of these disorders. Consequently, the issue of identifying and measuring potential conditions that may be associated with PCOS is a priority and should be the standard of practice in its management. Hyperhomocysteinemia has been shown as independent predictor of cardiovascular events in patients with atherosclerosis as a result of promotion of inflammation and direct injure of endothelial cells. The aim of our study was to determinate levels of homocysteine in woman with polycystic ovary syndrome compared with healthy woman. Thirty patients (age, 23.5 ± 5.5) with PCOS and twenty four (age, 25.5 ± 4.3) healthy woman were involved in the study. Blood samples were collected in early follicular phase. Total homocysteine was measured using fluorescent immunoas-

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say. Statistically significant differences in serum concentration of homocysteine were observed between groups. Mean homocysteine level we found as (10.2 ± 2.9 versus 7.0 ± 1.5) in PCOS and normal group respectively ($p < 0.05$). For Macedonian population we found statistically significant increased homocysteine levels in woman with PCOS. Although the mean homocysteine levels are within normal limits, there are significant higher mean homocysteine concentrations between these two groups. Because an increased concentration of tHcy has been shown as a independent risk factor for cardiovascular alterations, it is essential in this group of woman to be taken measures for early prevention.

Keywords: homocysteine, inflammation, Polycystic ovary syndrome.

SUN-188
Identification of complexes of antimicrobial proteins and peptides human milk

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Investigation of antimicrobial properties of minor human milk proteins and peptides is very important for understanding the role of breastfeeding in the development of infant immunity. In this research we analyzed human milk on the content of antimicrobial peptides and protein. Human milk was subjected to preparative continuous acid-urea polyacrilamide gel ectrophoresis. The main cationic fractions contained lysozyme and had antimicrobial activity against test bacteria *E. coli* (Gram-positive) and *L. monocytogenes* (Gram-negative). Moreover, human neutrophil peptides 1–4 were identified in low-mobility high molecular weight cationic fractions with myeloperoxidase, lactoperoxidase and lactoferrin by Dot-ELISA. That indicates availability the presence of protein complexes. We attempted destruction high molecular weight protein complexes by 2M sodium chloride and pH reduction to 2.5 with acetic acid. Then we fractionated human milk whey, high salt milk extract (native pH) and high salt with acetic acid milk extract (pH 2.5) by Vivaspin sample concentrators with a range of molecular weight cutoff (100, 50, 30, 10 kDa (GE Healthcare). As a result of ultrafiltration native human milk whey fractions more than 100 and 50–100 kDa had higher antimicrobial activity against L.monocytogenes compared to the native human whey. The 30–50 and 10–30 kDa fractions had not antimicrobial activity at all. Apparently, lysozyme is included in complex with other proteins with a total molecular weight of more than 50 kDa. In high salt extractions of milk there was a slight increase in antimicrobial activity against *L. monocytogenes* compared to native milk whey. 10–30 and 30–50 kDa high salt milk fractions had only small quantity of lysozyme compared to other proteins. There was the significant increase in antimicrobial activity against *E. coli* and *L. monocytogenes* in 30–50 and 10–30 kDa fractions of high salt with acetic acid extractions of milk. Milk proteins fractions with antimicrobial activity against the test bacteria were separated by RP-HPLC. As the results of chromatography the main component of the high salt with acetic acid 10–30 kDa fraction (pH 2.5) was lysozyme (about 70%) with high antimicrobial activity against *L. monocytogenes* and *E. coli*. The high salt with acetic acid 30–50 kDa fraction (pH 2.5) had about 30% of lysozyme, 60% of alpha-lactalbumin and a small quantity of proteins with a molecular weight from 6 to 29 kDa. Based on the obtained results we can assume that the main antimicrobial component of human milk is lysozyme, it is probably contained in milk in the form of macromolecular complexes, which are destroyed in the stomach in a low pH.

Keywords: antimicrobial proteins and peptides, innate immunity, lysozyme, human milk.

SUN-189
Identification of *E. coli* mimetics proteins that can inhibit phagocytosis mechanisms

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Introduction: Sepsis is a complex syndrome defined by systemic inflammatory response syndrome of infectious origin and characterized by multiple manifestations that can determine dysfunction or failure of one or more organs or systems. It is the leading cause of death in intensive care units in critically ill patients and has represented a constant source of concern for health systems around the world, mainly due to high rates of morbidity and mortality. The treatment of sepsis is challenging and remains a difficult task due to numerous interfering factors. Studies have demonstrated the link between *Escherichia coli* (*E. coli*) and CD16 provides an increase in the inflammatory response resulting from inhibition of induction of phagocytosis, therefore seek to identify peptides and their CD16 binding proteins involved in phagocytosis of infectious agents to finding a possible therapeutic target.

Methods and Results: Using the methodology Phage Display which is a cloning technique that allows the expression of various peptide sequences on the surface of bacteriophage (phage) and selecting these on the basis of affinity for a target molecule sought peptides that interact with CD16. By biopanning found that two peptides obtained this interaction. The expectation is that these peptides found (previously called for: Peptide 3 and Peptide 19) mimics CD16 binding proteins, in particular proteins of E. coli, involved in the interaction with CD16.

Conclusion: The identification of proteins capable of inducing inhibition of phagocytosis through the CD16 receptor, can be used as a new treatment of sepsis, as well as exploited in the treatment of autoimmune diseases, there is seen that these proteins also increase CD16-inflammatory dependent.

Keywords: CD16, *E. coli*, Sepsis.

SUN-190
Identification of endocytic proteins involved in IFN-α stimulated JAK-STAT signaling

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The role of endocytosis in signal transduction has been traditionally viewed as a mechanism for internalization and degradation of activated receptors in lysosomes. However, this model is changing with reports suggesting that endocytic proteins also participate in various aspects of cellular signaling and transcriptional regulation.

The aim of our study is to identify endocytic proteins involved in type-I interferon (IFN-α) stimulated signaling. IFN-α is a cytokine with potent anti-viral and anti-proliferative activities. It is produced in response to pathogenic antigens. In human cells, IFN-α induces the JAK-STAT signaling cascade after binding to the IFN-α receptor (IFNAR) complex, composed of IFNAR1 and IFNAR2 chains. It has been shown that, upon ligand binding, IFNAR1 receptor is internalized by clathrin- and dynamin-dependent endocytic pathway (Marchetti et al., 2006 Mol Biol Cell *17*, 2896–2909). Therefore, it is reasonable to suspect that IFNAR might exploit a broader spectrum of endocytic machin-

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ery to modulate its signaling. However, so far there is little evidence to support this idea.

We performed small-scale RNAi-based screening to identify endocytic proteins that regulate the JAK-STAT pathway. The methodology included silencing of pre-selected genes encoding individual endocytic proteins followed by analysis of transcriptional response using luciferase-based reporter assay upon IFN-α stimulation. Genes that upon silencing manifested more than two-fold change in the transcriptional activity were considered as potential regulators of the JAK-STAT pathway. We identified both positive and negative regulators of the pathway, including previously reported dynamin and clathrin. Subsequently, expression of IFN-α target genes (such as OAS1, IFI44 and IFI6) and Stat1 phosphorylation were analyzed upon silencing of the novel potential regulators using real-time PCR and western blotting, respectively. Ongoing experiments aim to address whether known interacting partners of the identified endocytic regulators are similarly involved in the regulation of IFN-α signaling. Further investigation will focus on eliciting the mechanisms through which selected endocytic proteins modulate the IFN-α signaling pathway and what the biological outcome of this regulation is.

Keywords: cell signalling, Interferon, TRANSPOL.

SUN-191
Identification of immunomodulators in liver infected with clonorchis sinensis by microarray and bioinformatics analysis

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Hosts secrete various immunomodulators when the parasite infection. Immunomodulators which secreted anti-inflammatory substances by acting on cells involved in innate immunity of the host to suppress the innate immunity mechanism. Based on this, it is possible to find the genes and proteins involved in immune suppression when parasitic infection in host. It is the basis for finding drug treatment of autoimmune diseases. In the present study, microarray profiling of gene expression was performed to identify the genes regulated by infected with *Clonorchis sinensis* in mouse livers. The DEG (differentially expressed genes) were further analyzed by bioinformatics tools for genetic functional clustering and gene to gene interaction. We selected the final immunomodulators candidate genes derived from *Clonorchis sinensis*. For the experiments, the mice were divided in two groups: C57BL/6 control, C57BL/6-*C. sinensis* infected. Thirty metacercaria of *C. sinensis* were infected orally to infected groups and extract liver tissue after maintained for 2 weeks. For gene expression profiling, microarray analysis using the Illumina MouseWG-6 v2.0 expression BeadChip. The differentially expressed gene were further analyzed for protein-protein interaction, integrative comparison among the biological function and genetic expression, pathway analysis using bioinformatics tools including STRING, DAVID and KEGG. The list of 329 genes (up regulated: 224, down regulated: 105) differentially expressed gene with a fold change>2.0, $p < 0.01$ was submitted to DAVID. Five annotation clusters were selected after gene functional annotation clustering with enrichment score>1.0. The 118 gene and interacting protein were submitted to STRING and explore the possible protein-protein interaction within these genes. In conclusion, we selected immunomodulators genes that expressed in a significant difference in the host when infected with *Clonorchis sinensis*. This would be the applied for candidate drugs for the treatment of autoimmune diseases.

Keywords: Bioinformatics analysis, immunomodulation, liver fluke.

SUN-192
Identification of regulatory mechanism of Src homology 2-containing protein tyrosine phosphatase 2 in brain astrocytes against ROS-mediated oxidative stress by using a systematic mutagenesis approach

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Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), and hydroxyl radicals (OH·), are well-known regulatory signal molecules in the brain. ROS are involved in a wide range of cellular processes by triggering signal transduction events, such as Src and mitogen-activated protein (MAP) kinases.

Src homology 2-containing protein tyrosine phosphatase 2 (SHP-2) is known to protects neurons from neurodegeneration during ischemia/reperfusion injury. SHP-2 is composed of two src homology 2 (SH2) domains at the amino-terminus, a single protein tyrosine phosphatase (PTP) domain and a carboxy-terminal tail. We have recently reported that ROS-mediated oxidative stress promotes phosphorylation of endogenous SHP-2 in brain astrocytes and lipid rafts and caveolin-1 are involved in astrocyte-specific intracellular responses linked to the SHP-2-mediated signaling cascade.

To evaluate the precise function of SHP-2 in brain astrocytes against oxidative stress, we systematically mutated or deleted SHP-2 domains. We compared the caveolin-1 binding affinity of wild-type and mutants SHP-2 using flow cytometric competitive binding assay and surface plasmon resonance (SPR). Deletion of N-SH2 domain of SHP-2 and mutant 542/580 had lower affinities for caveolin-1 compared with wild-type. Wild-type SHP-2 directly bound to caveolin-1 and increased Src kinase activity by inducing phosphorylation at Tyr 419. Further study is in progress to identify and characterize the function of each variant of SHP-2 in ROS-mediated signaling pathways in brain astrocytes in vitro.

Keywords: Reactive oxygen species, astrocytes, SHP-2.

SUN-193
IKKα promotes intestinal tumorigenesis by limiting recruitment of M1-like polarized myeloid cells

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Recruitment of immune cells into solid tumors comprises an essential prerequisite of tumor development. Depending on the prevailing polarization profile of these infiltrating leucocytes tumorigenesis is either promoted or blocked. Here we identify IκB kinase α (IKKα) as a central regulator of a tumoricidal microenvironment during intestinal carcinogenesis. Mice deficient in IKKα kinase activity are largely protected from intestinal tumor development that is dependent on the enhanced recruitment of IFNγ expressing M1-like myeloid cells. In IKKα mutant mice M1-like polarization is not controlled in a cell autonomous manner but depends rather on the interplay of both IKKα mutant tumor epithelia and immune cells. Because therapies aiming at the tumor- microenvironment rather than directly at the mutated cancer cell may circumvent resistance development we suggest IKKα as a promising target for CRC therapy.

Keywords: colorectal cancer, Macrophage activation, tumor microenvironment.

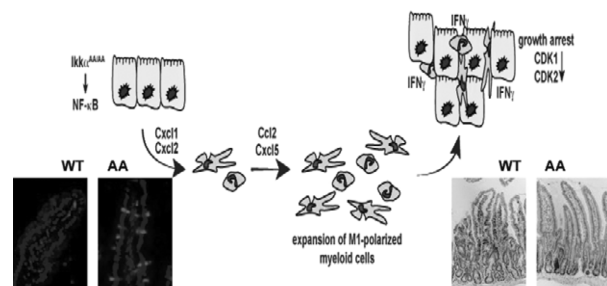

Fig. 1.

SUN-194
IL-10 gene knockout decreases the synthesis of spermine, changes the expression of mGlu receptor 1a/b, and reduces the glutamate-dependent production of nitric oxide in synaptoneurosomes

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IL-10 provides trophic and survival effects directly on neurons, promotes axonal outgrowth, and stimulates neuroregeneration. In this study, we analyzed the activities of arginase and nitric oxide synthase (NOS) in synaptoneurosomes derived from C57BL/6 IL-10 gene-knockout (KO) and wild-type (Wt) mice and determined that the synaptoneurosomes derived from KO mice present lower arginase II activity and lower spermine content than those derived from Wt mice, whereas the basal NOS activity in the KO synaptoneurosomes is higher than that observed in the control synaptoneurosomes. Moreover, our results indicates that the plasma membranes isolated from the KO mice brain exhibit significantly lower spermine-induced enhancement of [3H]MK-801 binding than the plasma membranes from the brain of Wt mice. Glutamate increases the production of nitric oxide (NO) in Wt synaptoneurosomes in a dose-dependent manner, whereas in the KO synaptoneurosomes, this amino acid does not affect the synthesis of NO. The glutamate-dependent acceleration of NO synthesis in Wt synaptoneurosomes was abrogated by LY367385, an antagonist of mGluR1a/b. The western blot analysis of the synaptoneurosomal proteins demonstrates that the expression of the subunits of NMDAR (NMDAR2A and NMDAR2B), the level of NMDAR-bound nNOS, and the expression of iNOS are not changed in KO mice and that only the level of mGluR1a/b is markedly reduced in the synaptoneurosomes of KO mice. We conclude that a neuroprotective and neuroregenerative property of IL-10, in addition to its effects on polyamine metabolism and the spermine-dependent modulation of NMDAR, may involve the regulation of mGluR1a/b expression.

Keywords: IL-10, polyamine biosynthesis, nitric oxide, metabotropic glutamate receptor.

SUN-195
IL-18 gene expression levels are associated with patients with carotid atherosclerosis

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Carotid atherosclerosis (CA) is the atherosclerotic stenosis of proximal internal carotid artery and is one of the main causes of stroke. Recent studies have indicated that levels of circulating pro-inflammatory cytokines are related to cardiovascular diseases. Interleukin-18 (IL-18) is a pleiotropic proinflammatory cytokine which plays an important role in the inflammatory cascade. IL-18 promotes Th1 immune response by inducing IFN-γ and also it enhances the expression of matrix metalloproteinases. Thus, IL-18 is thought to be a potent mediator of atherosclerotic plaque destabilization and vulnerability. Increased expression of IL-18 has been shown in human atherosclerotic plaque lesions where it is localized mainly in plaque macrophages. Thus, we aimed to determine the expression levels of IL-18 gene in both symptomatic and asymptomatic patients with CA.

The study is composed of 50 symptomatic and asymptomatic patients with CA and 52 healthy controls. IL-18 gene expression levels were determined by quantitative real-time PCR (qRT-PCR) from the venous blood samples of CA patients and healthy controls.

Fasting glucose, creatinin and CRP levels were found to be higher in CA patients compared to controls ($p < 0.05$). The IL-18 mRNA levels were significantly increased in CA patients compared to healthy controls ($p = 0.024$). The severity of internal carotid artery (ICA) stenosis was found to be higher in symptomatic patients compared to asymptomatic ones ($p = 0.016$). However, the mRNA levels of IL-18 did not show any significant difference between genders when CA patients and controls were compared ($p = 0.737$, $p = 879$). None of the samples of CA patient group showed any relation in IL-18 mRNA levels between symptomatic and symptomatic patients ($p = 0.965$). There was also no significant relationship between CA patients and controls when IL-18 mRNA levels were compared with ICA stenosis severity ($p = 0.096$). A positive correlation was found between IL-18 mRNA levels and serum CRP levels of CA patients ($r = 0.436$, $p = 0.029$). This study suggests that IL-18

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transcript may affect the pathogenesis of CA and serum CRP levels in carotid atherosclerosis.

Keywords: Carotid Atherosclerosis, gene expression, IL-18 gene.

SUN-196
IL-6 role in fibroblast-macrophage interplay in a fibrotic in vitro model

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The onset of fibrotic conditions in many autoimmune and chronic disorders is characterized by the activation of the cells in the mesenchyme and the secretion of excess extracellular matrix (ECM), growth factors and cytokines, resulting in the formation of a stiff and contractile tissue. While fibrotic responses are involved in many pathological conditions, the underlying mechanism of fibroblast activation is still poorly understood. We isolated fibroblasts from patients with trachoma, a fibrotic blinding disease and leading cause of preventable blindness worldwide, to study the underlying molecular and functional characteristics of the fibrotic tissue. We developed an in vitro model using 3D collagen matrices to measure tissue contraction in response to different environmental stimuli. We found that fibrotic fibroblasts are more contractile than controls and respond better to PDGF stimulation.

In order to identify the molecular mechanisms underlying the fibrotic phenotype, we performed a microarray and a microRNA analysis of 4 trachoma lines and 4 matching control cell lines. We identified a differential expression of 187 genes and 54 miRNAs in trachoma. Amongst those genes, we identified a number of molecules that could be involved in the fibrotic response. IL-6 gene expression was upregulated 4 times in the cases compared to controls, and this was matched with a higher secretion level in trachoma cells. IL-6 inhibition using function-blocking antibodies did not affect fibroblast-mediated standard matrix contraction, but significantly increased contraction in a macrophage-fibroblast co-culture model. In order to study the role of IL-6 in macrophage-fibroblast interaction, fibroblast derived conditioned medium was added to U937 monocytes before or after differentiation to macrophages upon PMA activation. We found that trachoma derived conditioned medium differentially stimulated macrophages and promoted secretion of matrix metalloproteinase (MMP) through IL-6 and STAT3-Akt signaling pathways.

Keywords: fibrosis, IL-6, Macrophages.

SUN-197
Immunogenicity of recombinant analog of lactaptin, a new potential anticancer peptide

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Lactaptin, the proteolytic fragment of human milk kappa-casein, induces the death of various cultured cancer cells [1]. Following treatment with the recombinant analogue of lactaptin (RL2) strong caspase -3, -7 activation was detected [2]. Earlier we observed that RL2 constitutively suppressed *bcl-2* mRNA expression and down regulated Bcl-2 protein expression in MDA-MB-231 cells. We demonstrated that RL2 penetrates cancer and non-transformed cells. Identification of the cellular targets of the lactaptin analogue revealed that α/β-tubulin and α-actinin-1 were RL2-bound proteins [3]. We demonstrated that the recombinant analogue of lactaptin significantly suppressed the growth of solid tumours. In this study we investigate the potency of recombinant analog of lactaptin to involve immune system in apoptosis realization. Anti-RL2 antibodies production was estimated in blood serum of mice treating with various doses of RL2 and by various schemes of therapy. Titres of antibody were very low after four serial injections of RL2. Real-time RL2 cytotoxicity assay was performed with cultured cancer cells (MCF-7 and MDA-MB-231) and primary culture of normal human endometrium using impedance-based iCelligence equipment. Also cells were treated with RL2 and dynamic changes in the mRNA level of *NF-kB* cascade genes, as well as *IFIT2* and *IFIT3* genes were observed. RL2 treatment of cancer and normal cells did not induce changes in *IFIT2* and *IFIT3* mRNA level. It is known that anthracyclines induce calreticulin/ERp57 surface-exposure which is recognized by dendritic cells and signals potential immunogenicity. *CRT* gene expression was estimated to examine RL2 as inductor of immunogenic cell death. We found that RL2 treatment of MCF-7 cells resulted in the induction of *CRT* gene transcription after 5 h. After 24–48 h of incubation the *CRT* mRNA level in treated MCF-7 and MDA-MB-231 cells was significantly lower relative to the mRNA level in control cells ($p < 0.05$). Calreticulin translocation was observed by fluorescent microscopy using anti-CRT antibodies. Our results may indicate that RL2 is low immunogenic and unlikely induces immunogenic death of cancer cells.

This work was supported by grants: RFBR # 13-04-01457, RFBR # 13-04-01313, Federal target program #16.N08.12.1009, FSM #13, SB RAS Integrated project # 94.

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Keywords: Apoptosis, anticancer, Calreticulin, milk proteins.

SUN-198
Immunoregulation studies for biotherapy purposes by using a larval amphibian model

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Live animals have been of crucial importance, through the centuries, to study physiology and more recently to ensure human care by developing and testing new therapeutic approaches. Mice are widely exploited for medical studies but their use has always been a subject of debate. On the contrary, larval amphibians represent undoubtedly a more ethically acceptable model.

Our project aims at studying the immune tolerance to fight intestinal inflammation and to better understand the regenerative capacity by using amphibian *Xenopus tropicalis* tadpoles.

We are focused on the role of regulatory T lymphocytes (Tregs), which play an indispensable role in maintaining the immune tolerance. Tregs represent a subpopulation of $CD4^+$ T cells (5–10% in mice and 3–5% in humans), which are characterized by the expression of the IL-2 receptor alpha chain (CD25) and of the transcription factor Foxp3 (*Forkhead/winged-helix protein 3*). Foxp3 is presently the more specific molecular marker for Tregs and its expression is essential for Treg differentiation and functionality.

The *foxp3* gene is conserved in vertebrates. However, the functional activity of Tregs is poorly understood in non-mammalian vertebrates. We have recently identified a high level of gene synteny of the *foxp3* locus between amphibians and mammals. Cur-

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rently, we are determining the expression patterns of Foxp3 RNA in tadpoles during their development.

The first objective of our work is to study, in a model of intestinal chronic inflammation induced in tadpoles and mimicking inflammatory bowel disease (IBD), the role of 'immunosuppressive' probiotics on the activation of Tregs in the intestinal mucosa. Lactic acid bacteria will be genetically engineered, by synthetic biology, to produce natural 'immunosuppressive' molecules.

Our second objective is to investigate the contribution of Tregs in tissue regeneration, a necessary phenomenon in any inflammatory process. This aspect has recently emerged as an innovative conceptual axis. The regenerative capacity will be evaluated following the tail amputation during the larval development.

To achieve our goals, we will generate fluorescent tadpoles for the *Foxp3* gene from *X. tropicalis* embryos.

In conclusion, our fundamental project on immunoregulatory mechanisms would open new opportunities: (1) to improve tolerogenic biotherapies, in humans, in which Tregs play a central role (autoimmune diseases, food allergies, IBD, tolerance to transgenes and vectors used in gene therapy, ...); (2) to increase the knowledge linking immunosuppression with regeneration capacity and tissue repair, that would be applied in the regenerative medicine field.

Keywords: Regulatory T cells, Synthetic biology, Xenopus.

SUN-199
Impact of hypokinesia on metabolic characteristics and gut microbiota composition of dairy cows

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In the field of animal-breeding the 'stable keeping' has a negative effect on the overall functional state of the animal's organism. Hypokinetic conditions not only produce problematic effects upon the animals' immune status and metabolism, but also generate a range of economic damages relating to productivity and meat product quality and taste.

The influence of hypokinesia on metabolic indexes and gut commensal bacteria from Caucasus brown cows and their calves has been investigated.

The investigations testify that the changes in gut microbiota and in metabolic characteristics of the animals under the conditions of physical inactivity can cause an increased risk of infectious diseases of those animals.

Keywords: None.

SUN-200
Impact of intracellular localization dynamics of invasive bacterial pathogens on host immune response transcriptional signatures in single cells

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Intracellular bacterial pathogens, such as *Shigella flexneri*, enter host cells through tightly regulated, conserved molecular mechanisms. During the invasion process *Shigella* localize at different subcellular niches such as the host cell membrane, pathogen-containing vacuoles or the cytosol. The individual subcellular localization of the bacteria are instantly sensed by the host cellular immune system, which activates diverse signaling pathways. However, it has been insuffiently understood, how the differential orchestration of the host immune transcriptional response is individually determined by the dynamics of bacterial infection and intracellular localization within cellular compartments. Host gene expression has been measured from whole cell populations masking valuable information on subtle transcriptional changes upon individual compartmental changes of the bacteria. To overcome these limitations, we set out to perform a comprehensive study that integrates spatial-temporal information and transcriptomic profiling of the host immune response on the level of small cell populations and of single cells. Combining fluorescence-based approaches and multiplex qPCR single cell analysis identified localization-dependent host transcriptional signatures to *Shigella* infection, that correspond to each stage of bacterial infection, i.e. the membrane-bound and even the cytosolic bacterial localization as well as to yet non-infected bystander cells. Further characterization of individual transcriptional profiles during infection with different *Shigella* mutants gave new insights into how bacterial effectors manipulate localization-dependent host gene expression signatures. Single cell analysis revealed how the invading bacteira affected the coordination of gene expression in a localization-dependent manner. This study contributes to novel understanding of the orchestration and coordination of the host immune response gene expression signatures, determined by the bacterial localization with respect to the cell as well as by different bacterial effectors.

Keywords: host-pathogen interaction, immune response, single cell analysis.

SUN-201
In silico anticancer drug discovery to find NFκB inhibitors

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Nuclear factor kappa B (NFκB) is a transcription factor that plays a significant role in the regulation of immune and inflammatory responses. It is constitutively activated in numerous diseases such as cancer, inducing the expression of genes that promote cell proliferation, regulate apoptosis, facilitate angiogenesis and stimulate invasion and migration of cancer cells. This makes NFκB an attractive target for drug intervention. In this study, computational methods were used to identify similar compounds to 6-Aminoquinazoline (6-Amino), a potent but toxic NFκB inhibitor.

Ligand Based Virtual Screening (LBVS) of the Zinc database was performed using vROCS and Pipeline Pilot. Prior to the similarity search the database was filtered with a drug-like filter to find chemically analogous compounds to 6-Amino. To further enrich our database with potential hits, structure based virtual screening was performed with the compounds identified in the LBVS. Firstly, the unknown target of 6-Amino was elucidated through a series of blind dockings on the available crystal structures of the NFκB pathway proteins. Control compounds with known targets in the NFκB pathway were used to validate our findings. Their targets correlated with what was known in literature, allowing us to identify a putative binding site on the p50/p65/IκBα complex. The binding site is in close proximity to the nuclear localisation signal of p65 and to the phosphorylation and ubiquitination site of IκBα. Binding of 6Amino may induce a conformational change that inhibits the translocation of p65 into the nucleus possibly by inhibiting the degradation of IκBα. Several compounds identified

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to have a high similarity score were also found to bind with a predictably higher affinity to the complex than 6-Amino.

In conclusion, results look promising and are being validated with in vitro inhibition assays. Validating the binding site by site directed mutagenesis or by obtaining a crystal structure will allow for a more targeted structure based drug design approach to inhibit NFκB.

Keywords: Cancer therapy, computer-aided strategy, NF-kB.

SUN-202
In vitro evaluation of proinflammatory effect of trivalent arsenical species at intestinal level

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Chronic exposure to inorganic arsenic (As) is associated to type 2 diabetes, cardiovascular diseases and cancer. Ingested inorganic As is transformed within the gastrointestinal tract, and can give rise to more toxic species such as monomethylarsonous acid [MMA(III)] and dimethylarsinous acid [DMA(III)]. Thus, the intestinal epithelium comes into contact with toxic arsenical species, and the effects of such exposure upon epithelial function are not clear.

The present study has evaluated the effect of 1 μM arsenite [As(III)], 0.1 μM MMA(III) and 1 μM DMA(III) upon the release of cytokines [interleukin-6 (IL6), IL8, tumor necrosis factor alpha (TNFα)], using a compartmentalized co-culture model with differentiated Caco-2 cells in the apical compartment and peripheral blood mononuclear cells (PBMC) in the basolateral compartment. In addition, the combined effect of arsenical species and lipopolysaccharide (LPS), both added into the apical compartment, has been analyzed.

The results indicate that exposure to the arsenical forms induces a proinflammatory response. An increase in cytokine secretion into the basolateral compartment was observed, particularly as regards TNFα (up to 1600%). The cytokine levels on the apical side also increased, though to a lesser extent. As/LPS co-exposure significantly affected the proinflammatory response as compared to treatment with As alone. Treatment with DMA(III) and As/LPS co-exposure increased the permeability of the intestinal monolayer. In addition, As/LPS treatments enhanced As(III) and MMA(III) transport through the intestinal monolayer.

Keywords: Arsenic, cytokines, intestinal epithelium.

SUN-203
In vivo Keap1 S-glutathionylation

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Parkinson's disease (PD) is a movement disorder resulting from severe dopamine (DA) deficiency arising from dopaminergic neuronal degeneration. The molecular mechanisms underlying DA cell death are still not fully understood, but mitochondrial dysfunction, oxidative stress, failure of proteolytic pathways and neuronal inflammation are involved in PD pathogenesis.

Glutathione S-transferase pi (GSTP) is a phase II drug metabolizing enzyme that catalyzes the conjugation of reduced glutathione to electrophilic groups on substrate molecules. GSTP expression is regulated by the nuclear factor-erythroid 2-related factor 2 (Nrf2) and plays an important defensive role against the accumulation of reactive metabolites that contribute to neuronal damage. Moreover, GSTP has been shown to protect cells from ROS by altering the levels of glutathione, by acting as an endogenous regulator of c-Jun N-terminal kinase catalytic activity and by modulating *S*-glutathionylation of proteins.

Aims: We hypothesized that in response to oxidative stress, increased GSTP expression may potentiate Kelch ECH associating protein 1 (Keap1) *S*-glutathionylation, leading to Nrf2 activation and neuronal protection.

Results: MPTP-induced oxidative stress leads to glutathione depletion and altered glutathione-dependent reactions including *S*-glutathionylation. Our results show that GSTP potentiates Keap1 *S*-glutathionylation in mice brain following MPTP administration with subsequent Nrf2 pathway activation, and increased expression of GSTP, in a positive feedback regulatory loop.

Innovation: Our data provide experimental evidence that Keap1 is modified by *S*-glutathionylation in vivo in a GSTP-dependent manner.

Conclusion: The presented results unravel a new mechanism contributing to GSTP-elicited neuronal protection.

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Keywords: Glutathione S-transferase P1, Keap 1, Nrf2.

SUN-204
Inflammatory milieu maintains hemangioma derived endothelial cells proliferative status and hinders regression in infantile hemangioma

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Infantile hemangioma is a vascular tumor that occurs in 5–10% of infants of European descent its defining feature being a dramatic growth of capillary endothelial cells and development into a disorganized mass of blood vessels. Afterward, a slow spontaneous involution begins around one year of age and continues for 4–6 years age. Acknowledging at molecular level the constitutively high expression levels of vascular endothelial cell growth factor (VEGF) and its specific receptor 2 (VEGFR2), we have searched the interrelation with other inflammatory factors that could positively modulate this VEGF-VEGFR2 couple. Our study focused on cellular characteristics of hemangioma-derived endothelial cells isolated from excised hemagiomas (HED). The primary cells isolated from hemangiomas were both in the proliferative and regression stage of the disease. From 21 children with age between 3 and 21 months several serum and urine markers were also evaluated (GLUT-1, MMP-1, TIMP-1, VEGF and MCP-1). Culture supernatants from hemangioma-derived endothelial cells isolated from HED were tested for angiogenic and inflammatory markers, morphological changes and apoptosis as well. As control cells we have

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used HUVEC cell line. Analyzing HED supernatants, GLUT-1 and MCP-1 were found in high concentrations. When applying ex vivo various concentrations of VEGF on HED there are a clear correlation of cell proliferation with the angiogenic factor concentrations, correlated moreover with decreased apoptosis, especially in the time range 3–24 hrs. When applying MMP-1 the effects are similar with the ones induced by VEGF in HED, while control cells display an inverse cellular behavior. When physiologically inhibiting MMP action with TIMP-1, there is a marked reduction of the cells proliferative capacity with an induction of early apoptosis mechanisms. Studying MCP-1 action, depending on the proliferative or regression tumor stage, its action is different. MCP-1 seems to be a factor that modulates positive the proliferative stage of the hemangioma. We can conclude that there is an intricate panel of pro-inflammatory factors displaying spatial and temporal action that converge towards the proliferative stages or to the regression one.

The study was partially supported by the following projects: POSDRU/159/1.5/S/141531 and PN. 09.33-01.01/2009.
Keywords: experimental model, hemangioma, inflammation.

SUN-206
Inhibition of MMP-9 gene expression and cancer cell proliferation by essential oils of *Ocimum sanctum*

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Matrix metalloproteinases (MMPs) released from inflammatory cells are involved in the development and progression of human cancers. Among the various MMPs, MMP-9 is found to be involved in metastasis of breast, colon and ovarian cancers. Natural products are effective in reducing inflammation and carcinogenesis. Essential oil from *Ocimum sanctum* was tested for its effect on inhibiting the proliferation of human breast cancer cells and reducing the expression of MMP-9 in human lymphocytes. Lymphocytes were treated with lipopolysaccharide to induce inflammation and then treated with essential oils. The expression of MMP-9 was analyzed using gelatin zymography and real-time reverse transcriptase PCR. Gelation zymography showed that MMP-9 expression was completely inhibited at 250 ug/ml of essential oil. A dose dependent decrease in the expression of MMP-9 was observed in real-time RT-PCR. The inhibitory effects of essential oils on the proliferation of breast cancer cells (MCF-7) were tested using the MTT assay and real-time PCR analysis. *Ocimum sanctum* essential oil (OSEO) inhibited proliferation (IC_{50} = 170 µg/ml) and migration (IC_{50} = 250 µg/ml) of MCF-7 cells in a dose-dependent manner. OSEO also induced apoptosis as evidenced by the increasing number of propidium iodide stained apoptotic nuclei. Flow cytometry analysis revealed that treatment with OSEO (50–500 µg/ml) increased the apoptotic cell population dose-dependently (by 16%> 84%) compared to the control. Gene expression analysis showed that OSEO upregulated the apoptotic genes p53 and Bid and elevated the ratio of Bax/Bcl-2. The results of our study indicate that OSEO has the ability to express both anti-inflammatory and anticancer activities.

Keywords: Anticancer, Essential oil, MMPs.

SUN-207
Innate immune response during NTM infections

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Background: As tuberculosis incidence declines in industrialized countries, nontuberculous mycobacteria (NTM) infections gained relevance. Human infection with NTM became relevant with AIDS pandemic, being currently recognized as a cause of pulmonary infection in humans. Despite this fact little is known about NTM pathogenesis. In the present work the role of innate immune response during NTM infection using THP-1 cells as a model of alveolar macrophages was evaluated.

Methods: *M. smegmatis* mc^2 155, 2 reference strains (*M. avium* ATCC25291; *M. fortuitum* ATCC6841) and 2 clinical isolates (*M. avium* 60/08; *M. fortuitum* 747/08) were used. Bacteria were grown until mid-exponential phase and stored at −80°C. Before each experiment an aliquot was thawed and diluted in RPMI with 10% HI- FCS in order to reach an $OD_{600\ nm}$ of 0.1. The inoculums were titrated by CFU enumeration on 7H10 medium supplemented with 10% OADC.

Briefly, 4×10^4 THP-1 cells were platted/well and incubated for 72 h with 100 nM PMA (37°C/5% CO_2) then fresh medium without PMA was added being the cells incubated for further 24 h. The cells were infected for 1 or 3 h for fast or slow growers, respectively. The intracellular persistence was evaluated by CFU enumeration at different time points from 1 to 24 h or 3–168 h for fast and slow growers, respectively.

Phagosome acidification was followed using confocal microscopy. The secretion of pro-inflammatory cytokines was assayed by ELISA, NO production using the Griess reagent and apoptosis was followed by flow cytometry and confocal microscopy. The ability of mycobacteria to persist at different pHs was evaluated using BACTEC-MGIT960.

Results: The mycobacteria experienced different fates within THP-1 macrophages. *M. smegmatis* and *M. fortuitum* ATCC6841 were cleared within 24 h, whereas 747/08 and the two *M. avium* strains were able to replicate. Despite this fact for the latest mycobacteria more than 50% of acidified phagosomes were present during the experience. Mycobacteria survival at acidic pHs (6.6; 5.4 and 4.6) was then evaluated. With the exception of *M. smegmatis* all strains grew at acidic pH showing that other factors than phagosome acidification were involved in mycobacteria killing.

Next, other components of the inflammatory response were evaluated. Measurable values of NO were present in supernatants of THP-1 infected for 3 days with 60/08 being this bacterium susceptible, to high concentrations of NO in vitro. Il-10 secretion was also assayed. For both fast growing NTM and *M. avium* ATCC25291 the production of IL-10 was not detectable. For 60/08 IL-10 production peaked at 3 days, decreasing afterwards until undetectable levels at 7 days.

Another factor being explored is apoptosis induction by NTM. Our preliminary results point to differential induction of apoptosis by different NTM.

Keywords: None.

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SUN-208
Insights into haptoglobin: an interesting biomarker for predicting the prognosis of prostate cancer patients

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Personal Peptide Vaccination (PPV), a newly established cancer treatment, makes good use of immune systems we naturally have, reinforces the CTL activity, and has few side effects, which seems to be an attractive way to deal with cancer, but the effects vary among patients. Predicting the prognosis of patients before injecting of PPV is needed, and we would like to reveal if the vaccination is effective or not and specify what is causing such differences, so we held DNA microarray analysis and SNP analysis using samples from patients with advanced castration-resistant prostate cancer (CRPC).

We obtained peripheral blood mononuclear cells (PBMCs) from patients with advanced CRPC, and examined the gene expression profile. PBMCs were collected from advanced CRPC patients both who survived for more than 900 days and who died within 300 days after the peptide vaccination. We found 41 up regulated genes in short-term survivors to be differently expressed in pre- and / or post-vaccination. Haptoglobin (HP), a well-known immune response gene, was listed on these genes. Recently, HP has been attracted attention in cancer researches, but it is still unknown how HP expression pattern in CRPC patients is like. We focused on its expression in PBMCs from CRPC. Analysis of HP expression in PBMCs by qPCR method indicated patients with poor outcome had higher levels of HP mRNA expression in PBMCs. Moreover, from nucleotide sequence analysis of HP promoter region, we found there was HP promoter polymorphism which was significantly associated with overall survival. These suggested HP could be a prognostic biomarker for PPV.

Previous reports showed that serum HP was elevated in various cancer patients including prostate cancer compared to normal subjects and benign tumors. Taken together with previous reports, this study suggests HP in peripheral blood of CRPC patients may have an impact on the immune response to PPV based on either or both of tumor progressive function and immune suppressive function. However, it is obscure how to regulate HP expression level in PBMCs. We examined HP expression in some cell lines derived from blood cell under various culture conditions and found HP expression was induced under stress condition. Intensive research on HP expression will reveal what is causing the difference in the HP expression level between patients with well or poor outcome and what function HP has in immune systems.

Keywords: None.

SUN-209
Intense interactions between TNF and glucocorticoids in sepsis

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Sepsis hits almost 20 million people yearly. The mortality is very high and there is an urgent need for novel therapeutics and new insights in the disease. Despite sepsis has a clearly inflammatory component, glucocorticoids (GCs) are not helpful in sepsis. The reason for this GC resistance is investigated in our work. We apply several mouse models for sepsis and septic shock and we focus on the mechanism by which GCs protect against TNF-induced acute inflammation and on the impact that TNF has on the anti-inflammatory activities of GCs.

Keywords: glucocorticoid, inflammation, TNF.

SUN-210
Interaction between glycans and the immune system: do glycans play a role in Crohn's disease?

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Introduction: Crohn's disease (CD) is characterized by loss of tolerance towards intestinal microorganisms, reflected by serologic responses towards fungal-related glycans such as mannan and beta-glucans.
Objective: To explore glycan-induced immune responses and their correlation with intestinal inflammation.
Methods: Peripheral blood mononuclear cells (PBMCs), isolated from CD and normal control patients, were stimulated by glycans or heat killed (HK) yeasts. Mucosal cells were freshly isolated from surgical specimens. Mucosal biopsies were obtained from CD and ulcerative colitis (UC) patients (inflamed/ non-inflamed) and controls. Expression of the beta-glucan receptor-Dectin1, and mannose receptor- MR, cytokine secretion, and signaling pathways were assessed using flow cytometry, immunofluorescence, and ELISA. Dectin-1 (*CLEC7A*) and MR (*MRC1*) mRNA levels were assessed by real time quantitative PCR.
Results: Mannan induced significantly higher pro-inflammatory cytokine secretion by CD versus normal PBMCs ($p \leq 0.05$). Significant inhibition of glycan-induced cytokine secretion was observed using Syk and Src inhibitors (up to 90% inhibition).

HK yeast induced higher TNF-α and lower IL-10 secretion by CD versus normal PBMCs. Dectin-1 is expressed in the lamina propria and by freshly isolated epithelial cells. Mucosal Dectin1 and MR expression was higher in inflamed CD, but not UC versus controls.
Conclusions: Glycans are capable of stimulating innate immune responses. Glycan receptors are expressed by peripheral and mucosal immune cells and are enhanced in intestinal inflammation, specifically in CD. CD is characterized by hyper-responsiveness towards yeast-characteristic glycans. Thus, glycans may have a role in the pathogenesis of CD.

Keywords: beta glucans, Crohn's disease, mannan.

SUN-211
Interactions of Cu(II) and Cu(I) ions with amyloid-β peptides

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Alzheimer's disease (AD) is a progressive neurodegenerative disease that is characterized by a wide array of pathological features, including amyloid plaque formation, oxidative stress and metal dyshomeostasis. Metal ions have been proposed to be, directly or indirectly, involved in the neurodegenerative mecha-

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nisms of AD. In the amyloid plaques, there is colocalization of metal ions such as Zn, Cu, and Fe ions, and the Aβ peptide coordinates the metals with relatively high affinity. However the role of metal ions in AD etiology remains unresolved. Redox-active copper ions have been proposed to contribute to AD neurodegeneration. Copper is over-represented in AD brains, and binding of copper to the amyloid beta (Aβ) peptide modulates the aggregation pathways. The amyloid plaques are present in the extracellular space where the stable form of copper is Cu(II), and therefore Aβ/Cu(II) is the predominant complex outside the cells. But it has been demonstrated that copper is redox active in the amyloid, generating reactive oxygen species by copper redox cycling between the +1 and +2 oxidation states. In addition due to the reducing environment of the cytosol, the Cu(I) form may dominate inside the cells, enabling the Aβ peptides to interact with Cu(I). Therefore studying Aβ interactions with Cu(I) can contribute to the understanding of the molecular mechanisms of AD. While Aβ/Cu(II) binding has received a lot of attention, Aβ/Cu(I) interaction has been less well studied.

Here, we use NMR, circular dichroism and fluorescence spectroscopy to compare the effects of Cu(II) and Cu(I) on the Aβ peptide. Cu(II) results were similar to previous data reported in the literature, showing a realatively strong specific interaction (1–3). All the experimental methods used demonstrate that Cu(I) displays a non-specific weak interaction with the Aβ peptide, and only mildly affects its aggregation. Earlier studies have observed chemical shift changes in histidine NMR signals upon addition of Cu(I), which were interpreted as proof of histidine ligandation. However, as histidine chemical shifts are sensitive to pH changes, such shifts alone should not be taken as proof for specific binding. In this work, no histidine chemical shift changes were observed in the presence of Cu(I).

Acknowledgments: This study was supported by a scholarship to A.T. from the Swedish Institute.

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Keywords: Alzheimer's disease, amyloid-beta aggregation, metal ions.

SUN-212
Interleukin-33 promotes intestinal barrier function by direct effects on epithelial proliferation and differentiation

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Background and Aims: The small intestine comprises an array of epithelial cells that serves distinct functions during its time course. Many pathways are known to regulate this programming of intestinal stem cell. The balance between various cells of absorptive lineage and cells of secretory lineage (Goblet, Paneth and Enteroendocrine) is highly necessary to maintain homeostasis. However, under pathological conditions the intestinal epithelium is forced to undergo some changes which is mediated by cells of the immune system. Recently, Innate Lymphoid Cell Type2 family cytokines have been shown to participate in the process where particularly IL-33 is associated with goblet cell hyperplasia. In order to investigate its role in controlling small intestinal epithelium differentiation we used the IL-33 overexpressing transgenic mice.

Methods: We generatedan inducible transgenic mouse expressing IL-33 specifically in gut. For expressionof the cytokine mice were injected intraperitoneallywith Tamoxifen. Cre-mediated recombination was genotyped by PCR on tail DNA.To analyse the role of IL-33 directly on epithelium we used the*in-vitro* cultivation of organoids from C5BL/6mice. Localization of IL-33 was studied in IL-33*LacZ/LacZ*reporter mice.

Results: We observed high expression of IL-33 in the gut of TLR-ligand challenged mice. IL-33 was specifically expressed by cells which are located around the intestinal crypt bottom, where stem cells are located. Overexpression of IL-33 in transgenic mouse led to increased expression of anti-microbial peptides and goblet cell markers. Signaling of IL-33 into intestinal epithelial cells in vivo and in organoid cultures in vitro was associated with altered intracellular signalling governing proliferation and differentiation of intestinal epithelial cells.

Conclusions: Our data demonstrate a novel mechanism of IL-33 signallingand its significant consequences on intestinal epithelial cell function. Therefore it plays a role in maintaining intestinal homeostasis and tackling foreign challenges.

Keywords: barrier function, IL-33, reprogramming.

SUN-213
Investigation of tumor necrosis factor-receptor 2 gene polymorphisms in Turkish periodontitis patients

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Periodontitis is a highly complex and multi-factorial disease. With the pooled knowledge about genetic polymorphisms that were claimed to play a role in the predisposition to and the progression of aggressive and chronic periodontitis, it is now possible to identify candidate genes that could act as potential risk or protective factors for the disease. Genetic researches in periodontitis were generally focused on inflammatory cytokines, cell surface receptors and enzymes and related factors. In particular, genetic polymorphisms for cytokines and their receptors have been proposed as potential markers for periodontitis. One of these cytokines Tumor Necrosis Factor-α (TNF-α); mediates its diverse biologic effects by binding two high-affinity, cell surface receptors, TNF receptor 1 (TNF-R1, p55 TNFR) and TNF receptor 2 (TNF-R2, p75 TNFR). TNF-R2 may modulate TNF-α mediated inflammatory responses in periodontal disease. Recent studies have shown that TNF-R2 (+587) T/G gene polymorphism is associated with periodontitis. The aim of this study was to analyze TNF-R2 (+587) T/G polymorphisms in chronic periodontitis (CP) and agressive periodontitis (AP) patients in a Turkish population.

CP ($n = 54$), AP ($n = 47$) and periodontally healthy individuals ($n = 54$) were included in the study. The participants had no systemic diseases. After clinical and radiographic examinations, blood samples were obtained and genomic DNA was extracted from peripheral blood using QIAamp Blood MiniKit according to the manufacturer's instructions. Single nucleotide polymorphisms (SNP) were analyzed by PCR (Polymerase Chain Reaction) and RFLP (Restriction Fragment Length Polymorphism) methods in DNA samples. For TNF-R2 (+587) T/G polymorphism; TT, TG and GG genotype and allele frequencies were analyzed in CP and AP patients and controls.

For TNF-R2 (+587) T/G polymorphism; TT, TG and GG genotype and allele frequencies were analyzed and the differences in genotype and allele frequencies were found significant between the groups ($p < 0.001$). G allele carriage rates were found 66% for AP, 63% for CP and 29.6% for the control group. Further

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studies including greater number of subjects and performing linkage analyses may provide more supporting results.

Keywords: genetic polymorphism, Periodontitis, TNF-R2.

SUN-214

In-vitro research of epidermal growth factor on the gene expressions associated with the apoptosis in the gastric epithelial cells of ulcerous patients

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Epidermal grow factor (EGF) has a major role on recovery process of gastroduodenal ulcers and organization of mucosal cell proliferation. Apoptosis is also an important period taking role in ulcer formation. In this study, EGF application to the biopsy samples of gastric and duodenal ulcer patients and the cells increased from gastric cancer cell line has been carried out in-vitro environment in three different doses (20, 50 and 100 ng/ml). Using the RNAs isolated from these cells, the expression levels of Bax, Fas and Bcl-2 genes have been determined both semi quantitatively and with Real-Time PCR by making 'Comparative C_T Analysis'. In consequence of statistical analyses, the EGF application was found not to change expressions of Bax, Fas and Bcl-2 genes in gastroduodenal ulcerous patients. As a result of statistical evaluation of the data got by making 'Comparative C_T Analysis with Real-Time PCR, separately from the EGF, only the expression level of Bax gene in the control group was determined to be higher in comparison with the patient group (p = 0.001)'. When the gene expression levels in the primer cell culture and gastric cancer line were evaluated in Real Time PCR, it was found that EGF doses had no effect on the expression levels in semi quantitative method; on the other hand, there was a clear increase I n the expression level of Bax gene in the cell line when one dose EGF (20 ng/ml) was applied (p = 0.001) and it was also observed that 2 doses EGF (50 ng/ml) application decreased the expression of Fas gene in the cell line (p = 0.033).Consequently, it was determined that EGF playing a role especially on the ulcer recovery had influence on the expression of Bax and Fas genes which have a function activating apoptosis and the expression of Bcl-2 gene also didn't change with the EGF application. Because this study is the first examining the apoptotic effect of EGF in ulcerous patients, it is thought that it will be a guide for the other studies about this subject.

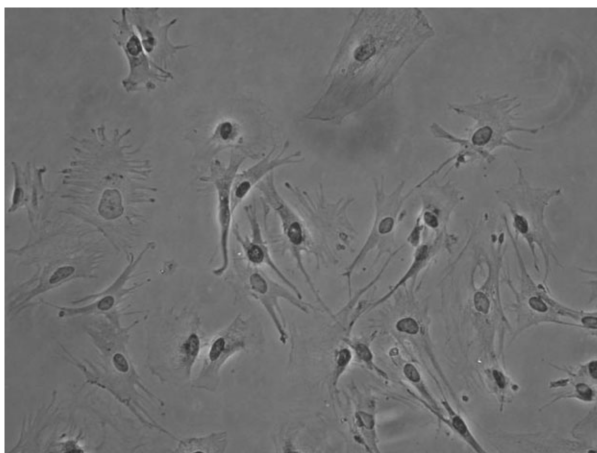

Fig. 1.

Keywords: EGF, gene, ulcer.

SUN-215

Is there an impact of von Willebrand factor and claudin-5 for reflecting disease activity in rheumatoid arthritis?

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Claudin family has an important role for endothelial cell permeability. Previous studies revealed that relationship existed between the vWF and Claudins. However there is no evidence about relationship between vWF and Claudins in patients with Rheumatoid arthritis (RA). The aim of the present study was to investigate possible relationships between von Willebrand factor (vWF) and Claudins with the level of disease activity in patients with RA.

A total of 35 frozen (−20°C) serum samples 25 of them belonged to patients with RA, and 10 sample belonged to healthy people, were enrolled prospectively. We used DAS-28 to evaluate disease activity. The following clinical data gathered from the original patients' charts. Serum VWF and Claudin-5 levels were measured by enzyme-linked immunosorbent assay.

The average DAS-28 score was found 3.43 ± 0.31 for RA patients in this study. The Claudin-5 levels in RA patient and healthy controls were found 44.9 ± 31.9 and 10.2 ± 4.1 respectively. Also the VWF levels in RA patient and healthy controls were found 41.1 ± 0.29 and 9.8 ± 3.9 The increase of VWF and Claudin-5 levels were found in high-level, correlation with disease activity (p = 0.009 and p = 0.011 respectively).

Our prelimenary results show that VWF and Claudin-5 contribute to pathological condition of RA. Thus, we believe that serum endothelial activity marker levels especially VWF and Claudin-5 can be as a marker in patients with RA, too. However other inflammatory diseases should be further investigated

Keywords: Claudin-5, rheumatoid arthritis, von Willebrand factor.

SUN-216

Kyotorphin analgesic derivatives and its anti-inflammatory effects: intravital microscopy and microcalorimetry studies

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Current pain research has not resulted in an optimal translation to the clinic where all analgesics – opioid agonists, anti-inflammatory steroids and non-steroidal anti-inflammatory drugs (NSAIDs) – have safety issues. In a new strategy we recently designed and tested the efficacy of new class of drugs derived from an endogenous neuropeptide, Kyotorphin (KTP, Tyr-Arg) combining C-terminal amidation and Ibuprofen (Ibp) conjugation. As the main bottleneck in the development of analgesic and anti-inflammatory drugs are the safety concerns, we evaluated the impact of the newly designed drugs on microcirculation and their undesired physiological effects. Their effective in vivo behaviour on microcirculation was studied by intravital microscopy (IVM) in the murine cremasteric muscle. We also evaluated if the conjugated drugs retained anti-inflammatory activity by its effects on LPS-induced leukocyte recruitment in vivo and the mechanisms involved in its anti-inflammatory activity in vitro by isothermal

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titration microcalorimetry (ITC). The combination of analgesia and anti-inflammatory activities with absence of toxicity is highly appealing from the clinical point of view and broadens the therapeutic potential and application of kyotorphin peptides. Our data show that KTP and conjugates do not cause damage on microcirculatory environment and efficiently decrease the number of leukocyte rolling induced by LPS challenging. The probable mechanism by which KTP conjugates peptides are able to decrease leukocyte rolling and adherent induced by LPS appeared to be correlated with their ability to bind/or perturb LPS micelles, revealed by ITC experiments. The selected drug leads in this work are expected to overcome the limitations of conventional analgesic peptides, resulting in candidates conjugating analgesic and anti-inflammatory activities, therefore bearing the potential to develop novel medicines.

Keywords: Intravital microscopy, Kyotorphin, microcalorimetry.

SUN-217
Lack of association of apolipoprotein E (Apo E) ε2/ε3/ε4 polymorphisms with depression among Turkish psoriatic patients

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Apolipoprotein E (ApoE) polymorphisms are also known as an important risk factor for several neurological diseases as well as depressive symptoms. Psoriasis is an immune-mediated skin disease with several comorbidities and psoriatic patients increased risk of depression due to the bodily manifestations related appearance. Aside, depression itself is also known as triggering factor for psoriasis development. In this reseach, we hypothesized that, Apolipoprotein E polymorphisms would be common triggering factor and have increased risk for depression among psoriatic patients with apolipoprotein E ε2-ε4 carriers.

Totally 134 psoriatic patients (69 with depression) were enrolled to this research. The genotypes were determined with melting curve analysis by using Real-Time polymerase chain reaction (Roche). Odds ratio were calculated to estimate the risk related to Apolipoprotein E variation and depression risk among psoriatic patients and Fisher's exact test was used to calculate the significance of OR.

The mean age of the cohort was 46.74 ± 16.5 and 59% were women. Psoriatic patients with depression did not shown significantly higher frequency of ApoE2/E3 [OR = 1.73, 95% confidence interval (CI) = 0.66–4.56; P = 0.27] or ApoE4/E3 [OR = 1.42, 95% confidence interval (CI) = 0.46–4.40; P = 0.54] than psoriatic patients without depression. Psoriatic patients who carriers of ApoE epsilon4 allele had not increased depression risk as compared to ApoE epsilon3 allele carriers [OR = 01.21, 95% confidence interval (CI) = 0.44–23.38; P = 0.71]. Also, epsilon2 allele carriers were not increased depression risk when compared with ApoE epsilon3 isoform [OR = 1.21, 95% confidence interval (CI) = 0.52–2.77; P = 0.66].

Neither the ApoE epsilon2 allele nor ApoE epsilon4 are sufficient risk factors for depression among psoriatic patients. The primary results of this study need to be confirmed by extensive study groups.

Keywords: Apolipoprotein E, Depressive disorders, Psoriasis.

SUN-218
Leptin-preconditioning induces cardioprotection against myocardial infarction through activation of AMPK and, induction of ROS

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Introduction: Leptin, a product of the *ob* gene, is a 16-kDa peptide synthesized primarily by white adipose tissue. It is also produced in the heart, suggesting that it has a cardioprotective effect. AMP-activated protein kinase (AMPK) is a serine/threonine protein kinase that plays a key role in cardioprotection in the abnormal heart. However, it is not clear whether AMPK-mediated cardioprotection influences the effects of leptin in cardiac muscle. Here, we investigated the mechanism of cardioprotection in leptin preconditioning and the relationship between leptin preconditioning-induced cardioprotection and the regulation of AMPK.

Methods: A rat ischemia model was constructed using left anterior descending coronary artery occlusion (CAO). Infarct size was measured to evaluate the cardioprotective effect. A rat cardiac myoblast cell line (H9c2) was used to investigate the cardioprotective mechanism of leptin.

Results: Leptin decreased myocardial infarct size in rat cardiac ischemic reperfusion model. The effect of leptin was abolished in combination with compound C, an AMPK inhibitor or NAC, a ROS scavenger. Leptin phosphorylated AMPK dose- and time-dependently in H9c2 cells. Leptin also induced generation of reactive oxygen species (ROS) dose- and time-dependently in H9c2 cells. AMPK phosphorylation by leptin was abolished in combination with NAC, however, leptin-induced ROS generation was not affected by compound C. Therefore, Leptin induced generation of ROS followed by activation of AMPK. Activated AMPK decreased cardiac ischemic damage.

Conclusions: AMPK activation plays a key role in leptin preconditioning-induced cardioprotection. Leptin-induced ROS generation results in AMPK activation.

Keywords: Leptin, cardioprotection, ischemia.

SUN-219
Lipid structures as biomarkers in septic shock: a new road to travel

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Purpose/Objetive: Sepsis is the first cause of death in Intensive Care Units (ICUs) and the molecular mechanisms underlying this disease remain poorly understood. Most of the times, it′s very difficult for the clinician to diagnose sepsis promptly, since many other inflammatory diseases present similar manifestations, while a specific sepsis biomarker is still lacking. The purpose of this study is to investigate lipid structures as potential sepsis biomarkers. These molecules, therefore, would be able to identify sepsis during its early phase, helping in its diagnosis and treatment.

Material and Methods: 11 patients who suffered severe trauma and were admitted in our ICU were included in the study. After 48 hours, their blood was collected and the plasma was sent for metabolomic analysis. At this time, any patient had manifested signs of infection yet. 7 of those patients didn′t infect during their

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whole ICU stay. 4 of them did infected early (after 48 hours, but before day 7) and developed septic shock.

Results: Metabolomic analysis identified 7 lipid structures that have the potential to be used as early biomarkers of sepsis. These structures are: homoserine lactone, sorbaldehyde, succinic acid, methylmalonic acid, bromo-decanoic acid, 4-hydroxy-3-methyl-but-2-en-1-yl trihydrogendiphosphate and many others.

Conclusion: Lipid structures may regulate unexpected molecular pathways during sepsis. Their role in inflammation, immunity and infection must be investigated, as well as their potential to help direct clinicians in the treatment of this devastating disease.

Financial Support: FAPESP (#2009/17731-2).

Keywords: inflammation, Lipidomics, sepsis.

SUN-220
Lipopolysaccharide induces pro-inflammatory cytokines and MMP production via TLR4 in nasal polyp-derived fibroblast and organ culture

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Nasal polyposis is characterized by persistent inflammation and remodeling in sinonasal mucosa. Toll-like receptors (TLRs) play a role in the innate immune response to microbes in the sinonasal cavity. The aim of this study was to evaluate whether nasal polyp-derived fibroblasts (NPDFs) and organ-cultured nasal polyps can synthesize pro-inflammatory cytokines and matrix metalloproteinases (MMPs) after exposure to lipopolysaccharide (LPS), a TLR4 agonist. NPDFs and organ-cultured nasal polyps were isolated from nasal polyps of 8 patients and exposed to LPS. The mRNA and protein expression levels of TLRs, cytokines, and MMPs were determined using a gene expression microarray, real-time RT-PCR, western blot analysis, enzyme-linked immunosorbent assay, and immunofluorescence staining. The enzymatic activities of MMPs were analyzed using collagen or gelatin zymography. The protein expression level of MMP-1 increased in nasal polyp tissues compared to inferior turbinate tissues. LPS induced mRNA expression of *TLR4*, *IL-6*, *IL-8*, and *MMP-1* and activated MAPK signaling in NPDFs. LPS promoted the release of interleukin (IL)-6 through extracellular signal-related kinase (ERK) and IL-8 through ERK and c-Jun N-terminal kinases (JNK). Production of IL-6 and IL-8 was induced by PI3K/Akt signaling in LPS-stimulated NPDFs. LPS increased the transcript and protein expression levels of MMP-1 and induced collagenase activity of MMP-1 via ERK and p38, but did not induce gelatinase activity of MMP-2 and MMP-9. LPS from *Rhodobacter sphaeroides* (LPS-RS) inhibited the stimulatory effects of LPS in NPDFs as well as in organ culture of nasal polyp. LPS triggers immune response via TLR 4 and activates MAPK and PI3K/Akt signaling pathway, which is involved in remodeling of nasal polyps.

Keywords: Cytokine, nasal polyp, toll-like receptor.

SUN-221
Liposomes for photodynamic therapy of vitiligo via pilosebaceous route

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Vitiligo is an acquired depigmentory skin disorder in which the pigment producing cells (melanocytes) are absent. It affects 0.1–4% of the population worldwide and is emotionally and socially devastating. Well established treatment modalities in today's therapeutic armamentrum have their own side effects and failure. Photodynamic therapy (PDT) an entirely new treatment modality which involves a photosensitizer and light. PUVA therapy used so far has marked failure in many of the clinical trials studies. Since no full therapeutic solution for vitiligo is available, PDT is the ray of hope. The aim of project was to develop and investigate the therapeutic efficacy of liposomes to produce immediate repigmentation (by melanin) along with correcting the cause simultaneously. Topical route has been chosen to directly target the diseased site and to minimize the systemic toxicity. Methoxsalen-melanin loaded liposomes were prepared by a lipid cast film method and were characterized *in-vitro* for their shape, size, percent antigen entrapment, Skin permeation and stability. Fluorescence microscopy was carried out to confirm the uptake of bilosomes. The *in-vivo* part of the study comprised of Induction of vitiligo by mushroom tyrosinase intradermally & photodynamic studies with different formulations. Cosmetic disfiguration and psychological sequel underlines the impact of vitiligo. Immunization with mushroom tyrosinase resulted in discoloration of the areas showing its effectiveness in inducing vitiligo. Sustained pigmentation resulted after application of formulation was suggestive of cure with fast repigmentation. Thus, pilosebaceous route is effective in targeting follicular melanocytes. Toxic manifestations of methoxsalen were also subsided when delivered in liposomes.

Keywords: liposomes, Methoxsalen, vitiligo.

SUN-222
Loading and release efficiencies of Bortezomib on chitosan coated magnetic nanoparticles

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Conventional chemotherapeutics are unspecifically distributed all over the body and they affect both cancerous and normal cells. This treatment results in excessive toxicities. Targeted drug delivery has emerged to overcome the lack of specificity of conventional chemotherapeutics. Nanotechnology opened a new door to the development of particles with nano sizes that can be fabricated from a multitude of materials in a variety of compositions, including quantum dots, polymeric magnetic nanoparticles, and

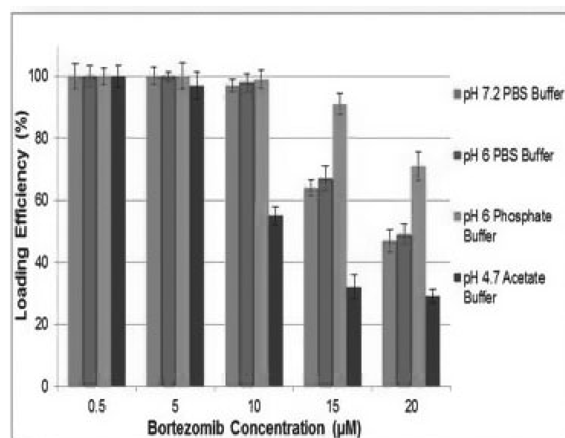

Fig. 1. Effect of initial Bortezomib concentration on loading efficiency in acetate buffer (pH 4.7), phosphate buffer (pH 6.0), phosphate buffered saline (pH 6.0 and pH 7.2).

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dendrimeric. Nanoparticles are promising to circumvent these challenges, by enabling high amounts of drugs to be loaded and targeted to the tumor site. Delivery of drugs via nanoparticles increases the half life and reduces toxic side effects of drugs, by improving their pharmacokinetic profile and therapeutic efficacy.

Chitosan magnetic nanoparticles (CS MNPs) were generated for targeting of tumor cells in the presence of magnetic field (Unsoy *et al*., 2012) and loaded with the anti-cancer drug Bortezomib (Velcade®). Bortezomib loading efficiency of CS MNPs was optimized (Figure 2). Highest loading capacity and Drug release characteristics of CS MNPs were identified. Stability of Bortezomib loaded CS MNPs were determined. Bortezomib, a highly potent proteasome inhibitor, was successfully loaded on CS MNPs and their release behavior was pH dependent. Bortezomib loaded pH sensitive CSMNPs were perfectly stable at physiological pH.

As a result, synthesized and Bortezomib loaded CS MNPs can be effectively used in magnetic targeted drug delivery as nanocarrier for the pH dependent release of Bortezomib in cervical cancer cells.

Keywords: Bortezomib, cervical cancer, nanoparticle, targeted drug delivery.

SUN-223
Local inflammatory response induced by CcH1, a P-I metalloproteinase isolated from *Cerastes cerastes* venom

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Envenomings induced by snakebite are characterized by local tissue damage, including, necrosis of skeletal muscle, edema, hemorrhage and inflammatory response, as well as by systemic alterations such as hemorrhage, cardiovascular shock, acute renal failure and coagulopathies. Local and systemic effects caused by snake venoms have been associated with diverse components of the venom, such as phospholipases A_2 and metalloproteinases. Snake Venom Metalloproteinases (SVMPs) play a relevant role in the complex multifactorial inflammatory response induced by snakebite. CcH1, an P-I class SVMP isolated from *Cerastes cerastes* snake venom, is a weak hemorrhagic protein, able to induce myonecrosis and to degrade fibrinogen, fibrin, type IV collagen and laminin. In this study, we analyzed the inflammatory reaction induced by CcH1 in gastrocnemius muscle, aiming to identify the cellular components involved in muscular cytokine release.

The inflammatory reaction induced by CcH1 in gastrocnemius muscle was assessed by tissue analysis and the release of pro-inflammatory mediators. Indeed, upon intramuscular injection, CcH1 induces formation of blisters and leukocyte infiltration into dermis, indicating an important pro-inflammatory effect of CcH1. Moreover, the injection of CcH1 in the gastrocnemius muscle, revealed a marked elevation of muscular levels of pro-inflammatory cytokines (TNF-α and IL-6), 50% higher than controls. The muscular concentrations of these cytokines returned to normal levels after 24 h of injection. Muscle injected with CcH1 resulted also the production of pro-inflammatory gelatinases, observed by zymography analysis. Two gelatinolytic bands were detected an apparent molecular weight of approximately 60 kDa and 100 kDa corresponding to latent forms of MMP-2 and MMP-9, respectively. These results suggest that the early production of cytokines induced by CcH1 may stimulate accumulation of leukocytes that will produce MMPs, which will enhance the levels of inflammatory mediators thus potentiating the local response and the tissue damage.

In conclusion, these results indicate that CcH1 is able to induce an inflammatory response characterized by a marked leukocyte infiltrate, MMPs and cytokines production in muscle tissue, indicating that multiple pathways may be involved in muscle inflammatory reaction. The characterization of the cell types and mediators involved with tissue damage and inflammatory response induced by metalloproteinases in snakebite accidents may contribute to the improvement of current therapies, adding to the currently available therapy with antivenoms.

Keywords: Inflammation, Metalloproteinase, Snake venom.

SUN-224
Macrophages engulfing apoptotic cells produce non-classical retinoids to enhance their phagocytic capacity

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Previous work in our laboratory has shown that transglutaminase 2 (TG2) acting as a coreceptor for integrin $\beta 3$ is required for proper phagocytosis of apoptotic cells. In the absence of TG2 SLE like autoimmunity develops in mice, similarly to other mice characterized by a deficiency in the clearance of apoptotic cells. In the present study we demonstrate that increasing TG2 expression alone in wild-type macrophages is not sufficient to enhance engulfment. However, during engulfment the lipid content of the apoptotic cells triggers the lipid sensing receptor liver X receptor (LXR), which in response upregulates the expression of the phagocytic receptor Mertk and the phagocytosis related ABCA1, and that of retinaldehyde dehydrogenases leading the synthesis of a non-classical retinoid. Based on our retinoid analysis this compound might be a dihydro-retinoic acid derivative. The novel retinoid then contributes to the upregulation of further phagocytic receptors including TG2 by ligating retinoic acid receptors. Inhibition of retinoid synthesis prevents the enhanced phagocytic uptake induced by LXR ligation. Our data indicate that stimulation of LXR enhances the engulfment of apoptotic cells via regulating directly and indirectly the expression of a range of phagocytosis-related molecules, and its signaling pathway involves the synthesis of a nonclassical retinoid. We propose that retinoids could be used for enhancing the phagocytic capacity of macrophages in diseases such as systemic lupus erythematosus, where impaired phagocytosis of apoptotic cells plays a role in the pathomechanism of the disease.

Keywords: efferocytosis, Macrophages, phagocytosis.

SUN-225
Maresin improves protective role of EPA in A549 cell line treated with benzo(a)pyrene

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Fatty acid metabolites – eicosanoids are important signalling molecules and regulate a variety of physiological and pathophysiological processes including inflammation.

The aim of our study was to assess the effect of eicosapentaenoic acid (EPA) supplementation with added Maresin and/or

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benzo(a)pyrene (BaP) on A549 human lung carcinoma epithelial cells by using a UHPLC/MS-TOF method. Additionally, we use Western blot technique to visualise the impact of Maresin, EPA and BaP on the enzymatic activity of cyclooxygenase 2 (COX-2) and prostaglandin E synthase (PES) in the A549 cells.

In the cells supplemented with EPA + Maresin + BaP we did not observe the presence of 8-iPGF3α, PGF3α, 8-isoPGF2α and 5-iPF2α in contrast to other treatment groups. Also, less activity of COX-2 and PES were observed in this group compared to the results for cells incubated with BaP. In our work, we have shown for the first time, protective role of EPA and EPA + Maresin in the model of epithelial cells exposed to BaP.

This project was possible through the support given by National Science Centre, Poland DEC-2011/01/B/NZ7/00038.
Keywords: Maresin, EPA, BaP.

SUN-226
Mesuagenin C attenuated LPS-stimulated BV-2 and NG108-15 cells by modulating NF-κβ, CCL21 and cytokines through PI3K-Akt/GSK-3β

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Background: The aetiology of neurodegenerative diseases remains vague, albeit growing empirical evidences implicate the role of microglial mediated neuroinflammation in the pathogenesis of the diseases. Mesuagenin C (MC) was found to protect NG108-15 cells against neuronal apoptosis which involved the modulation of CCL21. Therefore, we investigated the anti-neuroinflammatory effects of MC in LPS-stimulated BV-2 and NG108-15 cells through the modulation of chemokines and cytokines.

Material and Methods: The nitric oxide and ROS level were determined in the BV-2 cells and the expression of iNOS, COX-1, COX-2, mTORC1/2, PI3K-Akt and GSK-3β were examined. The production of TNF-α, IL-6 and IL-10 was first measured by cytometric cytokines bead array (CBA). The CCL21 gene in NG108-15 cells was silenced by using siRNAs, subjected to LPS and evaluated by chemotaxis and customized cytokines protein array. BV-2 and NG108-15 were co-cultured and stimulated with either LPS or recombinant CCL21 and compared to transfected NG108-15 cells. The translocation of p65NF-κβ was evaluated and its regulation with chemokines and cytokines was also determined.

Results: Pretreatment with MC decreased the iNOS expression which concomitantly attenuated both NO and ROS level. This was followed with the attenuation of COX-2 but not COX-1 expression. CBA revealed that MC significantly suppressed the production of TNF-α and IL-6 and reciprocally augmented the anti-inflammatory cytokines, IL-4 and IL-10. MC was found to activate mTORC1/2 and PI3K-Akt which led to the inactivation of GSK-3β and p65NF-κβ translocation. Furthermore, cytokines array revealed that treatment with MC, lithium chloride and ethyl 3,4-dihydroxychromate abated the production of most pro-inflammatory chemokines and cytokines (CCL21, IFN-γ, IL-1β, IL-2, MIP-1A, MIP-3B, RANTES) which accentuated the involvement of GSK-3β and NF-κβ in modulating LPS-induced neuroinflammation. Moreover, following CCL21 knockdown, both transfected and wild-type NG108-15 co-cultured with BV-2 cells pretreated with MC displayed suppression of microglial chemotactic activity and microgliosis in BV-2 which further proved that modulation of CCL21 was crucial in the initiation of microgliosis and hence, neuroinflammation.

Conclusion: MC suppressed LPS-induced neuroinflammation in BV-2 and NG108-15 by modulating GSK-3β and NF-κβ reciprocal regulation of CCL21, chemokines and cytokines through PI3K/Akt pathway. This finding advocates the MC potential for the treatment of neuroinflammation with a specific role of CCL21 in suppressing initiation of neuroinflammation.

Keywords: Cytokines, neurodegenerative diseases, Neuroinflammation.

SUN-227
miR-146a deficiency in Ly6Chigh monocytes contributes to pathogenic bone loss during inflammatory arthritis

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Monocytes represent a prototypic cell type when investigating the interplay between immune and skeletal systems as they can give rise to different cell types including dendritic cells, macrophages and osteoclasts (OC), which play key roles in immunity and bone homeostasis. Circulating monocytes consist of at least two main functional subsets, Ly6Chigh and Ly6Clow monocytes. It has been suggested that OC might develop preferentially from the Ly6Chigh monocyte subset which excessive and prolonged activation is a hallmark of many inflammatory diseases. Among key molecular rheostats of cell fate, micro(mi) RNAs are a class of regulatory RNAs that control basic biological functions and orchestrate inflammatory responses. Few miRNAs have been involved in osteoclastogenesis. The present study aimed at investigating the role of miRNAs in osteoclastogenesis in the context of monocyte subsets, under steady state and inflammatory conditions.

Genome-wide miRNA expression study identified 8 miRNAs and putative targeted pathways that are differentially expressed between Ly-6Chigh and LyC6low FACS sorted mouse monocytes, and common to their human counter parts CD14+CD16– and CD14dimCD16+ monocytes, respectively. Among these, miR-146a showed higher expression in Ly-6Clow monocytes when compared to Ly6Chigh monocytes. Under inflammatory arthritis conditions, expression of miR-146a in Ly6Chigh monocytes was down regulated as compared to healthy controls. Deficiency for miR-146a increased OC differentiation and bone erosion both in vitro and in vivo, respectively. Enforced expression of miR-146a both in vitro and in vivo led to decreased TRAP positive cells and was associated with decreased RelB expression.

Overall, our results show that specific over-expression of miR-146a in Ly6Chigh monocytes altered OC differentiation and decreased bone erosion in inflammatory arthritis. These data suggest a novel role for miR-146a in controlling osteoclast fate of Ly6Chigh monocytes and that reduced miR-146a expression in Ly6Chigh monocytes under arthritic conditions contributes to pathogenic bone loss.

Keywords: miR-146a, Monocyte subsets, Osteoclasts.

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SUN-228
Mitigating the inflammatory response by TiO2 nanotubes

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Introduction: Macrophages (MF) recruited at the biomaterials implantation site are the primary mediators of the non-self response. They play a key role in modulating the tissue healing around the implant and in mediating the foreign body reaction (FBR). FBR is characterized by the accumulation and activation of MF and the formation of foreign body giant cells (FBGCs). Surface nano-topographical cues of the endo-osseous implants proved to positively influence the osteoblasts behavior but little is known about the MF response. Hence, the aim of our study was to evaluate the effects of TiO_2 nanotube modified Ti surface (TNT) on inflammatory activity of MF with regard to cell activation, expression of the pro-inflammatory mediators and FBGCs formation.
Materials and Methods: TNT were elaborated by electrochemical anodization. Cell culture model was represented by murine MF cell line, RAW 264.7. These cells were maintained in contact with the test surfaces up to 7 days, under standard and pro-inflammatory (stimulation with LPS) culture conditions. Cell viability and proliferation were evaluated using MTT and Live/Dead assays and the cell morphology was assessed by fluorescent visualization of actin. The gene expression and protein secretion of the pro-inflammatory cytokines/chemokines were evaluated after 24 h and 48 h using qPCR and ELISA assays. Furthermore, the LPS-mediated induction of multinucleated cells was assessed at 7-days post-seeding by fluorescent labeling of actin and nuclei with FITC-phalloidin and DAPI, respectively. Further, the percentage of MF fusion was estimated by determining a 'multinuclear index'.
Results: The results regarding MF viability and proliferation did not show significant differences between the two analyzed surfaces. Instead, the microscopy studies, as well as the evaluation of gene expression and protein secretion levels exhibited by the pro-inflammatory mediators revealed a mitigation of the MF inflammatory activity on TNT. Thus, on cpTi surface was observed an increased number of modified cells, as a sign of the pronounced initial activation, fact that was also confirmed by low levels of pro-inflammatory factors. Likewise, less FBGCs were noticed on titania nanotubes.
Conclusions: These studies demonstrate a strong correlation between the nanotopography of TNT and MF behavior, nanotube modified surface eliciting a weaker inflammatory response.
Acknowledgement: This work was funded by UEFISCDI (project PNII-ID-PCE 188/2011).
Keywords: Inflammatory response, Macrophages, TiO2 nanotubes.

SUN-229
Modification of fibrinogen function and structure in post acute myocardial infarction patients

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Cardiovascular diseases are one of the most important causes of death globally and atherothrombosis is the underlying cause of most cardiovascular events. There is widespread evidance that oxidative stress contributes to vascular damage.

Among plasma proteins, fibrinogen represents the major target of oxidative modifications.

In postmyocardial infarction (postAMI) patients (6 months after the acute event) fibrinogen oxidative modification (protein carbonyls) and fibrinogen function were estimated by an in vitro and an ex vivo approach. Fibrinogen structural features and clot architecture were also explored.

In 39 postAMI patients and in 28 controls comparable sex and risk factors, oxidative stress markers (in plasma and in purified fibrinogen fractions), thrombin-catalyzed fibrin polymerization and plasmin-induced fibrin lysis were estimated. Not only the functional aspects were assessed, but also the structural ones. Circular Dichroism (CD) spectra of purified fibrinogen extracts, electron microscopy analysis and differential interference contrast microscopy analysis of fibrin clot were also performed. Differences in terms of oxidative stress were found between plasma of patients and controls ($p < 0.01$ versus Controls). In particular, an increased fibrinogen carbonylation, among patients (3.5 folds over control values), respect controls ($p < 0.01$ versus Controls) was evident.

As regards function, purified fibrinogen fractions from postAMI patients showed significantly reduced clotting ability and less susceptibility to plasmin-induced lysis ($p < 0.01$ versus Controls).

Not only fibrinogen function, but also structure was altered. In fact in patients, both fibrinogen secondary structure as suggested by CD spectroscopy, and fibrin clot architecture analysed by electron and confocal microscopy were modified.

In conclusion, post AMI patients an overall imbalance in redox status and a marked fibrinogen carbonylation associated to altered fibrinogen function are evident. These features occur along with modifications in protein structure and in clot architecture.
Keywords: Fibrinogen, Carbonylation, Fibrin clot.

SUN-230
Modulation of TRPA1/TRPV1-mediated responses in trigeminal sensory neurons by ADM_12

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The Transient Receptor Potential Vanilloid 1 (TRPV1) and Ankyrin 1 (TRPA1) ion channels belong to the TRP superfamily and are both integrators of a variety of noxious stimuli. Both channels are non selective cation channels which exert pleiotropic functions in a variety of cells; more specifically they colocalize in

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primary sensory neurons of the trigeminal, vagal and dorsal root ganglia (DRG). TRPA1 is activated by a number of pungent and irritant reactive chemical compounds including allyl isothiocyanate (AITC, mustard oil), cinnamaldehyde (cinnamon oil), allicin (onions), carvacrol (oregano), polygodial (Tasmanian pepper) and formaldehyde (formalin); all of these molecules elicit a painful burning and a prickling sensation. TRPV1 is activated by noxious stimuli including high temperature, pH, and vanilloid compounds such as capsaicin.

In this context, we describe a new water soluble derivative of lipoic acid [1], ADM_12 (Patent n. PCY/IB2014/059289) which proved to block TRPA1 channels activated by well-known TRPA1 agonists (AITC, Menthol, oxaliplatin); selectivity studies showed no appreciable block by this molecule of TRPM8, hERG and NaV channels.

Moreover we showed that ADM_12 was able to activate TRPV1 channel in TRPV1-expressing cells, whereas it acted as an antagonist of capsaicin- and AITC- induced responses in TRPA1/TRPV1 coexpressing cells.

Similarly we found that ADM_12 elicited ionic current in the TRPV1-positive/TRPA1-negative subset of trigeminal neurons, whereas it was able to block capsaicin- and AITC- induced responses in sensory neurons that express both TRPV1 and TRPA1 channels.

Based upon these findings, we speculated that ADM_12 could act as modulator of the heteromeric complex in sensory neurons between TRPA1 and TRPV1 channels, suggesting an interesting strategy for the design of a novel class of analgesic drugs.

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Reference

1. Nativi C., Gualdani R. et al. (2013) Scientific Reports, 3, 1–10.

Keywords: Trigeminal neurons, TRPA1 antagonist.

SUN-231
Molecular and cellular responces to titanium dioxide nanoparticles

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The increased use of nano-sized materials in the past several years has compelled the scientific community to investigate the potential hazards of these unique and useful materials. One of the most widely used nanoparticles is titanium dioxide. TiO2 is a very versatile compound that has many uses, depending on its particle size.

The objective of the research is to investigate the alterations in molecular and cellular responses in culture of primary lymphocytes to titanium dioxide nanoparticles.

Human lymphocytes isolated from heparinized blood samples of healthy individuals were exposed to TiO2 nanoparticles. Viability, ROS generation, the changes in the expression of genes encoding proinflammatory mediators TNF-α, Il-1β and Il-8 and DNA damage were assessed.

To examine the toxic effects of titanium dioxide nanoparticles, human lymphocytes were incubated with different concentrations of nanoparticles and viability was determined 24 and 48 h after treatment, respectively. Cell viability was decreased by treatment with nanoparticles in both a time- and concentration-dependent manner.

The ability of TiO2 to induce ROS formation in lymphocytes was evaluated using DCF (2',7'-dichlorofluorescin) fluorescence as a reporter of oxidant production. The fluorescence intensity of oxidized DCF was increased in cells when treated with nanoparticles. This means that ROS generation was occurring in response to the treatment with titanium dioxide nanoparticles in a concentration- and time-dependent manner, while fluorescence was insignificant in the control group.

To investigate the expression level of mRNA related to the inflammation responses in human lymphocytes real-time PCR was performed. The expression of interleukin-1β, interleukin-8 and TNF-α genes were increased by the exposure to nanoparticles (10, 20 and 40 μg/ml) for 24–48 h.

TiO2 nanoparticles were shown to induce the dose-dependent fragmentation of DNA strands.

Much evidence of hazardous health effects of nanoparticles has been reported in toxicological studies. In this study, viability was reduced under the exposure to TiO2. Oxidative stress was revealed by the treatment with titanium dioxide nanoparticles. Consequently, cytotoxicity in human lymphocytes seemed to be caused by oxidative stress. Oxidative stress may also trigger inflammation signals. Induced by exposure to nanoparticles it may cause the translocation of transcription factors to the nucleus, which regulate pro-inflammatory genes, such as TNF-α, Il-1β, Il-8. ROS is an important factor in the apoptotic process, and the excess generation of ROS induces mitochondrial membrane permeability and damages the respiratory chain, to trigger the apoptotic process.

Keywords: gene expression, reactive oxigen species, titanium dioxide nanoparticles.

SUN-232
Molecular basis for the heterointeractions between human guanylate-binding proteins (hGBPs)

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The interferon gamma-induced guanylate-binding proteins (hGBPs) are key players of the cellular response to pathogens like hepatitis C virus, influenca A virus and *Chlamydia trachomatis*. Although the molecular function of the hGBP family is still unknown, guanine nucleotide-dependent homo- and heterointeractions of hGBPs seem crucial for their anti-pathogenic function. Cellular localisation studies revealed a hGBP network in which the translocation of hGBPs is regulated in a GTP-hydrolysis dependent, hierarchical manner (Britzen-Laurent et al., 2010). Nevertheless, quantitative heterointeraction studies characterising the hGBP heterocomplexes are required as the molecular basis for understanding hGBP's cellular, and in particular, anti-pathogenic function. Therefore, we established an assay to investigate the formation of hGBP homo- and heterocomplexes in vitro, which is based on the proteins' GTPase activities. We showed earlier that GTPase activity of hGBP1 as well as hGBP5 is stimulated upon formation of homocomplexes in a protein concentration-dependent manner. Intriguingly, we observed a stimulation of the GTPase activity when combining different hGBP isoforms. For instance, we obtained maximal GTPase activities even for low hGBP5 concentrations, where hGBP5 is monomeric, upon addition of hGBP1 which leads to the formation of a hGBP1/hGBP5 heterocomplex. Beside this stimulating effect, we observed that the heterocomplex is tighter than the homocomplexes. Our studies provide quantitative data for better understanding the networking of hGBPs.

Keywords: GTPase, Guanylate-Binding Proteins, Heterocomplex.

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SUN-233
Molecular basis of the digestion-resistant peptides from the major peanut allergen Ara h 2

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Peanut allergens can trigger a potent and dangerous immune response in an increasing number of people. The molecular structures of these allergens form the basis for understanding this response. Conglutin isoforms Ara h 2 are considered the most relevant peanut allergens, because they are frequently recognized by patient's serum, and highly potent in IgE-binding. This may be explained by the fact that Ara h 2 is highly resistant to digestion. The aim of this work is to investigate the effect of digestion on the structure and immunogenicity of the different Ara h 2 isoforms.

Purified Ara h 2 isoforms Ara h 2.02, Ara h 2.01 were digested with immobilized trypsin. Digestion resistant peptides were analysed by SDS PAGE, Far UV CD spectroscopy, competitive IgE inhibition ELISA and immunoproteomics (2D PAGE, 2D immunoblot, and mass spectrometry).

Prolonged digestion with trypsin resulted of mixture of digestion-resistance peptides (DRPs), with masses close to the masses of the undigested forms. Under reducing conditions these DRPs contains lower masses which is association with arrangement of disulphide bonds. The secondary structure of the DRPs compared to the native counterparts was not changed. DRPs exhibited virtually the same IgE-binding potency as the native protein.

Digestion of separated Ara h 2 isoforms result in digestion-resistant peptides that resemble structure and IgE-binding from the native allergens, even although the peptide backbone is cleaved. The fact that allergic proteins or peptides survived proteolytic treatment and remains allergenic in vitro it's likely that they will be secreted into biological fluids. This indicates that they are most likely the sensitizing or tolerating agents within an allergic food, and may contribute to original development of allergic disease.

Keywords: digestion-resistance peptides, food allergy, immunoproteomics.

SUN-234
Molecular insights into interactions between isoforms of human guanylate-binding proteins – antipathogenic players of the immune systems

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During evolution, human organism has developed a variety of strategies to protect against pathogens. The innate immune system is the first line in defense. Upon infections the innate immune system upregulates a high number of proteins that in turn can eliminate pathogens and, therefore, inhibit disease progression. Several of the seven human guanylate-binding proteins (hGBP1-7), being members of the dynamin-superfamily and strongly upregulated in response to interferon-γ, have been shown to provide anti-pathogenic actions, among others, against Influenza A virus, Hepatitis C virus, and *Chlamydia trachomatis*. Homo- and heterointeractions of isoforms hGBP1-5 were qualitatively shown to define the subcellular localization of the proteins and intracellular hGBP translocation was shown to be regulated in a nucleotide-dependent, hierarchal manner, whereby prenylated hGBPs (hGBP1, 2 and 5) dominates over non-prenylated GBPs (hGBP3, 4, 6 and 7). However, the understanding of hGBPs networking demands quantitative data on the formation of heterocomplexes of the isoforms.

We used recombinant hGBPs to identify their individual biochemical properties. Specific nucleotide binding affinities, GTPase activities as well as the ability to form nucleotide dependent polymers defined a unique pattern for each isoform. Further, we established two different assays employing either GTPase activity or the polymerization of the hGBPs as a measure for heterocomplex formation. All the investigated hGBPs yielded a stimulated GTPase activity upon homointeractions, meaning low GTP turnover at low concentrations where the protein is expected to be monomer, but maximum activity at higher concentrations where the protein is shown to be dimer. When the hGBP isoforms were pairwise combined, remarkable effects on the GTPase activity were obtained. For instance, hGBP1 made hGBP5 to exhibit maximal GTPase activity even at low concentrations indicating that heterocomplex formation is not only tighter but also capable of stimulating the partner. Further quantifications could be made within the absorbance-based polymerization assay. We monitored that GTP-hydrolysis induces polymerization of prenylated hGBPs, which in turn is inhibited by non-prenylated hGBPs in a concentration dependent manner.

Taken together, the hGBPs interact and mutually influence each other in a finely tuned system, which engages on two fundamental molecular mechanisms: firstly, the GTP hydrolysis properties and secondly, the polymerization and depolymerization. Both are involved in defining the subcellular localization and the function of the hGBPs.

Keywords: GTPase, Guanylate-Binding Proteins, Heterinteractions.

SUN-235
Molecular interactions and possible polyfunctionality of serum blood protein which is associated with inflammation, tumors and autoimmune conditions

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The name of pregnancy associated alpha2-glycoprotein- α2-PAG (also is pregnansi zone protein – PZP) is empirical name evidence only that he was first discovered in this physiological state. Shortly it was found a small concentration in the blood of healthy persons. Next, in a large clinical material we have found that the synthesis of 2-PAG significantly increases not only during pregnancy, but the inflammation and swelling. This phenomenon is definitely associated with its function.

For establishing function of the α2-PAG, we studied its immunomodulatory properties in vivo on mice. His condition in the blood were studied by immunochemical and physico-chemical properties – by biochemical methods.

Had been revealed communication between rising of α2-PAG level and decreasing of IgA, IgG, TNFα, IL4 and lymphocytes in a blood of patients with inflammatory diseases. Were obtained data on affinity of α2-PAG to estrogen. We have also identified a discrepancy physicochemical parameters α2-PAG in the blood to its molecular weight. In chromatography elution volume α2-PAG coincides with a peak output of alpha2-macroglobulin- MG (fraction 'heavy blood proteins'). MG twice as heavy PAG. Use of detergents indirectly confirms the intermolecular interactions between two proteins with different molecular weights.

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To justification the interactions α2-PAG and MG we used the database of proteins SwissModel and Swiss PDB Viewer program for forming putative conformations and studying coinciding for interaction positions non polar amino acids (single and multiple), which are responsible for interaction.

Analysis showed that the location of nonpolar amino acids is often the same position in the range of 1–2. It is possible that certain amino acids may remain on the surface of the tertiary structure of the protein and to be a basis of intermolecular interactions due to of hydrophobic bonds. Is possible that these effects can provide intermolecular interactions (or adhesion effect) which determine the function of the protein.

This work was supported by RFFR grant N Nk 14-04-01075/14.

Keywords: 2-PAG or PZP, associated with inflammation protein, molecular interactions.

SUN-236
Molecular modeling and virtual screening to identify new antifungal compounds against the drug target thioredoxin reductase

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The prevalence of invasive fungal infections has increased steadily worldwide in the last few decades. The development of drugs that act selectively in target pathogenic fungi without producing collateral damage to mammalian cells is a daunting pharmacological challenge. Indeed, many of the toxicities and drug interactions observed with contemporary antifungal therapies can be attributed to non selective interactions with homologous enzyme or cell membrane systems found in mammalian host cells. We selected the potential drug target *trr1*, a gene that encodes for thioredoxin reductase and is conserved in eight human fungal pathogens and absent in humans. The recombinant protein Trr1 of *Paracoccidioides lutzii* was produced by heterologous expression in *Escherichia coli* system. The Trr1 recombinant protein was used for enzymatic assay and antifungal activity in vitro. Moreover, we performed the homology modeling of *P. lutzii* Trr1to predict 3D model and the virtual screening in chemical libraries for select the main small molecules that interact with Trr1 model. Initially, compounds from Life Chemicals was docked with the model by virtual screening simulations. The small molecules that interact with the model were ranked and, among the best hits, 12 molecules were finally selected as putative inhibitors of Trr1. These molecules were synthesized for validation and in vitro antifungal activity assays for *Paracoccidioides lutzii*, *P. brasiliensis*, *Candida albicans* and *Cryptococcus neoformans* were performed. From the molecules tested, 3 of which turned out to harbor inhibitory activity against *Paracoccidiodes* spp. Corroborating these findings we have also detected inhibitory activity of those molecules against the purified recombinant enzyme TRR1 in biochemical assays. Therefore, a rational combination of molecular modeling simulations and virtual screening of new drugs has provided a cost-effective solution to an early-stage medicinal chemistry problem. Consequently, news perspectives were generated for technological development and innovation for new antifungal agents against human pathogens. Financial Support: CNPq (*Conselho Nacional de Desenvolvimento Científico e Tecnológico*) and FAP-DF (*Fundação de Apoio a Pesquisa do Distrito Federal*).

Keywords: human pathogenic fungi, new antifungal compounds, tioredoxin reductase.

SUN-238
Native stability and the pathway of α1-antitrypsin polymerisation

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Serpins are a family of protease inhibitors whose active conformation is a kinetically stabilised folding intermediate, and includes the abundant plasma protein, α_1-antitrypsin. Upon cleavage of an exposed loop on α_1-antitrypsin by elastase, the central five-stranded β-sheet undergoes a transition to an extremely stable six-stranded form. Mutations can promote this transition in the absence of a proteinase; when accompanied by an intermolecular domain swap, the result is a growing polymer chain of inactive molecules. Polymers result in pathologies with various degrees of severity, and can also be generated experimentally under destabilising conditions, such as at elevated temperatures. Various mutations – both naturally-occurring and rationally-designed – have been described that alter the rate of heat-induced polymerisation, and in some studies these data have been used to infer features of the polymerisation mechanism. However, this approach is overly simplistic, as it does not account for general effects on native stability. Here we show that the temperature midpoint of thermal denaturation reflects the transition of α_1-antitrypsin to an intermediate species, and determine the relationship with polymerisation rates in the presence of stabilising additives and mutations at several different temperatures. This study shows that the effects of mutations on polymerisation are primarily manifested through global changes in native state stability, and seldom through specific structural perturbations of the polymerisation mechanism.

Keywords: polymerisation, serpinopathies, stability.

SUN-239
Nesfatin-1 levels in polycystic ovary syndrome

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Nesfatin-1 is an hormon synthesized mainly in hypothalamus and several other specific organs regulating eating habits, appetite and is thought to be related to ovarian functions. In our study, we aimed to evaluate nesfatin-1 levels with the other metabolic parameters in the polycystic ovary syndrome(PCOS), a condition that is known to be related to both ovarian functions and obesity. Study subjects were chosen from the women who applied to the Obstetrics and Gynecology Department of Istanbul Bilim University Avrupa Florence Nightingale Hospital. Thirty-five healthy control subjects and 55 PCOS patients were included. Blood samples were obtained in the 3rd-4th days of the menstrual cycle. Luteinizing hormone (LH), Follicle-stimulating hormone (FSH), Free testosterone (FT), Dehydroepiandrosterone sulfate (DHEA-S), Insulin, Fasting blood glucose (FBG), High density lipoprotein (HDL), Low density lipoprotein (LDL), Triglycerides (TG), Sex hormone binding globulin (SHBG) levels were measured; homeostatic model assessment – insulin resistance (HOMA- IR) value was calculated. Nesfatin-1 levels were measured by a competetive inhibition ELISA method. Due to our results, PCOS patients were having lower nesfatin- 1 levels compared to the control group. Additionally, the groups were divided into subgroups according to their BMI and the nesfatin-1 results

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were found to be independent of the BMI, we emphasize the necessity of further studies with larger groups to be able to understand the mechanism of nesfatin-1 decrease in PCOS cases.
Keywords: metabolic profile, Nesfatin-1, Polycystic Ovary syndrome.

SUN-240
Neuropathy-associated mutations of alpha-crystallin domain affect the structure and properties of human small heat shock protein HspB1

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Charcot-Marie-Tooth disease (CMT) is peripheral distal neuropathy affecting motor and sensory neurons of peripheral nervous system. About twenty mutations of small heat shock (sHsp) protein HspB1 correlate with development of CMT. HspB1 belongs to the large family of sHsp which are able to prevent aggregation of denatured proteins and demonstrate the chaperone-like activity. The small heat shock proteins possess multiple activities; however, molecular mechanisms underlying induction of neuropathy by mutated HspB1 remain obscure. In order to understand effect of HspB1 mutations in the onset of neuropathy we analyzed structure and some properties of four mutants (G84R, L99M, R140G, K141Q) carrying a single point mutation located in the alpha-crystallin domain.

The data of size-exclusion chromatography, analytical ultracentrifugation and dynamic light scattering indicate that the wild type HspB1 and its K141Q mutant form large stable oligomers with apparent molecular weight of ~560 kDa. At the same time G84R, L99M and R140G mutants form much less stable oligomers tending to dissociate at low protein concentration. In addition large oligomers formed by R140G mutant are very unstable and tend to aggregate. In response to different stimuli HspB1 undergoes phosphorylation by MAPKAP 2 kinase inducing dissociation of large oligomers. Mutations G84R and L99M promote phosphorylation-induced dissociation of HspB1 large oligomers. All mutants analyzed possessed lower in vitro chaperone-like activity than the wild type protein with two model protein-substrates (a-lactalbumin and subfragmet-1 of skeletal muscle myosin). Thus, all mutations analyzed significantly modify the structure and properties of HspB1 and this is probably one of the reasons of development of CMT disease. This research was supported by the Russian Foundation for Basic Research (13-04-00015).
Keywords: mutations, neuropathy, small heat shock proteins.

SUN-241
Neurotrophin 3 (NT-3), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) in various human pathologies

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Introduction: Chronic obstructive pulmonary disease (COPD), ischemic stroke (IS) and viral meningitides (M_VIR) have been associated with systemic inflammation. Pro-inflammatory cytokines and neurotrophins have been recognized as mediators of neuroprotective mechanisms or in airway remodeling processes. In the present work we investigated the serum levels of NT-3 and pro-inflammatory cytokines TNFα and IL-6 in order to identify the similarities and particularities of systemic inflammatory response in these human different pathologies.
Patients and Methods: Serum levels of NT-3, TNFα and IL-6 were investigated by ELISA (kits EuroClone) in the three groups of patients: with chronic disease COPD (n = 30) and acute diseases, IS (n = 30) and M_VIR (n = 30), 2–6 days after admission. The study was conducted in three types of comparative clinical courses: favorable (F), medium (M) and severe (S). Data were statistically analyzed by Student t test; the threshold of significance was considered for $p < 0.05$.
Results: The mean level of NT-3 (pg/ml) was increased in COPD compared to the IS and M_VIR in all clinical courses: (F) 19.2 ± 3.5 versus 14.4 ± 4.9 and 8.3 ± 0.8 / (M) 33.7 ± 31.2 (#) versus 21.8 ± 9.9 and 8.8 ± 0.7 ($p < 0.05$) / (S) 44.2 ± 16.1 (#) versus 14.2 ± 3.5 ($p = 0.016$) and 7.9 ± 0.6 ($p = 0.01$). On the contrary, the mean level of IL-6 (pg/ml) was increased in IS compared to COPD and M_VIR in all the clinical courses: (F) 26.9 ± 8.6 versus 9.4 ± 3.5 ($p = 0.001$) and 2.1 ± 0.7 ($p = 0.05$) / (M) 55.5 ± 24.6 (#)versus 15.1 ± 14.4 and 2.2 ± 0.7 / (S) 35.3 ± 24.3 (#) versus 19.5 ± 16.7 (#) and 11.8 ± 0.9 ($p = 0.0038$). The dispersion of NT-3 and IL-6 responses in a large range of values (#) has been accompanied by persistent responses of TNFα [IS: (M) 158.4 ± 76.5 and (S) 103.2 ± 45.6 / COPD (M) 24.5 ± 17.6 and (S) 40 ± 16.8 / M_VIR (S) 58 ± 17].
Conclusion: Although the pathologies investigated by us are different, the increased mean level of NT-3 and IL-6 shows a strong systemic inflammatory response in IS and COPD as opposed to M_VIR where the inflammatory response is mainly locally. The trend of increasing of systemic NT-3 level in COPD is associated with the progression of chronic disease, while the increasing of systemic IL-6 level in IS is accompanied with acute course complicated. The TNFα persistence indicates the worsening clinical condition and imminent death.
Keywords: Cytokines, inflammation, neurotrophin 3.

SUN-242
Neurotrophin-3 lung synthesis as a possible fibrosis inducing mechanism in chronic obstructive pulmonary disease

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Introduction: Chronic obstructive pulmonary disease (COPD), estimated to be the 3rd cause of death in 2020, is characterized by an irreversible and progressive airflow limitation, mucus hypersecretion, inflammatory changes and remodeling of the airway wall. Immunocytochemistry and other evidence suggest that both neurotrophins and their receptors are expressed by a wide variety of component lung cells (nerves, immune cells, epithelium, smooth muscle, fibroblasts and endothelium). As with many other diseases, there is limited information on the role of neurotrophins in pulmonary fibrosis. Neurotrophin-3 (NT-3) is elevated during airway inflammation, because alveolar macrophages, mastocytes and eosinophils expresss NT3, as well as NT3-receptor. This work studies the role of NT-3 in inflammation and regeneration modulation in COPD, the purpose being to emphasize the potential of NT-3 in improving our understanding of the pathophysiology of COPD.
Patients and Methods: NT-3 serum and bronchoalveolar lavage levels were determined in 43 patients with COPD using

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ELISA. Inflammatory cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6), Tumoral necrosis factor α (TNF-α) and interleukin10 (IL-10) were determined, too.

Results: A significant increase of serum and bronchoalveolar lavage NT-3 and IL-6 levels was obtained, as well as a positive correlation between these two parameters.

Conclusion: Bronchial smooth muscle cells, local macrophages and neutrophils secrete cytokines, chemokines and growth factors. The expression of NT-3 may be regulated by IL-6, suggesting a dynamic interplay that might have a potential role in airway inflammation. Previous in vitro studies found that human pulmonary fibroblasts constitutively secrete nerve growth factor (NGF) and that NGF secretion is enhanced by proinflammatory cytokines (IL-1β and TNF-α). Our in vivo results suggest that IL-6 enhances NT-3 lung synthesis in COPD, which should result in enhanced production of extracellular matrix components, thus contributing to fibrosis. NT-3 could be used to quantify lung fibrosis in COPD.

Keywords: interleukin6, lung fibrosis, neurotrophin3.

SUN-243
NFATc1 is required for imiquimod-induced psoriasis-like skin inflammation in mice

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Psoriasis is a systemic chronic immunological disease characterized primarily by abnormal accelerated proliferation of the keratinocytes in the epidermal layer of the skin. In psoriasis patients, the precipitating event leads to keratinocytes activation followed by immune cell activation. Also, there is recent evidence that in psoriasis the tolerance towards self antigens is defective. The main players investigated in psoriasis are the effector T cells. However, there is recent evidence that B cells play some role in the disease.

Bregs are a special type of B cells that are believed to have a regulating influence on other B cells and T cells, tuning the immunological response through immunomodulatory cytokines. They serve to keep the immune response in check in case of auto immune diseases and in case of fulminate inflammatory conditions. Their effects are reported on Th1, Th17, $CD4^+$ T cells and monocytes.

NFATs are a family of transcription factors that are highly expressed T cell and B cells. The typical NFAT members have different isoforms due to the use of multiple promoters, several polyadenylation sites and alternative splicing events. NFATc1 is part of the signal transduction pathways that regulates B cell activation and function.

We used the mouse model of IMQ-induced psoriasis-like inflammation, which was first reported by Gilliet in 2004. We induced the psoriasis-like inflammation in wild type (WT) mice, mice that lack NFATc1 in their B cells ($NFATc1^{f/f}$ x mb1-cre) and mice that lack B cells (mb1-cre homozygous mice). The $NFATc1^{f/f}$ x mb1-cre mice showed significantly less IMQ-induced psoriasis-like inflammation than the WT mice. This pointed that NFATc1 is needed for the development of psoriasis. The mb1-cre homozygous mice showed a flared up psoriasis-like inflammation in comparison to the WT mice. This suggested an important role of B cell in psoriasis. Analysis of the $NFATc1^{f/f}$ x mb1-cre mice showed that both Bregs and germinal center B cells were affected favoring the dampened clinical picture in case of mice that lack NFATc1 in their B cells. Investigation of in surface expression of B cells showed that IL-10 is highly expressed upon NFATc1 ablation especially with IMQ treatment. This suggested that IL-10 is an important link between NFAT ablation and less aggressive psoriasis-like inflammation in IMQ treatment.

Keywords: B-cells, Psoriasis, Autoimmunity.

SUN-244
NLRC3 as a novel regulator of the cryopyrin inflammasome

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Inflammasomes are protein complexes which recognize pathogens and activate inflammation. Special attention has been attributed to the Cryopyrin inflammasome due to its wide range of activator molecules and its implication in the physiopathology of several auto-inflammatory diseases. To avoid accidental induction of inflammation, the Cryopyrin inflammasome activation is tightly regulated in the cells. Control mechanisms started to be identified but still other Cryopyrin inflammasome modulators remain to be characterized.

NLRC3, another member of the NLR family, was found to be a negative regulator of T-cell activation, NFκB pathway and STING-dependent cytokine secretion. However, the effect of NLRC3 on the Cryopyrin inflammasome or other inflammasomes is still unknown. We propose that NLRC3 could be a novel regulator of the Cryopyrin inflammasome. For this purpose, we tested NLRC3's effect on Cryopyrin inflammasome activation under overexpression and endogenous conditions and in *vivo* systems.

HEK293FT cells were transfected with different components of the Cryopyrin inflammasome and IL-1β secretion was measured. Whereas transfection of Cryopyrin induced IL-1β secretion in a dose-dependent manner, co-transfection of Cryopyrin and NLRC3 resulted in significantly reduced IL-1β release. Similarly, co-transfection of HEK-ASC-GFP cell lines with Cryopyrin and NLRC3 led to a significant decrease in ASC speck formation. These results were further confirmed in THP-1 cells lines, where IL-1β secretion in response to known Cryopyrin inflammasome activators such as MSU, ATP and Imiquimod was significantly higher in NLRC3 KD stable lines compared to control lines. Moreover, injection of NLRC3 protein in rat's eye treated with LPS decreased IL-1β levels compared to the ones only treated with LPS. In conclusion, we have found that NLRC3 interferes with ASC speck formation and inhibits IL-1β secretion in all systems tested. Thus, NLRC3 is a novel negative regulator of the Cryopyrin inflammasome. The exact molecular mechanism of this inhibition is still under investigation.

Keywords: NLRC3, Cryopyrin, Inflammasome.

SUN-245
Noninvasive NIR and PET imaging of the functional pancreatic beta-cells mass using the Fab fragment of the monoclonal autoantibody IC2

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Keywords: Imaging, monoclonal antibodies, Pancreatic beta-cells.

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SUN-246
Novel Kv1.3 blockers for the treatment of autoimmune diseases

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The subset of inflammatory T and B cells with elevated expression level of voltage-gated potassium Kv1.3 channel is a functional marker of autoimmune diseases (multiple sclerosis, type 1 diabetes, rheumatoid arthritis). Inhibition of Kv1.3 channel is an attractive strategy for the treatment of autoimmune diseases without generalized immunosuppression. Peptide toxins found in animal venoms are potent inhibitors of Kv1-family channels, and some of them possess high selectivity to Kv1.3 channel.

The original fluorescent analytical systems developed by us were used to search for Kv1.3, Kv1.1 and Kv1.6 channel blockers in arthropod venoms. The analytical systems are based on (i) expression of hybrid potassium channel KcsA-Kv1.x (x = 1,3,6) in the plasma membrane of *E.coli.*, (ii) fluorescently labeled ligand and (iii) confocal microscopy as a measurement technique. New ligands are recognized and characterized a competitive binding assay. Using these analytical systems we studied dozens of spider and scorpions venoms for the presence of peptides possessing affinity to Kv1 channels. We confirmed the presence of active compounds in *Leiurus quinquestriatus* and *Orthochirus scrobiculosus* scorpion venoms. Six new high affinity Kv1.x (1,3,6) blockers were discovered in addition to 3 known ligands in *Mesobuthus eupeus* scorpion venom. Functional studies of novel peptide toxins are in progress now. They are aimed to clarify pharmacological value of the new blockers.

Keywords: None.

SUN-247
Novel nanoscale carriers as alternative in further cancer therapy

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Severe toxic side effects and drug resistance are the major limitations of doxorubicin (Dox), one of the most potent anticancer agents in clinical use. Nanocarrier preparations offer the opportunity to overcome these drawbacks, which is reflected in the clinical approval of two liposomal Dox preparations. Additionally, there are many attempts to enhance the activity of Dox against multidrug resistant (MDR) cancer cells. However, most of these strategies resulted in the increased uptake of Dox in resistant cells, only, while it remained unchanged in chemo-sensitive cells.

Here, we present a new polymeric-phospholipidic hybrid delivery system which distinctly enhanced the accumulation and activity of Dox in all tested cancer cell lines including several MDR cell models. Notably, the resistance levels against Dox were reduced from about 6-fold to about 2-fold. Moreover, the new nanocarriers were shown to rapidly (within 10 min) and effectively transport Dox into resistant as well as sensitive cancer cells. Consequently, treatment with the new Dox-containing nanocarriers resulted in effective cell cycle arrest in G2/M phase and ROS-induced cell death induction.

Finally, the new nanocarriers were tested against NK/Ly lymphoma and L1210 leukemia cells in vivo. Since rather low drug concentrations were used in these tests (0.1 mg/kg), the treatment schemes were well tolerated and the mice did not exhibit any symptoms of toxicity, such as fatigue, or significant weight loss even in the Dox-receiving groups. Treatment with Dox at these concentrations led to transient stop of tumor growth (indicated by increased body weight) and, thus, prolonged the mean survival of NK/Ly bearing mice from 21 to 39 days. The nanocarrier distinctly increased this activity resulting in disease remission and cure, which was indicated by restored body weight of the NK/Ly-bearing mice to the initial levels. Notably, even after end of PC-Dox treatment no increase in body weight was observed. Thus, at day 60 (end of experiment) all of the PC-Dox-treated mice were still alive and, thus, classified as 'cured animals'. Similar results were observed in the L1210 model as depicted in, where a dose of 0.1 mg/kg PC-Dox resulted in complete loss of tumor-mediated animal death. In both cell models, the nanoformulation of Dox resulted in 100% cured animals already at low concentrations (0.1 mg/kg), while free Dox solely extended survival time.

Keywords: Cancer therapy, Doxorubicine, Nanoparticles.

SUN-248
Novel sea anemone peptide inhibitor of acid-sensing ion channel 3 (ASIC3)

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The ASIC3 channel belongs to a family of sodium-selective acid-sensing ion channels (ASIC), activated by a drop of extracellular pH. ASIC3 channels are predominantly expressed in peripheral sensory neurons and play an important role in acidic and inflammatory pain stimuli during pathological conditions. ASIC3 channels generate a transient current followed by a sustained component in response to a drop of extracellular pH. Animal venoms already proved themselves as useful tools for the study of structural and functional features of various ion channels. Only two selective peptide inhibitors of ASICs is known to date. It is Psalmotoxin 1 (PcTx1), a potent and specific inhibitor of homomeric ASIC1a channels from a tarantula venom, and APETx2, an inhibitor of homomeric and heteromeric ASIC3 channels from a sea anemone venom.

Three novel peptides of 29 amino acid, cross-linked by two disulfide bridges, were isolated from the sea anemone *Urticina grebelnyi* venom. The structure of the gene encoding the shared precursor protein of the identified peptides was determined. One peptide, Ugr 9-1, produced a reversible inhibition effect on both the transient and the sustained current of human ASIC3 channels expressed in *Xenopus laevis* oocytes. Recombinant Ugr 9-1 was produced through the prokaryotic expression system with the with the final yield of about 8 mg/liter of cell culture. The identity of the recombinant Ugr 9-1 and the natural peptide was confirmed by the chromatographic as well as the electrophysiological methods. Ugr 9-1 completely blocked the transient component (IC_{50} 10 μM) and partially (~50%) inhibited the amplitude of the sustained component (IC_{50} 1.5 μM) of the ASIC3 current. Also Ugr 9-1 showed no activity on the other ASIC isoforms: ASIC1a, ASIC1b, and ASIC2a Using in vivo tests in mice, Ugr9-1 significantly reversed inflammatory and acid-induced pain. It is *worth noting that* the structure and the mechanism of action of this peptide differ from the well known sea anemone toxin APETx2.

NMR spectroscopy revealed that Ugr 9-1 has an uncommon spatial structure, stabilized by two S-S bridges, with three classical β-turns and twisted β-hairpin without interstrand disulfide bonds. This is a novel peptide spatial structure that we propose to name boundless β-hairpin. *It is interesting to note that* two other peptides, Ugr 9-2 and 9-3, don't modulate ASIC3 activity but have

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significant homology with the active Ugr 9-1 peptide and evidently have the same fold type. All together, the three novel peptides are a visual confirmation of the combinatorial biochemistry model, according to which several sequence alterations of peptides with similar folds result in selectivity to different cellular receptors.
Keywords: acid-sensing ion channel, inflammatory pain, peptide structure.

SUN-249
Nutritional modification of endoplasmic reticulum stress and inflammasome activity by a bioactive lipokine prevents atherosclerosis

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The prevalence of obesity has increased epidemically, with little hope for effective treatments on the horizon. Rising along with obesity is an aggregation of co-morbidities including insulin resistance, diabetes, hepatosteatosis, dyslipidemia, hypertension and atherosclerosis, collectively known as the metabolic syndrome. Signaling pathways that lie at the interface of metabolism, inflammation and stress are altered by excessive exposure to lipids and other nutrients and profoundly influence these chronic diseases. Despite intense research into the crosstalk between metabolic, inflammatory and stress pathways, there remains a gap in our knowledge regarding the nutritional modification of such crosstalk. What are the nutritional cues that can beneficially modify this crosstalk? Bioactive lipokines offer tools for nutritional modification of the crosstalk between metabolism, inflammation and stress pathways that are major contributors to the formation of atherosclerosis.

Recent studies showed that bioactive lipid species have profound effects on metabolism. For example, palmitoleate (PAO) can act as a lipokine – a lipid that is generated from the adipose tissue through *de novo* lipogenesis and exert endocrine effects in distant tissues such as the liver and skeletal muscle. PAO can suppress inflammation in the adipose tissue and improve insulin signaling in skeletal muscle and liver. The impact of PAO on atherosclerotic lesions is also highly relevant to cardiovascular diseases; the links between nutrients, stress pathways and inflammation are central to atherogenesis and its complications, but very little is known about nutritional modification of this crosstalk. Our studies show that PAO can inhibit lipid-induced endoplasmic reticulum stress, inflammasome activity, pro-inflammatory cytokine secretion in macrophages and prevent atherosclerosis in mouse models. Our findings have important implications for unraveling the pathogenesis of metabolic and inflammatory diseases and can facilitate generation of novel therapeutic approaches utilizing bioactive lipokines in these diseases.

Acknowledgement: This study is supported through a Marie Curie Reintegration Grant FP7.
Keywords: None.

SUN-250
Obesity protects heart but increases lung injury by endotoxin inflammation

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Purpose/Objective: Sepsis is a severe disease that represents a significant healthcare burden worldwide, while obesity has reached epidemic proportions over the last few decades. Although the mechanism is uncharted, it is known that obesity increases morbidity and mortality in sepsis through its multiple effects on many organ systems, including heart and lungs. Our aim was to investigate the effects of obesity in heart and pulmonary inflammatory process in an experimental model of endotoxemic shock.
Material and Methods: Animals were fed a high fat diet (30% of fat) for 6 weeks and then injected with 15 mg/kg LPS i.p. They were euthanized after 6, 24 and 48 hours. Inflammation was characterized by measurement of plasma and tissue cytokines. Quantification of adipokines and adhesion molecules were performed by ELISA.
Results: Obesity decreased the survival rate of the animals 24 hours after LPS injection. There was higher plasma concentration of IL1-beta, IL-6 and TNF-alpha in these animals. Furthermore, LPS increased resistin plasma levels, and this effect was more evident in obese mice. Adiponectin levels were higher in obese mice, and this difference was abolished by LPS injection. Endotoxemia resulted in increased concentration of IL-6, IL-1beta and MIP2 in obese mouse lungs, but decreased IL-6 and IL-1beta in their heart. The protective effect in the heart may be related to decreased expression of adhesion molecules (ICAM and VCAM). Although lung inflammation was exacerbated in obese mice, no difference was observed in collagen deposition.
Conclusion: Obesity may be an additional complication factor in sepsis induced pulmonary inflammation.
Financial Support: FAPESP (#2009/17652-5).
Keywords: endotoxemia, lung injury, Obesity.

SUN-251
Oligopeptidase B from *Serratia proteamaculans*: structural basis for cold adaptation

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Oligopeptidase B (opdB) is a ‘processing peptidase’ from the prolyl oligopeptidase family of serine peptidases present in bacteria, protozoa and plants. OpdB is known to be an important virulence factor of bacterial and protozoan pathogens, causing severe diseases that are difficult to diagnose and treat: Chagas disease, sleeping sickness and other trypanosome infections as well as leishmaniasis that make the enzyme a promising therapeutic target.

OpdB from the nonpathogenic, psychrotolerant gram-negative microorganism *Serratia proteamaculans* (PSP) is the first known psychrophilic enzyme of this class. As a result this protein may serve as a model enzyme for the study of the pathogenesis of many infectious diseases or for drug development, since unlike its highly pathogenic mesophilic counterparts PSP poses no danger to researchers due to its thermal inactivation at 37°C.

The aim of the study is to reveal the mechanism of cold adaptation of PSP in the context of its structure-function features. Here we applied experimental and bioinformatic approaches to highlight the different aspects of the PSP structure associated with cold adaptation and studied the impact of different external and internal factors on thermostability and activity of recombinant PSP.

The positive effect of glycerol on PSP thermostabilization was revealed using the Thermofluor Stability Assay, though ionic strength and calcium ions caused the acceleration of PSP unfolding. The findings were in good agreement with the results of high-sensitivity differential scanning calorimetry and the residual enzymatic activity determination following heat treatment at 37°C. The full-sized PSP and its truncated form lacking an N-terminal 100 amino acid loop were produced in *E. coli* and used for crystallization screening and structural studies.

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Keywords: Oligopeptidase B, Psychrophilic enzymes, Thermofluor Stability Assay.

SUN-252
Palmitoylation of proteins in LPS-stimulated cells analyzed by click chemistry

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Lipopolysaccharide (LPS) is a component of the outer membrane of Gram-negative bacteria. During bacterial infection, LPS is bound in the serum by LPS-binding protein (LBP) which delivers LPS to CD14 protein of the plasma membrane in macrophages. CD14 transfers LPS to the TLR4/MD-2 complex which subsequently triggers two signaling pathways, MyD88- and TRIF-dependent one. Production of pro-inflammatory mediators follows. Our previous studies indicated that Lyn kinase is among key factors controlling TLR4-triggered signaling. Taking into account that both CD14 and Lyn kinase are anchored in sphingolipid- and cholesterol-rich rafts of the plasma membrane, these membrane regions are likely to contribute to TLR4 signaling. Among few factors facilitating interaction of proteins with rafts is their acylation with palmitic acid (C:16). Therefore, we aimed to examine whether palmitoylation of Lyn, and possibly other proteins, affects pro-inflammatory responses to LPS.

To examine the importance of protein palmitoylation in LPS-induced signaling, RAW264 cells were incubated with 2-bromopalmitic acid (BPA), an inhibitor of palmitoyl transferase. An exposure of RAW264 cells to 50–250 μM BPA inhibited in a dose dependent manner the production of TNF-α and RANTES in cells stimulated with 100 ng/ml LPS. These two cytokines were chosen for analysis because they are generated in MyD88- and TRIF-dependent manner, respectively. BPA at 250 μM inhibited also significantly activation of NFκB indicated by IκB phosphorylation in LPS-stimulated cells. In an attempt to identify proteins palmitoylated in LPS-stimulated cells, a click chemistry was applied. For this, cell were metabolically labeled with ω-alkynyl-palmitate analog of palmitic acid, and after lysis, subjected to 'click reaction' with azido-azo-biotin. Subsequently, labeled proteins were immunoadsorbed on streptavidin beads, eluted with sodium dithionite which cleaves azido-azo-biotin. Eluted proteins were separated by SDS-PAGE, transferred onto nitrocellulose and probed with antibodies against selected proteins, including Lyn. We detected palmitoylation of Lyn in resting cells which was increased after stimulated with 100 ng/ml LPS for 1 h. LPS-induced palmitoylation of proteins was inhibited in the presence of 125 μM BPA.

Taken together our data indicate that palmitoylation of proteins, including Lyn kinase, can control TLR4-triggered signaling.

Keywords: click chemistry, lipopolysaccharide, protein palmitoylation.

SUN-253
Pancreatic β-cell glycotoxicity: alterations in ERp46 expression and the role of glucagon-like peptide analogues

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Background: Diabetes Mellitus is characterized by peripheral insulin resistance, hyperglycaemia and defective insulin secretion. Insulin producing pancreatic beta cells are equipped with a highly developed endoplasmic reticulum (ER) and thus are affected by ER stress under hyperglycaemic conditions. We have previously studied the influence of high glucose on cultured β-cells in vitro. Proteomic analysis revealed a number of proteins involved in glucose toxicity, while further biochemical analysis identified the endoplasmic reticulum protein ERp46 as a molecule with a possible role in insulin production at the post-translational level. In addition, the involvement of incretin hormone glucagon-like peptide 1 (GLP-1) in diabetes proposes that incretin-mimetic compounds may be among the optimal choices in future therapeutic interventions; therefore their effects on various aspects of the pathogenesis of diabetes mellitus should be explored in detail.

Aim: Based on the above, we examined the possible involvement of ERp46 in insulin production and the effect of the GLP-1 analogue liraglutide on the expression of ERp46 in vitro, in β-cells cultured under high glucose conditions and in vivo, in the mouse db/db diabetic model, where pronounced hyperglycemia is a key characteristic.

Results: Confocal microscopy revealed areas of co-localization of ERp46 and pro-insulin in pancreatic islets. In order to explore the possible interaction between ERp46 and insulin immunoprecipitation was used. In extracts from cultured β-cells, antibodies against insulin co-precipitated ERp46 and antibodies against ERp46 co-precipitated insulin, as shown by Western blotting. Furthermore, β-cells cultured in high glucose conditions exhibited a 2-fold decrease in ERp46 expression, while treatment with the GLP-1 analogue liraglutide restored ERp46 levels, leading to a 2.5-fold increase of ERp46. In the diabetic mouse model db^{-}/db^{-}, ERp46 expression was reduced in pancreatic islets, as documented by morphological and biochemical techniques. This decrease was abolished after treatment with the GLP-1 analogue in a dose-dependent manner.

Conclusions: We propose that since ERp46 is a member of the disulfide isomerases family, it is likely to play a key role in insulin biosynthesis and its reduction under high glucose conditions may be a novel contributor to the glycotoxicity of β-cells. In addition, the GLP1 analogue liraglutide seems to interfere in this process and may exert its beneficial effects in diabetes by affecting insulin production via restoration of ERp46 expression.

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Keywords: endoplasmic reticulum, diabetes, GLP-1 analogues.

SUN-254
Paraoxonase activities in depending on the gender and tobacco smoking

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Paraoxonase (PON) is high density protein-attached (HDL-attached) extracellular esterase, which contributes the protection of HDL against peroxidation. PON is known to have antioxidative and anti-inflammatory properties against oxidative stress from tobacco smoke.

The aim of the study was to investigate a possible association between PON activities and lipids peroxidation in persons exposed to tobacco smoke.

The investigations were performed in the serum of 58 healthy persons, who were in similar age (23.73 ± 4.83 years) and body mass index (21.49 ± 2.87 kg/m^2). Data about smoking were verified by the determination of cotinine concentration – the metabolite of nicotine (ELISA method). The concentrations of total cholesterol, HDL and triglycerides were determined by spectrophotometric methods. Low density protein (LDL) concentration was calculated using Friedewald formula. PON

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activity was measured by spectrophotometric method using paraoxon (phosphotriesterase activity, PON-P), phenyl acetate (aryloesterase activity, PON-A) and dihydrocoumarin (lactonase activity, PON-L) as substrates.

It was shown a statistically significant an increase in PON-P activity in the group of non-smoking women compared to non-smoking men (122.5 ± 58.7 and 80.9 ± 41.2 U/l, respectively). An increased PON-A and PON-L activities in men compared to women (PON-A: 200.5 ± 29.2 and 167.1 ± 34.3 U/l, PON-L: 12.7 ± 3.0 and 9.6 ± 2.1 U/l, respectively) in smokers groups were noted. It was shown a decreased PON-P activity in smokers (65.8 ± 21.6 U/l) compared to non-smokers in the groups of women. An increase in PON-A and PON-L activities in smokers compared to non-smokers (PON-A: 164.4 ± 31.3 U/l, PON-L: 10.2 ± 1.8 U/l) in men groups were shown. The links between LDL concentration and PON-P activity (r = 0.8214, p = 0.0234 in the group of non-smoking women) was observed.

PON is a sensitive maker, which activity is dependent on the gender and it can be modulated by oxidative stress resulting from tobacco smoke. Depending on the gender, various activities of PON are engaged in the protection against oxidative stress induced by tobacco smoke.

Keywords: gender, paraoxonase, tobacco smoke.

SUN-255
Pathogenic and non-pathogenic Simian immunodeficiency virus (SIV) infections induce host DNA methylation changes

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Introduction: In human immunodeficiency virus (HIV) infection, a chronic state of immune activation leads to a progressive depletion of $CD4^+$ T cells, culminating in AIDS in the absence of treatment. SIV infection in macaques (MAC) leads to a disease profile resembling that of humans, while SIV infection of its natural hosts, such as African green monkeys (AGM), is non-pathogenic. AGM downmodulate the excessive immune activation by the end of acute infection, unlike MAC and humans, and maintain this control their entire lifespan. This control is especially pronounced in lymph nodes and associates with low levels of viral replication locally. Here, we investigated whether DNA methylation is involved in the immune and/or viral control of SIV-infected AGMs.

Materials and Methods: We intravenously infected five AGM and five MAC with SIVagm.sab92018 and SIVmac251, respectively. Before infection and at days 14 and 65 post infection (p.i.), lymph nodes were collected. $CD4^+$ cells were isolated from these lymph nodes, DNA was extracted and a bisulfite-treated DNA library was constructed for each of the 30 samples. Samples were sequenced on a Hiseq2000 (Illumina) and BSMAP was used to align reads to reference genomes and estimate methylation levels. Differentially methylated regions (DMRs) were defined using windows of 20 base pairs, q-value < 0.01 and a methylation difference of at least 40%.

Results: For each sample, 85% of all cytosines were covered. SIV infection induced methylation changes in both species. For AGMs, 84 hyper- and 35 hypo-DMRs (19 and 11 genes affected (DMGs)) were identified at day 14 p.i. At day 65 p.i., 55 hyper- and 111 hypo-DMRs (22 and 23 genes) were identified. For MACs, 411 hyper- and 193 hypo-DMRs (96 and 71 genes) were

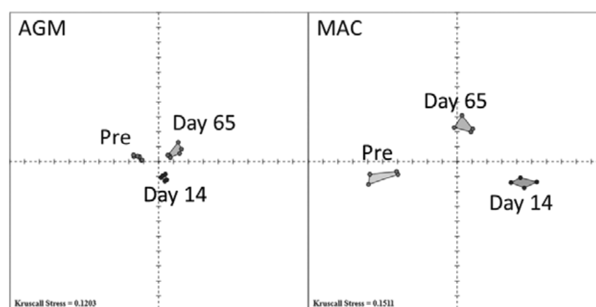

Fig. 1. Multidimensional scaling profiles of the DMRs in AGM and MAC. Each dot is the methylome profile of a biological sample. Panels are scaled to size.

identified at day 14 p.i. and 98 hyper- and 166 hypo-DMRs (42 and 41 genes) at day 65 p.i. The samples segregated per time-point based on the list of DMRs (Fig 1), showing DNA methylation was dynamic during infection. Among the DMGs in MAC were immune genes, e.g. *IRF4*, *RUNX3*, *IL6R* and *NLRP11*, as well as other genes as *MAP4K5*, *IGF2R* and *USP2*. For AGM, *CD37*, *TRAC* and *MAP3K11* were among the DMGs. Proviral genomes were not methylated. We are currently integrating these results with transcriptomic analysis to analyze the effect of these different methylation profiles on gene expression. Concluding, SIV infection modifies the host methylome more deeply in pathogenic than in asymptomatic lentiviral infection.

Keywords: DNA methylation, host-pathogen interaction, inflammation.

SUN-256
Pathological changes in hepatic tissue following scorpion envenomation: involvement of oxidative stress and inflammation-related mediators

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Hepatic inflammation is a common finding during liver diseases including scorpion venom-induced liver damage. The inflammatory phenotype can be attributed to the innate and adaptive immune response generated by Kupffer cells, monocytes, neutrophils, and lymphocytes. The exact mechanism of the triggering of the inflammatory response following scorpion envenomation is still not completely understood and several related events have been suggested to be involved.

The purpose of this study is to assess *in-vivo* hepatic inflammatory response and to evaluate organ toxicity and oxidative damage induced by scorpion venom in an experimental model *envenomated* with Algerian scorpion venom *Androctonus australis hector* (Aah).

This study showed that envenoming with Aah venom caused an inflammatory reaction, characterized by vasodilatation and increased the permeability of the hepatic vessels. This event was accompanied by edema-forming as assessed by the increased liver index and also by an increase in cell infiltration, phagocytes including neutrophil and eosinophil cells as indicated by the increase of myeloperoxidase and eosinophil peroxidase activities. Increase of these immune cell levels results in an important release of reactive oxygen intermediates such as H_2O_2 and nitrogen reactive species. The excessive release of nitric oxide and reactive oxygen species, leads to severe imbalanced redox status with altered or insufficient antioxidant systems, demonstrated by

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the reduction of the hepatic glutathione level, superoxide dismutase and catalase activities.

Our findings showed also that the immune response induced by scorpion venom is characterized by *increased lymphocyte count* associated with highly production of percentages of Thelper Th1 (IL-2, IFN-γ) and Th2 (IL-4, IL-10) cytokines-in peripheral blood. These results suggest that cytokines produced during the inflammatory process intensify the level of oxidative stress caused by leukocytes, which may have serious consequences for liver membrane integrity.

Envenomed mice displayed also hepatic alterations marked by hemorrhage, edema and inflammatory cell infiltration. These tissue alterations caused by Aah venom are correlated with severe concomitant increase of metabolical enzymes in sera indicated by the increased plasma levels of alanine aminotransferase and aspartate aminotransferase activities which are usually correlated with the intensity of tissue damage.

In conclusion, pathophysiological changes occurring in the liver may be mediated, in part, by cytokines and cytotoxic leukocyte-derived product release and possibly reactive oxygen/nitrogen species.

Keywords: Inflammatory response, Liver injury, Scorpion envenomation.

SUN-257
Peptidome profiling of gingival crevicular fluid by MALDI-TOF/TOF mass spectrometry

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Mass spectrometry (MS) represents a promising tool for novel biomarker and eventually new druggable targets discovery [1]. As a part of an ongoing project aimed to the detection of biomarkers in human bodily fluid [2–4], we are now investigating gingival crevicular fluid (GCF), an exudate that can be collected from the gingival sulcus by a minimally invasive procedure. The composition of the GCF is highly diversified and the analysis of its specific constituents provides a quantitative biochemical indicator of the local cellular metabolism that reflects a person's periodontal health status [5]. Because of its sample size, limited to sub-microliter volumes, identification of GCF protein composition by classical biochemical methods has always been limited. To ensure optimized protein extraction from GCF, a novel protocol was developed. After informed consent was obtained, GFC was collected from 5 healthy and 5 gingivitis subjects by paper strips. MALDI-TOF peptidomic profiles of healthy and gingivitis subjects were then compared. Five peptides were found to be significantly more intense in gingival subjects in comparison to healthy individuals.

The pattern of five peptides was identified by both MALDI-TOF/TOF MS and 1-DE followed by MALDI-TOF/TOF MS for molecular weights over 4000 Da. The identified biomarkers included proteins involved in inflammation and immunological processes.

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Keywords: Biomarkers, Gingival crevicular fluid, Inflammation.

SUN-259
PI3K associated with the IgG mediated phagocytic receptor complex in mouse RAW 264.7 macrophages

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The circulating blood ligand Immunoglobulin G (IgG) binds to foreign antigens via its variable domain and its Fc domain is recognized by its Fc receptor on macrophages that trigger phagocytosis, which is required for engulfment of bacteria and microscopic particles. The IgG-Fc receptor complex from RAW 264.7 macrophages was analyzed using Live cell Affinity Receptor Chromatography (LARC) that resulted in the detection of phosphoinositide 3-kinase (PI3K) with the activated receptor complex by liquid chromatography and tandem mass spectrometry (LC-ESI-MS/MS). Computation with SQL, statistical analysis with R revealed specific proteins that overlayed the previously existing genetic and biochemical literature using STRING that revealed the network of PI3K associated proteins of the Fc receptor complex. PI3K were shown to be specifically associated with the activated receptor complex and was detected 78 times compared to about three times in the non-specific controls or other ligands such as BSA, and oxLDL that served as controls. The PI3K proteins captured by LARC were compared to immunoaffinity chromatography (IAC) of the Fc receptor complex from crude homogenates that was not as effective. Staining of RAW cells by anti-PI3K antibody resulted in the detection of staining on the plasma membrane during phagocytosis compared to secondary antibody alone as shown with a laser scanning confocal microscope. Western blotting was used to confirm the specificity of the PI3K antibodies. We screened the effect of the receptor specific PI3K isoforms with silencing RNA (siRNA) that was transiently transfected into RAW macrophages alongside scrambled siRNA controls to assay their effect on the phagocytosis of IgG opsonized microbeads. Treatment of RAW cells with range pharmacological inhibitors including wortmannin, LY24002, and ZSTK474 prevented the phagocytosis of IgG microbeads as measured by laser confocal microscope. The dose of inhibitor required to achieve knockdown was consistent with the siRNA data that indicated PI3KC2G is the key isoform for IgG mediated phagocytosis in RAW macrophages.

Keywords: phagocytosis, PI3K, RAW marcophages.

SUN-260
Plant proteins with medically important properties: structural studies of three members of the β-trefoil family that function as serine protease inhibitors and/or as lectins

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Three related plant proteins that belong to the β-trefoil family, which includes Kunitz-type serine protease inhibitors, have been investigated by X-ray crystallography as well as by biochemical and biophysical techniques. Two of them are potent inhibitors

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of trypsin-related enzymes. EcTI, isolated from the seeds of *Enterolobium contortisiliquum*, inhibits the invasion of gastric cancer cells through alterations in integrin-dependent cell-signaling pathway. BbKI, found in *Bauhinia bauhinioides* seeds, is a kallikrein inhibitor with a reactive site sequence similar to that of kinins, the vasoactive peptides inserted in kininogen moieties. A much weaker protease inhibitor isolated from the bark of *Crataeva tapia* tree (CrataBL) also functions as a lectin. We determined high-resolution crystal structures of free EcTI and in complex with bovine trypsin, in the process re-determining the amino acid sequence. A comparison of the EcTI-trypsin complex with the complexes of related Kunitz inhibitors has shown that rigid body rotation of the inhibitors by as much as 15° is required for accurate juxtaposition of the reactive loop with the active site while preserving its conformation. Modeling of the putative complexes of EcTI with several serine proteases and a comparison with equivalent models for other Kunitz inhibitors elucidated the structural basis for the fine differences in their specificity, providing tools that might allow modification of their potency towards the individual enzymes. The structure of free BbKI was determined at high resolution, showing that the presence of disulfide bonds is not necessary for stabilization of the fold of the members of this family. We have also determined the high-resolution crystal structures of two different crystal forms of glycosylated CrataBL and identified dimers of the protein forming the crystals. CrataBL shows relatively weak inhibitory activity against trypsin and is more potent against Factor Xa, but not active against a number of other serine proteases. We have shown that, as a lectin, CrataBL binds only sulfated oligosaccharides, most likely heparin and its derivatives. We have observed that addition of CrataBL to DU145 and PC3 prostate cancer cell lines leads to their apoptosis, with release of mitochondrial cytochrome *c* through activation of caspase-3. The viability of these cell lines was also impaired by BbKI. Further studies aimed at more complete elucidation of the structural basis of the anti-cancer activities of these β-trefoil family members are in progress.

Keywords: inhibitor, lectin, protease.

SUN-262
Potential novel biomarker from iTRAQ analysis between pre-chemotherapy and post-chemotherapy serum of metastatic osteosarcoma patients

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Osteosarcoma (OS), a malignant bone tumour occurs commonly in children and young adults between the ages of 10–30. Although the standard treatment for OS advances has significantly improved OS patient survival rate, its poor prognosis continues to remain as major problem in managing the disease. In this study, we have conducted a series of systematic analysis to identify the novel proteins associated with the metastatic progression of human OS using a 4-plex iTRAQ analysis. Pooled serum samples were collected from patients diagnosed with metastatic osteosarcoma at two stages (pre- and post-chemotherapy) and they were actively monitored for at least 5 years. Approximately 217 proteins with 104214 spectra were analysed. The PANTHER analysis revealed presence of many plasma proteins involved in biological processes such as cellular component organization or biogenesis (39.4%), cellular process (35.4%), biological regulation (20.0%) and immune system process (29.3%). In addition, these proteins have also shown to be significantly altered in their expression at pre- and post-chemotherapy patients as compared to the control such as C-reactive proteins, vascular adhesion molecule-1 (VCAM-1) and gelsolin. To date, this study is the first differential protein expression study on metastatic osteosarcoma patients' serum at different stages for proteome comparison. We have successfully generated a comprehensive data on the differentially expressed protein. The comparative study results showed a significant amount of proteins expression that has been altered. The results could provide a new insight in the OS biological processes and can be used to identify potential biomarkers for better OS prognosis.

Keywords: chemotherapy, iTRAQ, osteosarcoma.

SUN-263
Prevalence of HCV infection among Thalassemia patients; a perspective from a multi-ethnic population of Islamabad region

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In Pakistan consanguineous marriages, high birth rates, and large population result in high number of thalassemic patients and according to an estimate, each year approximately 5000 newborns are affected due to this genetic disorder. Management of thalassemia is primarily based on regular blood transfusions that results in iron overload and transfusion transmitted infectious diseases. Presented here is a study based on 95 thalassemic cases, a formal consent of the patients was obtained and necessary data was collected including demographic location, clinical representation and family history of these individuals. Altogether, 96% of these patients were of beta thalassemia major, based on serological findings 49% were confirmed as anti-HCV positive. Upon dividing data into two groups, thalassemic only and thalassemic with HCV infection, mean ferritin levels were calculated for both groups, statistical analysis showed a random distribution of ferritin levels, though gender wise analysis showed female patients have relatively higher ferritin levels in comparison to male patents however, the differences were not statistically significant (t-test = 1.203; $p = 0.2319$). Similarly, a random distribution of Hepatitis C was observed regarding ethnic background, demography, socioeconomic status and ABO blood grouping of these patients. Prevalence of HCV was found more common among males 53% in comparison to females 45% and a direct association of age, number of blood transfusion and the prevalence of HCV among these patients was confirmed. Overall, associated clinical complications in these patients included hepatomegaly, splenomegaly and splenectomy. Despite the fact that in many countries standardized screening procedures for the blood related products were already outlined in 1990s higher prevalence rates of HCV among thalassemic patients requires greater attention.

Keywords: None.

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SUN-264
Preventing pathogenic ordered aggregation of α1-antitrypsin using an engineered single chain antibody fragment

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Alpha-1-antitrypsin (AAT) is a protease inhibitor that is mainly produced by hepatocytes and secreted into the circulation. It prevents excessive proteolytic damage by the enzyme neutrophil elastase in the lung. Most individuals have a normal 'M' allele of the SERPINA1 gene that encodes AAT. The Glu342Lys (Z) mutation is the most common severe deficiency allele in Europe and causes the synthesized AAT to self-associate into ordered polymers. These polymers are sequestered within the endoplasmic reticulum of hepatocytes and are associated with liver disease. Nearly half of AAT-deficient adults over 50 have pathological features of liver cirrhosis and occasionally hepatocellular carcinoma. The concomitant lack of circulating AAT predisposes to early onset emphysema. Severe cases of emphysema are treated with AAT replacement therapy where purified AAT is given as a weekly intravenous infusion. No other therapies or alternatives to this costly and partly controversial approach are currently available. In seeking new therapeutic strategies to prevent AAT caused pathologies, we recently developed a monoclonal antibody (4B12) that blocks polymer formation in cell models and *in vitro*. In order to characterise the structural basis for this activity, we have mapped the epitope of the 4B12 antibody. This was done by creating various single cysteine mutants that were conjugated to a 5 kDa PEG molecule. Impaired 4B12 binding to these modified AAT variants was assessed by ELISA and gel shift assays, and three critical positions were identified that are involved in 4B12 binding. Characterisation of the binding site and the definition of a binding pocket will form the basis for subsequent screening of small molecules with similar activity that could be further developed into drugs.

Keywords: Epitope mapping, protein aggregation, misfolding, serpinopathies.

SUN-265
Production of IL-33 and Galectin-3 by primary glial cells in response to myelin basic protein charge isomers

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Myelin basic protein (MBP) is one of the candidate autoantigens of the human inflammatory demyelinating disease – multiple sclerosis (MS), which is characterized by the active degradation of the myelin sheath. By a wealth of post-translational modifications MBP is presented as different charge isomers. The charge effects modulate MBP functions and may play an important role in pathogenesis of MS. The functions of IL-33 and its producing cells in the CNS are still uncertain.

In this study, we investigate the production of IL-33 and Galectin-3 in response of MBP Charge isomers by primary glial cells. We have found that primary astroglia release IL-33 in response to myelin basic protein charge isomers. The continuous exposure (12, 24, 72 h) of primary glial cells to 0.5 mM MBP C8 (the most modified, citrullinated form) and C1 isomers (the most cationic form) differently induce the release of IL-33 protein.

Besides we have shown that MBP charge isomers induce the secretion Galectin-3 from primary glia. Remarkable effects were seen for C1 isomer. As galectin-3 acts as a specific binding partner of activated k-Ras and that this interaction promotes strong k-Ras activation, we have investigated k-Ras activation in the lysate of the same cells. C1 isomer effect on the Galectin-3 secretion was correlated with the elevation of cytoplasmic k-Ras content. Our results suggest that myelin basic protein charge isomers have immunomodulatory properties and besides effects on Galectin-3 and IL-33 production, can change intracellular signaling through k-Ras translocation.

Keywords: IL-33, inflammation, myelin basic protein.

SUN-266
Prognostic value of IFN-γ, IL-6 and TNF-α in the radiation response of patients diagnosed with local advanced nonsmall cell lung canser and glioblastoma multiforme

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Objective: To investigate the effect of radiotherapy (RT) on plasma levels of Interferon gamma (IFN-γ), Interleukin 6 (IL-6) ve tumor necrosis factor alpha (TNF-α) which are critical for the clinical radiation response of patients with local advanced non-small cell lung cancer (NSCLC) and Glioblastoma multiforme (GBM).
Material and Methods: This study was performed 20 patients with NSCLC, 10 patients with GBM, all of whom was treated with RT and 30 healthy controls. Heparinised blood samples were taken from the control group for once and from the patients for twice before RT and after the completion of RT.Circulating cytokine levels were measured in patients.IFN-γ, IL-6 and TNF-α plasma levels were measured by ELISA prosedures.Post-RT and pre-RT levels compared.
Results: Post-RT TNF-α levels in NSCLC and IFN-γ levels in GBM were significantly lower compared to the pre-RT levels. Statistical analysis did not show any significant difference in IL-6 levels between post-RT and pre-RT. But pre-RT IL-6 levels in GBM, post-RT IL-6 levels in NSCLC were significantly higher compared with control group.
Conclusion: We observed that TNF-α levels in NSCLC and IFN-γ levels in GBM associated with RT.TNF-α levels in NSCLC and IFN-γ levels in GBM were significantly decreased after radiation exposure.

One question arising from our results is why we did not observe the same changes of cytokine for patients with GBM and NSCLC.The total volume of irradiated tissue usually is assumed to have an influence on the development of tissue injury. In our study, IL-6 levels were higher in post-RT NSCLC group versus the post-RT GBM group.

Interleukin-6 expression is linked to irradiation and radiation resistance, as demonstrated by in vitro and in vivo experiments. Interleukin-6-silencing vectors induces more tumor inhibition and DNA damage after irradiation. IL-6 can be important in determining the radiation response of tumor cells. Irradiation-induced IL-6 can be responsible for tumor regrowth. Therefore, treatment with concurrent IL-6 inhibition could be a potential therapeutic strategy for sensitizing nonsmall cell lung tumors to irradiation in the clinic.

Keywords: Cancer, Cytokines, Radiotherapy.

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SUN-267

Pro-inflammatory cytokines TNF-α, IL-6 and IL-1β exhibit differential effects on the expression and transcriptional activity of HIFs in human liver cancer cells

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During inflammation, the tissue microenvironment is characterized by high levels of inflammatory cytokines and chemokines, but also by decreased levels of O_2. Adaptive responses to this hypoxic microenvironment are mediated by the family of Hypoxia Inducible Factors (HIFs). It has been found that HIFs mediate the growth of tumors that are initiated by inflammation. Moreover, it has been proposed that HIFs, in addition to hypoxia, are also induced by inflammatory mediators. A complicated relationship exists between chronic inflammation and cancer, since inflammatory cytokines that induce chronic inflammation also play significant roles in oncogenesis. However, the precise mechanisms that mediate activation of HIF1 and HIF2 and the signaling pathways initiated by the inflammatory cytokines are elucidated neither under normoxia nor hypoxia. Here, we investigate the expression and transcriptional activity of HIF-1 and HIF-2 in response to the pro-inflammatory cytokines TNF-α, IL-6 and IL-1β in a human hepatoma cell line (Huh7) expressing both HIF-α isoforms. The expression of the hypoxia target genes PGK and EPO as well as plasmids expressing *PGK* or *SOD2* promoter constructs were used to specifically monitor HIF-1 or HIF-2 transcriptional activity, respectively. Our results show that HIF-2α protein levels are not affected by any of the pro-inflammatory cytokines tested, while HIF-1α protein levels are decreased by TNF-α and increased by IL-6 or IL-1β under hypoxia. While no effect on the HIF-1 transcriptional activity could be observed when cells were treated with TNF-α, IL-6 or IL-1β, HIF-2 transcriptional activity was significantly reduced only by TNF-α under hypoxia. In support of this result, we also observed that TNF-α reduced both the mRNA levels of EPO, a HIF-2 specific target gene, and EPO secretion under hypoxia. Our findings show that the expression and transcriptional activity of HIFs are differentially modulated by the pro-inflammatory cytokines TNF-α, IL-6 and IL-1β in human liver cancer cells. We are currently investigating the underlying molecular mechanisms, clarification of which will improve our knowledge on carcinogenesis and contribute to better therapeutic strategies.

The project '3129 Pro-inflammatory cytokines TNF-α, IL-6 and IL-1β exhibit differential effects on the expression and transcriptional activity of HIFs in human liver cancer cells'. Is implemented under the 'ARISTEIA II' Action of the 'OPERATIONAL PROGRAMME EDUCATION AND LIFELONG LEARNING' and is co-funded by the European Social Fund (ESF) and National Resourses.

Keywords: HIFs, Hypoxia, Inflammation.

SUN-268

Protease inhibitors and lectins from mushrooms accentuate the plasticity of the β-trefoil fold

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Mushrooms are a source of proteins that show unique characteristics and are exclusive to basidiomycetes (1,2). We have characterized several protease inhibitors and lectins with the β-trefoil fold that affords them resistance to proteolytic degradation and tolerance to extreme pH and high temperatures (3). Two families of cysteine protease inhibitors, mycocypins, including clitocypin from *Clitocybe nebularis* (family I48) and macrocypins from *Macrolepiota procera* (family I85), displayed a distinct mechanism of cysteine protease inhibition, and showed inhibitory profiles differing from those of protease inhibitors from other sources. Two representatives of trypsin specific protease inhibitors (family I66), cnispin from *C. nebularis* and cospin from *Coprinopsis cinerea* (4), further showed that different loops of the β-trefoil fold can be recruited for inhibition of the same protease and the same loop can be recruited for inhibition of different proteases. Antiproliferative activity specific to human leukemic T cells was demonstrated for a *N,N'*-diacetyllactoseamine (GalNAcβ1-4GlcNAc)-specific lectin, CNL, from *C. nebularis* (5). Another ricin B-like lectin that specifically binds *N*-acetyllactoseamine (Galβ1-4GlcNAc), MpL, was isolated and thoroughly characterized from *M. procera* fruiting bodies. These protease inhibitors and lectins were suggested to have a defensive role in basidiomycetes, protecting spore-bearing fruiting bodies against parasites and predators that cause tissue damage, such as insect larvae and nematodes. In addition to a stable molecular scaffold, specific features of defensive β-trefoil proteins include lack of endogenous targets, cytoplasmic localization and genetic heterogeneity. These features can also be exploited for numerous potential applications of these proteins in biomedicine, biotechnology and agriculture. They could find use in drug development and design as well as in research leading to understanding of the role of individual proteases or glycoreceptors in cancer, neurodegenerative, cardiovascular and autoimmune diseases.

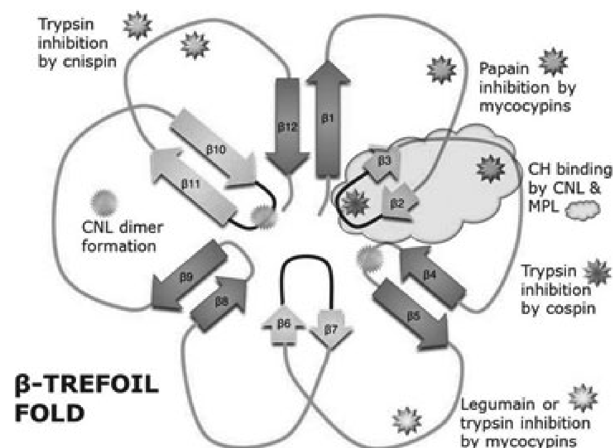

Fig. 1.

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Keywords: immunomodulation, lectin, protease inhibitor.

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SUN-269
Protective effect of lidocaine against glycocalyx damage secondary to lung ischemia reperfusion

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Background: Increased vascular permeability is a characteristic feature of ischemia reperfusion injury (IRI) that has been ascribed to a malfunction of endothelial cells. Recently it has become evident that the endothelial glycocalyx (EGX) is of considerable importance concerning various aspects of vascular physiology. It is involved in inflammatory and immune reactions and it mediates the release of vascular regulatory agents such as nitric oxide. Another important biological property of the EGX is the ability to create a barrier between the endothelium and the blood cells to prevent cell adhesion. The degradation of the EGX is associated with increased circulating levels of the major EGX components heparan sulfate (HS) and syndecan-1 (Synd-1). Lidocaine is a commonly used local anesthetic agent which has also been found to possess anti-inflammatory activity in several tissues including lung. However, the influence of lidocaine on glycocalyx structure has not been investigated.
Aim: To investigate a possible protective effect of lidocaine on lung glycocalyx injury secondary to IRI.
Animals and Methods: 2 groups (control and lidocaine) of 6 large-white pigs were submitted to left lung auto-transplant. Both groups received the same anesthetic induction (fentanyl 3 μg/kg, propofol 3 mg/kg, atracurium 0.5 mg/kg). In addition animals of lidocaine group received a continuous IV of lidocaine 1.5 mg/kg/h during surgery. Blood samples were taken in four different moments, 1) pre-pneumonectomy (PPn) (5 min before pulmonary artery clamp), 2) pre-reperfusion (PRp) (5 min before reperfusion), 3) 30 min post-reperfusion (PR30) and 4) 60 min post-reperfusion (PR60), in order to measure the levels of glycocalyx markers Synd-1, HS and Cathepsin B. Intercellular adhesion molecule-1 (ICAM-1) was also measured.
Results: Levels of Synd-1 were markedly higher ($p < 0.05$) at PR30 compared to PPn and PRp values. The increase was even higher at PR60. IRI also increased HS and ICAM-1 concentrations in plasma ($p < 0.05$). On the contrary, decreased syndecan-1 levels were observed in the lung ($p < 0.05$) after reperfusion. All these effects were partially blocked by lidocaine.
Conclusions: Our findings demonstrate the contribution of the endothelial glycocalyx to the lung IRI. Lidocaine prevented the deleterious effect on glycocalyx markers suggesting that lidocaine administration may aid to protect the glycocalyx.
Keywords: glycocalyx, ischemia reperfusion injury, lung.

SUN-270
Protective effects of (R)-(+)-α-lipoic acid against MPP+-stimulated microglia cells and toxicity in dopaminergic SH-SY5Y cells through PI3K-Akt/GSK-3β pathway

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Microglia are primary immune cells of the brain whose uncontrolled activation can lead to aberrant production of inflammatory factors that contribute to neuronal injury and progressive degeneration of neurons. Numerous empirical evidences have supported the use of LA as anti-inflammatory nutraceutical since it evokes an exclusive array of cellular and molecular mechanisms, albeit its conclusive molecular mechanisms in Parkinson's disease are still not completely understood. We investigated the neuroprotective effects of (R)-(+)-α-Lipoic Acid(R-LA) on 1-methyl-4-phenylpyridinium (MPP^+)-induced activation of microglia BV-2 and neurotoxicity on dopaminergic SH-SY5Y cells. We then investigated the protective effects of R-LA in co-cultured BV-2 and dopaminergic SH-SY5Y cells stimulated with MPP^+. R-LA mitigated MPP^+-induced cell death in both dopaminergic SH-SY5Y and activated BV-2 cells. Pretreatment with R-LA significantly attenuated MPP^+-induced overexpression of inducible nitric oxide synthase (iNOS) and subsequent production of nitric oxide in BV-2 cells. R-LA treatment induced the activation of PI3K-Akt which then reduced the cyclooxygenase (COX)-2 expression, inactivated GSK-3β(Ser9) and supressed the p65NF-κβ translocation in BV-2 cells. Following this, R-LA aggrandized the level of anti-inflammatory cytokine IL-10 which concomitant diminution of pro-inflammatory cytokines TNF-α, IL-Iβ, IL-2 and IL-6. Furthermore, R-LA independently decreased MPP^+-induced oxidative stress in SH-SY5Y by reducing intracellular ROS level, aggrandizing the GSH level and the expression of heme oxygenase-1. Following co-culture of BV-2 and SH-SY5Y cells, neuronal cell death was inhibited where nuclear condensation and apoptotic bodies was abated with intact mitochondrial membrane potential which led to the suppression of caspase-dependent apoptosis as compared to MPP^+-treated co-cultured cells. Moreover, the addition of lithium chloride and triciribine hydrate (API-2) resulted in the prolonged BV-2 activation, elevation of pro-inflammatory cytokines which led to neuronal cell death in SH-SY5Y cells. This further confirmed R-LA protected the BV-2 and dopaminergic SH-SY5Y against MPP^+ through PI3K-Akt/GSK-3β pathway. In conclusion, neuroprotective effects of R-LA against MPP^+ are mediated, at least in part, through suppression of neuroinflammation and oxidative stress-associated factors in BV-2 cells. This further justifies the rational use of R-LA as nutraceutical for neurodegenerative diseases.
Keywords: neurodegenerative diseases, Neuroinflammation, Neuronal cell death.

SUN-271
Protective effects of 2-benzoxazolinone derivatives with anti-oxidant and anti-inflammatory activities on experimental acute pancreatitis in rats

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New derivatives designed and synthesized by molecular hybridization of benzoxazolinone, triazole and thiadiazole have been previously reported as novel compounds with potent anti-inflammatory and analgesic activity and with no ulserogenic risk (1). Acute pancreatitis is a disease with high morbidity and mortality, but its complete mechanism has not been established. Present study was designed to evaluate the protective effect of the two new anti-inflammatory agents, substituted hydrazide (**4**) and 4-methoxybenzylidenehydrazine (**8e**), on experimental acute pancreatitis in a rat model of cerulein-induced acute pancreatitis.

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Male Wistar rats were randomly divided into control group, pancreatitis group and study groups. In the control group, normal saline was replaced by cerulein. Pancreatitis was induced using four i.p. injections of cerulein (50 μg/kg) in 1 ml of saline at 1-h intervals within 4 h. In the study groups, rats were pretreated orally with compounds 4 and 8e (100 mg/kg in 0.5% sodium carboxymethyl cellulose) 12 hours before cerulein induction. Twelve hours after cerulein or saline injections, the animals were killed by decapitation. Blood samples were collected to analyze serum levels of LDH, transaminases, amylase, lipase, and proinflammatory cytokines (TNF-α, IL-1ß and IL-6). Reduced and oxidized GSH, lipid peroxidation level and myeloperoxidase activity were determined in pacreatic tissues. Tissue samples were also examined histologically. Acute pancreatitis markedly increased serum LDH, AST, ALT, amylase and lipase activities as well as serum TNF-a, IL-1ß, and IL-6 levels. Acute pancreatitis caused a significant decrease in tissue reduced GSH levels, increase in oxidized GSH levels while pancreatic MDA level and MPO activity were also increased. On the other hand, pretreatment with the novel compounds reserved all these biochemical parameters as well as histopathologic alterations that were induced by cerulein. According to the results, newly synthesized compounds protected the pancreatic tissues from inflammation and oxidative damage induced by cerulein, and this effect possibly involves the inhibition of neutrophil infiltration and lipid peroxidation. However, more detailed in vivo studies are needed to clarify whether these prodrugs could provide a therapy of pancreatitis.

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Keywords: acute pancreatitis, anti-inflammatory activity, benzoxazolinone.

SUN-272
Protective effects of vitamin U on amiodarone-induced liver injury of rats

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Amiodarone is a currently used drug for the treatment acute life-threatening arrhythmias and for chronic suppression of arrhythmias. Beside beneficial effects, it has several side effects in organs such as lung, thyroid, kidney, liver, eye, brain and skin. S-methionine sulphonium, which is a derivative of the methionine amino acid, defined mostly as vitamin U (Vit U) and antiulcer properties, antiinflammatory action, wound-healing and cell protective effects of Vit U have been demonstrated. The present study was undertaken to determine whether the vitamin U exhibits preventive effects on amiodarone-induced liver toxicity. Male Sprague-Dawley rats were randomly divided into four groups. Group I; control animals receiving corn oil. Group II; control animals receiving Vit U (50 mg/kg) for 7 days orally. Group III; animals receiving 100 mg/kg amiodarone for 7 days orally. Group IV; animals receiving Vit U orally for 7 days (in the same dose and time) 1 h prior to the administration of amiodarone. On the 8th day, all the animals were sacrificed. Liver tissue was taken from animals for histopathological and biochemical studies. Pretreatment with Vit U particularly regressed mild degenerative morphological changes such as picnotic nucleus in hepatocytes, sinusoidal dilatation, hyperemia, dark eosinophilic cells, rupturings in endothelium of central vein seen in amiodarone treated rats. On the other hand, liver aspartate transaminase, alanine transaminase and alkaline phosphatase activities were increased, catalase, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase activities were decreased in amiodarone group. Administration of Vit U reversed these effects in amiodarone group. In conclusion, pretreatment with Vit U may decreases liver injury induced with amiodarone treatment.

Keywords: amiodarone, Arrhythmia, vitamin U.

SUN-273
Protein phosphatase 2A in lipopolysaccharide-induced cyclooxygenase-2 expression in murine lymphatic endothelial cells

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The lymphatic endothelium plays a crucial role not only in maintaining interstitial fluid balance, but also in modulating immune response. Many lines of evidence demonstrated that alterations in lymphatic functions are associated with edema and inflammation. However, the underlying mechanisms by which lymphatic endothelium responses to inflammatory stimuli remain poorly understood. In addition, cyclooxygenase (COX)-2 plays a major role in inflammatory processes, and its expression has been linked with several diseases associated with inflammation. We thus interested to investigate the signaling cascade involved in inducing COX-2 expression in murine lymphatic endothelial cells (LECs) exposed to proinflammatory stimuli, lipopolysaccharide (LPS). The COX-2 mRNA and protein levels were increased in LECs exposed to LPS. LPS time-dependently induced phosphorylation of JNK1/2 and p38MAPK. Treatment of cells with a p38MAPK inhibitor (p38MAPK inhibitor III) and a JNK signaling inhibitor (JNK inhibitor II) significantly inhibited LPS-induced COX-2 expression in LECs. In addition, okadaic acid, a protein phosphatase 2A (PP2A) inhibitor, abrogated LPS's effects on JNK1/2 and p38MAPK phosphorylation. LPS exposure also led to increases in COX-2 promoter-luciferase activity as well as C/EBPβ- and κB-luciferase activities. COX-2 promoter luciferase activity induced by LPS was attenuated in cells transfected with the COX-2 reporter construct possessing the C/EBP- or κB-binding site mutation. C/EBPβ- and κB-luciferase activities were suppressed by okadaic acid despite presence of LPS. LPS-increased p65 and C/EBPβ binding to the COX-2 promoter region was attenuated in the presence of okadaic acid. LPS also caused an increase in PP2A phosphatase activity in LECs. In conclusion, we demonstrated in this study that PP2A may contribute to LPS-induced p38MAPK and JNK1/2 activation, leading to increase in p65 and C/EBPβ binding to the *cox-2* promoter region and subsequent COX-2 up-regulation in LECs.

Keywords: lymphatic endothelial cells, cyclooxygenase-2 (COX-2), protein phosphatase 2A (PP2A).

SUN-274
P-selectin functionalized lipid nanoparticles specifficaly target activated endothelium in acute and chronic inflammation mice models

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Background: Inflammation is a common occurrence associated, involved and/or responsible for many pathologies. P-selectin is a

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cell adhesion molecule highly expressed by endothelium in inflammation, and therefore a potential target for nanotherapy. We hypothesize that functionalization of nanoparticles with P-selectin high affinity peptide will increase their binding to activated endothelium in inflamed tissues. Thus, the aim of this study was to develop nanoparticles loaded with curcumin (Cm, a hydrophobic anti-inflammatory polyphenol) that are able to target the inflammatory process in both, acute and chronic inflammation.

Materials and Methods: Curcumin-loaded lipid nanoparticles (CmLN) functionalized with a P-selectin affinity peptide (P-sel_CmLN) have been developed. The CmLN were characterized for size and structure (by DLS and TEM), entrapment efficiency and curcumin release capacity (by HPLC). In vitro studies using human endothelial cells (HEC) were performed to investigate the cytotoxicity of CmLN (by MTT), their cell binding and internalization (by flow cytometry and confocal microscopy), the signaling pathways involved (Western Blot) and the effect on monocytes adhesion to HEC (fluorescence microscopy). In vivo studies were performed using IVIS Caliper live imaging system on an acute [C57BL mice injected with lipopolysaccharide (LPS) for 5 h] and chronic inflammation mice models [atherosclerotic ApoE-/- mice fed a high cholesterol diet for 4 months].

Results: In vitro studies on HEC revealed that CmLN have: (1) low cytotoxicity; (2) anti-inflammatory effect by down-regulating ERK1/2 and p38 MAPK signaling pathways and (3) impaired adhesion of monocytes to activated endothelial cells. Functionalization of CmLN with P-sel peptide induced (4) an increased binding to activated HEC. In vivo studies (after 1 h of nanoparticles administration) revealed (6) an increased accumulation of Psel_CmLN in the lungs of LPS-injected C57BL mice compared to controls (i.e. non-targeted CmLN and PBS-injected C57BL mice) and (7) an increased binding of Psel-CmLN to the aorta (in atherosclerotic plaque-prone areas) of ApoE-/- mice, compared to non-targeted CmLN binding.

Conclusion: P-selectin coupled CmLN efficiently bind to activated endothelial cells in vitro and in vivo in acute and chronic experimental inflammation. Therefore, P-selectin exposed on activated HEC surface represent a reliable target for delivery of nanoparticles-carrying drugs.

Acknowledgements: This work was supported by Romanian Academy and UEFISCDI, *contract no. 4_001* of EuroNanoMed, PNII-ID-PCCE-2011-2-0028 and PN-II-ID-PCE-2011-3-0928 and by the CARDIOPRO Project ID: 143.

Keywords: Inflammation, Nanoparticles, P-selectin.

SUN-275
Purification and characterization of a fibrinolytic metalloprotease from exiguobacterium indicum isolated from tapioca flour waste

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Cardiovascular diseases as the leading cause of death in the world can be triggered by thrombosis. Discovery of novel fibrinolytic enzymes as potential therapeutic agents for thrombosis becomes the focus of research investigation. Tapioca flour waste is a potential source to isolate protease-producing microbe because the use of cassava, which contains >1% of crude proteins. In addition, microbes that isolated from waste may produce novel enzymes since they have to survive in extreme conditions during the process of tapioca flour production. The aim of this study was to purify and characterize a fibrinolytic enzyme from selected tropical microbe isolated from tapioca flour waste. Isolate Tap 3.1 was identified through biochemical and molecular screening as *Exiguobacterium indicum*. Crude enzyme was produced by *E. indicum*. The enzyme was purified through several steps of purification, including ammonium sulphate precipitation, dialysis, concentration, and ion exchange using FPLC. Fibrinolytic activity was characterized by fibrin zymography. The apparent molecular mass of the purified enzyme was estimated to be 66.5 kDa by SDS-PAGE. The precipitation with 50% ammonium sulphate resulted in a 2.6-fold purification of the enzyme. The optimal temperature for fibrinolytic enzyme was 37°C, and its activity was still observable at extreme temperatures (4 and 70°C). Interestingly, the optimal pH for fibrinolytic enzyme was reached at broad ranges of pH (4–10). The enzyme effectively hydrolyzed fibrinogen as well as gelatin, elastin, and casein, suggesting its catalytic specificity in broad protein substrates. Fibrinolytic activity was potently inhibited by EDTA, indicating that the enzyme is a metalloprotease. In summary, a fibrinolytic enzyme from *E. indicum* isolated from tapioca flour waste may have potential to be developed as an alternative therapeutic agent for thrombosis.

Keywords: *Exiguobacterium indicum*, Fibrinolytic enzyme, purification.

SUN-276
Raft-located Lyn kinase serves as a negative regulator of TLR4 signlaing triggered by LPS

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Toll-like receptor 4 (TLR4) is localized in the plasma membrane of macrophages and is activated by lipopolysaccharide (LPS) of Gram-negative bacteria. Activation of TLR4 involves a sequential engagement of serum LPS-binding protein (LBP), plasma membrane CD14 and the TLR4/MD2 signaling complex. CD14 is a GPI-anchored protein enriched in sphingolipid- and cholesterol-rich microdomains of the plasma membrane, rafts, and associates with Lyn kinase, a member of the Src tyrosine kinase family in macrophages. As protein tyrosine phosphorylation controls pro-inflammatory signaling of TLR4, we undertook studies to address an engagement of Lyn kinase in these events. In RAW264 macrophage-like cells LPS induced activation of Lyn in a dose- and time-dependent manner, as indicated by phosphorylation of tyrosine residue 396 of the catalytic domain of Lyn and simultaneous dephosphorylation of inhibitory tyrosine residue 507. Activated Lyn was enriched in the raft fraction of the plasma membrane and the integrity of rafts was crucial for the enzyme activity. Inhibition of protein palmitoylation under the influence of 2-bromoplmitic acid also reduced the amounts and activity of Lyn kinase found in the raft fraction. These data indicate that raft-anchored Lyn is involved in LPS-induced signaling pathways. We undertook studies to reveal the role of Lyn in two signaling pathways of TLR4 which depend on the engagement of either MyD88 or TRIF adaptor proteins. For this, we obtained a series of Lyn constructs fused with GFP at the C-terminus: wild type Lyn, constitutively active Y507F, so-called up/up Lyn, kinase-dead K275R Lyn and Lyn with point mutations in either SH2 (R155A) or SH3 (W98A) domains. The proteins were overexpressed in RAW264 macrophage-like cells with transfection efficiency reaching 40% of cell population. The Lyn-GFP construct were anchored in the plasma membrane as shown by confocal microscopy. Overexpression of the wild type and constitutively active forms of Lyn inhibited NFκB activation, reduced TNFα and MIP-2 production and nearly abolished RANTES generation induced by 100 ng/ml LPS, thus affected

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both signaling pathways of TLR4. The wild-type and constitutively active Lyn became enriched in the raft fraction under the influence of LPS. In contrast, an expression of kinase-dead Lyn (K275R) increased TRIF-dependent production of RANTES by 50%, moderately up-regulated NFκB activation and increased MyD88-dependent production of TNFα and MIP-2 both at mRNA and protein levels. Similar effects were exerted by overexpression of R155A and W98A Lyn kinase mutated in its SH2 and SH3 domains, respectively. Taken together, our results indicate that raft-anchored Lyn functions as a negative regulator of TLR4 signaling pathway in macrophages.

Keywords: Lyn kinase, Toll-like receptor4 (TLR4), lipopolysaccharide (LPS).

SUN-277
Recognition of tumor cells by Dectin-1 orchestrates innate immune cells for anti-tumor responses

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The eradication of cancer cells requires communication between cells which constitute the complex immune system. Natural killer (NK) cells are essential tumor-killing effector cells of the innate arm of the immune system; however, little is known about whether or how other immune cells recognize tumor cells to assist NK cells. In addition, although signal-transducing pattern recognition receptors are known to play important roles in innate immune responses against invading pathogens through recognition of pathogen-associated molecular patterns (PAMPs), it is still enigmatic whether and how these receptors contribute to anti-tumor immune responses. In this study, we show that the innate immune pattern recognition receptor Dectin-1 expressed on dendritic cells (DCs) and macrophages is critical to NK-mediated killing of tumor cells. We also show that tumor cell-mediated Dectin-1 signaling is instigated by the receptor recognition of N-glycan structures on tumor cells, which we term tumor-associated molecular patterns (TAMPs). As such, the TAMP-Dectin-1 signaling causes activation of the Interferon Regulatory Factor 5 (IRF5) transcription factor and subsequent induction of genes required for the full-blown killing activity of NK cells. The importance of these events is underscored by the observation of massive tumor metastasis in mice genetically deficient in either Dectin-1 or IRF5. Thus, these results reveal a hitherto unrecognized facet of pattern recognition receptors in the orchestration of anti-tumor innate immune responses; recognition of TAMPs. This study is the first demonstration that an innate immune pattern recognition receptor potentiates anti-tumor immune responses, offering new insight into anti-tumor activity of the innate immune system with implications for anti-tumor immunotherapy.

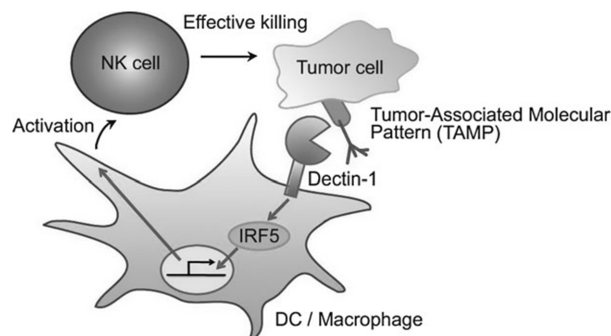

Fig. 1.

Keywords: innate immunity, pattern recognition receptor, tumor immunity.

SUN-278
Recombinant TMV-based virus carrying hydrophobic hemagglutinin influenza fusion peptide moves via plant vascular system and forms stable particles

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Influenza is one of the world's most common and deadly viruses. The inherent disadvantages associated with the preparation of conventional vaccines suggest discovering of the universal Influenza vaccine. Given the importance of neutralizing antibodies against hemagglutinin (HA) in controlling Influenza infection, the conserved regions in the HA proteins have received great attention in recent years. The HA2 N-terminal amino acids (aa) are conserved among all Influenza subtypes. This highly hydrophobic peptide serves for fusion of viral and endosomal membranes inside an infected cell. Recently it was shown that monoclonal antibodies against this so-called fusion peptide (fp) are capable of reacting with all types of Influenza HA proteins; therefore, it might be a perspective candidate for production of the broad-spectrum vaccine. Lately a new approach for plant super-expression of another conservative Influenza epitope M2e has been developed in our research group. Chimeric TMV-M2e particles based on *Tobacco mosaic virus* (TMV-U1, wt) genome proved high-level protective efficacy against lethal homologous and heterologous challenge (Petukhova *et al*., 2013, *Curr. Pharm. Des*., 19, 5587–5600). Similarly, we constructed TMV-fp vector designed for *Agrobacterium*-mediated delivery into the plant cell nucleus. TMV coat protein (CP) contained 14 aa of fusion peptide (GLFGAIAGFIEGGW). The codon usage of foreign antigen was optimized for expression in plants. Infection of *Nicotiana benthamiana* with TMV-fp vector caused systemic symptoms on 11th day post inoculation differing from symptoms caused by TMV-wt and TMV-M2e viruses. We observed severe necrosis and deformation of upper leaves and necrotic fracture of the stem. Western blot analysis of plant extracts revealed the protein (22–23 kDa) that coincided with the predicted molecular weight of CP-fp, reacted with antiserum (AS) against TMV CP and was not found in negative control. One may conclude that recombinant virus TMV-fp is able to replicate and systemically transport in *N. bentamiana* plants. Following the purification, we found two proteins in viral preparation corresponding to CP-fp and CP without epitope (approximate ratio 30:70). Electron microscopy of TMV-fp preparation represented the rod-shaped particles similar in morphology to TMV-wt virions. RT-PCR analysis of total RNA fraction from infected plants and genomic RNA from purified viral preparation showed the presence of fp-sequence in the CP gene. Direct sequencing of PCR product did not reveal any mutation thus confirming genetic stability of TMV-fp vector. Thereby, this is the first report of hydrophobic Influenza fusion peptide expression using plant viral particles expected as a carrier.

Keywords: Influenza virus, hemagglutinin, fusion peptide, Tobacco mosaic virus, recombinant virus, systemic movement, plant.

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SUN-280

Relation of protein oxidation parameters in patients with Behçet's disease

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Background: Behçet's disease (BD) is a chronic inflammatory vasculitis characterized by endothelial dysfunction, elevated reactive oxygen species (ROS), and neutrophil hyperfunction production including acute attacks and remission periods. Ischemia modified albumin (IMA), advanced oxidation protein products (AOPP), prooxidants-antioxidants balance (PAB), and ferric reducing antioxidant power (FRAP) were evaluated in regard to their role in the pathogenesis of BD as well as their relation to clinical presentation, uveitis attacks and remission periods, and healthy volunteers.
Methods: The study included 28 BD cases and 27 healthy volunteers as the control group. Blood samples were taken twice from each patient; first during an attack and second about three months after an attack, during remission period.
Results: AOPP, IMA and PAB levels were significantly increased in active periods of patients with BD compared with healthy control and remission periods of patients with BD ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$, respectively). FRABP levels were found to be lower in active periods of patients with than healthy controls and remission periods of patients with BD ($p < 0.001$, $p < 0.05$, respectively). The AOPP levels were negatively correlated with the levels of FRAB in patients ($r = -0.468$, $p = 0.012$; $r = -0.394$, $p = 0.038$, respectively). The PAB levels were positively correlated with the levels of CRP in patients ($r = -0.606$, $p = 0.001$).
Conclusions: Our results show that these parameters play a major role in the inflammatory reactions observed in BD. Increased levels of IMA and PAB are likely to be a result of inflammation-induced oxidative stress and hence its potential significance as a new marker of oxidative stress in BD.
Keywords: Behçet's disease, AOPP, IMA.

SUN-281

Relationship between C-reactive protein, glucose, HbA1c and age

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Objective: After understanding the importance of inflammation in the pathogenesis of Cardiovascular diseases (CVD), CRP usage has been increased for the determination of cardiovascular risk. As an inflammation marker, the relationship between CRP levels and CVD risk and age has already been shown. In this study our purpose is to investigate the relationships between CRP, glucose, Haemoglobin A_{1c} (HbA1c) and age.
Materials and Methods: CRP, HbA1c, glucose levels and ages were analysed retrospectively in subjects admitted to the laboratory in 2013. Correlations were examined between CRP, HbA1c, glucose levels and ages. In the present study no patient had CRP value higher than 10 mg/dL.
Results: CRP, HbA1c, glucose levels and ages of 53 females, 67 males, overall 120 cases were 3.26 ± 2.59 mg/dL (mean $\pm$ SEM), 7.07 ± 1.818, 153.18 ± 83.54 and 55.66 ± 15.33 respectively. Positive correlation was found between CRP and HbA1c in whole group ($r = 0.250$, $p < 0.05$). Cases were divided into two groups, according to their HbA1c values (Group A: HbA1c ≥ 6.5, Group B: HbA1c < 6.5). Glucose and HbA1c levels were found to be significantly higher in group A ($p < 0.05$). In group B, significant positive correlations were determined between age and HbA1c; and glucose and age ($r = 0.366$, $p < 0.05$ and $r = 0.269$, $p < 0.05$, respectively). In group A, significant negative correlation between age and HbA1c and positive correlation between glucose and HbA1c were determined ($r = -0.337$, $p < 0.05$ and $r = 0.796$, $p < 0.05$, respectively).
Conclusion: In patients with CVD, Diabetes mellitus (DM) leads to functional and structural vascular alterations of the peripheral vasculature which are determined by the control of the disease underlining the relevance of a strict control of the DM to prevent accelerated atherosclerosis. CRP is also associated with macrovascular events in patients with DM. In our study, positive correlation was found between HbA1c and CRP. It showed that a higher level of circulating HbA1c was related to elevate inflammatory markers such as CRP. We conclude CRP levels, add significantly to the prediction of macrovascular events and mortality in individuals with DM who have baseline CVD or risk factors.

In this retrospective study significant negative correlation in Group A and positive correlation in Group B were found between HbA1c and age. HbA_{1c} levels in Group B increase with age. Therefore, see a need for age-specific reference ranges for HbA_{1c} in normal subjects.
Keywords: C-reactive protein, glucose, Haemoglobin A1c.

SUN-282

Resistance mechanisms in silver-citrate nanoparticles treated colon cancer cells

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Despite the significant progress in cancer treatment over the last decades, multidrug resistance (MDR) continues to be the biggest challenge in tumor therapy. The various resistance mechanisms and their combinations represent the adaptation capacity of MDR cancer cells to toxic insults when structurally and functionally unrelated chemotherapeutic agents are applied. Induction of drug efflux pumps, overexpression of P-glycoprotein encoding MDR1 gene, all contribute to the development of a resistant phenotype, since through these membrane proteins cytotoxic drugs are exported from the cells.

Recently, novel nanotechnology-based therapeutic strategies gathered grounds because they target different biochemical pathways and provide the potential to overcome MDR. Silver nanoparticles (AgNPs) are broadly used nanomaterials in medicine due to their unique chemical, physical properties and their outstanding antiviral and antibacterial features. Our recent results indicate that AgNPs induce apoptosis in various cancer cells but their effect on MDR cells is still unknown. The potential application of AgNPs in cancer therapies, as single agents or in combination with targeted and conventional drugs, has raised our interest to investigate their activity on MDR cancer.

In this study we used sodium-citrate coated ~35 nm sized quasi-spherical AgNPs for treating drug-sensitive (Colo 205) and MDR1 overexpressing drug-resistant (Colo 320) colon adenocarcinoma cells. We found that AgNPs killed cancer cells in a concentration dependent manner, however drug resistant Colo 320 cells showed higher viability compared to drug sensitive Colo 205 cells. Cleaved caspase 3 staining indicated apoptosis during AgNP mediated cell death which proved to be more prominent

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in drug sensitive cells. We detected elevated levels of reactive oxygen species in nanoparticle treated MDR cells compared to control suggesting the induction of the mitochondrial apoptosis pathway. Immunofluorescent staining revealed the disappearance of mitotic cells after AgNP treatment, indicating a cell cycle arrest. Examination of mRNA levels of apoptotic and stress markers verified programmed cell death in Colo 205 cells however elevated expression of the antiapoptotic survivin was observed in Colo320 cells.

Our results show that although AgNPs kill both drug resistant and sensitive cells, MDR Colo 320 cells manifest several resistance mechanisms to fight the applied stressor and survive.

Keywords: nanoparticles, inflammation, cell death.

SUN-283
Reversing effect of vitamin U on valproic acid-induced renal damage through its anti-oxidant, anti-inflammatory and anti-fibrotic properties

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Valproic acid (VPA) is extensively used as anti-epileptic drug for decades. However, it has many side effects such as renal damage. It has recently become prominent with its inhibiting effect of histone deacetylases. Several reports have indicated that anti-epileptic drugs affect the oxidant/antioxidant status, immune system and fibrosis, but the mechanism is not clear. Vitamin U (Vit U), S-methyl methionine sulfonium chloride, is a water soluble vitamin. The aim of the present study was to investigate the effect of Vit U on the oxidative stress, inflammation and fibrosis within the context of VPA-induced renal damage.

In this study, female Sprague Dawley rats were randomly divided into four groups. Group I was intact control animals. Group II was control rats given Vit U (50 mg/kg/day, by gavage). Group III was given only VPA (500 mg/kg/day, intraperitonally). Group IV was given VPA+ Vit U. The animals were treated by Vit U one hour prior to treatment with VPA every day. On the 16th day of experiment, animals were sacrificed under anesthesia. Kidney tissue was taken from animals for biochemical and microscopic analysis.

The following results were obtained in Vit U + VPA-treated rats; *i.* The reversing effect of Vitamin U on renal damage was showed by decreased histopathological changes and increased activity of Na^+/K^+-ATPase *ii.* Antioxidant property of Vit U was determined by decreased malondialdehyde level; increased glutathione level and activities of catalase and superoxide dismutase. *iii.* Anti-inflammatory property of Vit U was determined by decreased TNF-α, IL-1β, MCP-1 levels and activities of adenosine deaminase and xanthine oxidase in Vit U+VPA-treated rats. *iv.* Anti-fibrotic effect of Vit U was showed by decreased TGF-β1, Collagen-1 and activity of arginase.

Collectively, these data show that VPA is a promoter of inflammation, oxidative stress, and fibrosis which resulted in renal damage. Vit U can be proposed as a potential candidate for reversing renal damage which arose during the therapeutic usage of VPA.

Keywords: Renal Damage, Valproic acid, Vitamin U.

SUN-284
Role of adipokines in insulin synthesis and secretion

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Introduction: The adipose tissue has become a central player in the pathogenesis of metabolic disease. The aim of this study was to evaluate whether preadipocytes from different depots, could change differently synthesis and secretion of insulin in beta-pancreatic cells.

Methods: We performed isolation, cultivation and differentiation of human preadipocytes using either abdominal subcutaneous or mesenteric adipose tissue obtained from bariatric interventions. Oil Red O staining was used as marker of differentiation. The media from day 0, 4, 7, 10, 15 and 20 was added over PANC-1 cells ($2X10^4$ cells) and the insulin and proinsulin levels were measured by ELISA. We also used a transwell system in which the PANC-1 beta-pancreatic cells grown in the lower well of the 6-well culture plate were co-cultured for 24 hours with differentiated adipocytes (day 0, 4, 7, 10, 15 and 20) in the transwell inserts (with 0.4 μm porous membrane). The leptin, adiponectin, insulin and proinsulin levels in co-cultured PANC-1 cells (media and cell lysates) were determined by ELISA as well. The experiments were done in duplicate and repeated four times.

Results: Intracellular proinsulin level was higher then in the media while insulin was lower in the cell lysates and these was maintained with preadipocytes differentiation. The expresion level of leptin increases steadily with the degree of differentiation only intracelular while levels of adiponectin dit not differed. When the two types of cells were cocultured in the transwell system except for proinsulin all parameter did not differ from those obtained in experiment 1. Adiposse tissue origin does not influence neither synthesis nor secretion of insulin.

Conclusion: Our results suggest that the ratio between pro/anti-inflamatory adipokines during adipocytes differentiation has an important rol in both production and secretion of insulin in beta-pancreatic cells.

Acknowledgements: This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS-UEFISCDI, project number PN-II-ID-PCE-2011-3-0429'. Dr. Daniela Lixandru was supported by the postdoctoral program POSDRU/89/1.5/S/60746, from European Social Fund and Alexandru Petruta by the program POSDRU/107/1.5/S/82514 from European Social Fund.

Keywords: adipo/cytokines, bariatric surgery, diabetes mellitus.

SUN-285
Role of an aspartic acid cluster within the internal cavity of endoplasmic reticulum aminopeptidase 1 in regulating enzyme activity and substrate specificity

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Endoplasmic reticulum aminopeptidase 1 (ERAP1) trims N-terminally extended peptide antigenic precursors to produce mature epitopes that are presented by major histocompatibility complex

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class I molecules. The internal sequence of peptide precursors highly affects the N-terminal trimming rates by ERAP1. Specifically, ERAP1 shows preferences towards peptides that contain positively charged aminoacid residues. In an effort to understand the atomic determinants of this preference we investigated the contribution of an aspartic acid cluster located within the enzyme's extended substrate binding cavity. We produced recombinant ERAP1 in which aspartic acids at positions 406, 435 and 439 are replaced by alanine and we compared enzymatic activity with the wild-type enzyme. The mutant enzyme was found to be more active than the wild-type using model fluorigenic substrates. Arginine scanning using the model antigenic epitope LSIINFEKL revealed that this cluster can affect substrate trimming rates but this was highly dependent on the position of the Arginine residue in the substrate. Surprisingly, the mutant was much more efficient in further trimming the LSIINFEKL epitope to smaller peptides that would not be expected to bind onto MHC class I, suggesting that the aspartate cluster may be important for length selection. This phenomenon was however, sequence-dependent since the length selection properties of ERAP1 versus a series of poly-Glycine peptides remained unaltered.

Keywords: None.

SUN-286
Role of antoxidants on RANKL and OPG production in apoptotic osteocytes

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Osteocytes are the major mechanosensory cells in bone, and their apoptosis due to microdamage is related to increased local bone turnover and resorption observed in various bone diseases. Previously, we showed in a osteocyte-like cell line, MLO-Y4, that the starvation-induced-apoptosis, that mimics the apoptosis due to microdamage, is an redox-regulated process related to JNK activation. The aim of this study was to investigate in starvation-induced-apoptosis in osteocytes the role of antioxidants, such as glutathione (GSH), NAC and lipoic acid (LA), on cytokines, such as the receptor activator kB ligand (RANKL) and osteoprotegerin (OPG), involved in bone remodelling. These factors are produced by osteoblasts and osteocytes, RANKL increases bone resorption, whereas OPG inhibits osteoclastogenesis activating bone formation. These events are maintained in equilibrium by the regulation of RANKL/OPG ratio. The involvement of the redox-regulated kinases, ERK1/2 and JNK, on the production of these cytokines was also studied.

The results show that in MLO-Y4 all antioxidants inhibited RANKL expression and release which increased in apoptotic cells, in particular, RANKL levels returned to the control values after treatment with all antioxidants. Whereas, OPG expression remarkably decreased in apoptotic cells, and only NAC and LA were able to significantly increase the OPG levels. No OPG release has been detected in our experimental conditions. Therefore, RANKL/OPG ratio, measured by using expression values, significantly enhanced in apoptotic cells as compared with controls, whereas the ratio decreased in cells treated with the antioxidans. The results show that JNK and ERK1/2 were activated in apoptotic osteocytes but all antioxidants inhibited their activation. Experiments performed with SP600125 and U0126, specific inhibitors of JNK and ERK1/2 respectively, show that the activation of both kinases up-regulated RANKL expression and release. On the contrary, only JNK activation down-regulated OPG expression. However, RANKL/OPG ratio decreased in SP600125 or U0126 treated apoptotic cells indicating the involvement of both kinases on the ratio regulation.

These results suggest that the osteocytes, JNK and ERK1/2 may be potential therapeutic targets for treatments of bone diseases related to oxidative stress. It should be also interesting to evaluate the association of these antioxidants and/or JNK and ERK1/2 antibodies with anti-resorptive drugs to enhance the therapeutic efficacy and/or to decrease toxic effects.

Keywords: antioxidant activity, Osteocytes, redox homeostasis.

SUN-287
Role of EGF and TGF beta in modulating the expression of the stearoyl CoA desaturase 1 in association with metastatic breast cancer development

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Despite significant scientific progress in prevention, detection and treatment of breast cancer, it remains the leading cause of women cancer deaths in the world. The percentage of mortality significantly increases when the cancerous cells acquire a metastatic potential. We previously showed that high stearoyl CoA desaturase 1 (SCD1) expression, a key lipogenic enzyme, was associated with increased metastatic potential of breast cancer cells. SCD1 desaturated long chain fatty acids forming monounsaturated fatty acids. EGF (Epidermal Growth Factor) and TGFβ (Transforming Growth Factor -beta) are also associated with the acquisition of a metastatic potential. In breast cancer cells, EGF activates JAK / STAT, PI3K, Src kinase, PLCγ, ERK dependent signaling pathways while pathways activated by TGFβ involved the Smad proteins.

Project's Objectives: The aim of our study was to analyze the role of EGF and TGFβ in SCD1 expression and to characterize the signaling pathways induced by the two hormones using the MDA-MB 231 breast cancer cells.

Methodology: MDA-MB 231 cells were treated with or without EGF (10 ng/ml) or TGFβ (0.2 nM) and cytosolic proteins were analyzed by Western blotting. The same hormonal treatment was made on cells transfected with a construct containing 3.7 kb of the SCD1 promoter cloned upstream of the luciferase reporter gene. Finally, specific inhibitors of kinases involved in signaling pathways induced by EGF or TGFβ, were added for 1 h before addition of the hormones. 24 h latter, the effect of each inhibitor on SCD1 expression was evaluated by RT -PCR.

Results and Conclusion: Our results showed that both EGF and TGFb increased SCD1 protein expression in MDA-MB-231 cells. This hormonal effect is probably due to a regulation at the transcriptional level as both hormones increased SCD1 promoter activity by more than 3 fold. In addition, the use of specific inhibitors allowed us to determine the pathways involved in SCD1 regulation by both EGF and TGFb. Our study describes for the first time a new link between EGF and TGFb with SCD1 expression associating lipid metabolism with metastasis development in breast cancer cells.

Keywords: Breast cancer, EGF and TGF beta, Stearoyl CoA desaturase 1 (SCD1).

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SUN-288
Role of EGF and TGF beta in modulating the expression of the stearoyl CoA desaturase 1 in association with metastatic breast cancer development

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Keywords: Breast cancer, EGF and TGF beta, Stearoyl CoA desaturase 1 (SCD1).

SUN-289
Role of interleukin-1β in antifungal immunity – differential host responses induced by *Candida albicans* and *Candida parapsilosis*

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Candida parapsilosis is an emerging fungal species causing invasive candidiasis worldwide. However, little is known about the molecular background of immune responses induced by this pathogen. In this study, our aim was to investigate the host responses induced by *C. albicans* and *C. parapsilosis* using human peripheral blood mononuclear cells (PBMCs) and PMA-induced THP-1 monocytes. We have previously shown that heat killed *C. parapsilosis* induces similar Tumor necrosis factor α (TNFα) and Interleukin-6 (IL-6), but slightly lower Interleukin-1β (IL-1β) production in human PBMCs compared to *C. albicans*. Furthermore, we have shown that *C. parapsilosis* induces significantly lower T helper 17 (Th17) differentiation in comparison to *C. albicans*. In this study, we found that PBMCs stimulated with live *C. parapsilosis* produced similar quantities of TNFα and IL-6, and much lower amounts of IL-1β, compared to *C. albicans*-stimulated cells. As IL-1β has been shown to be important for Th17 differentiation, we further examined the details of IL-1β production upon *C. albicans* and *C. parapsilosis* infection using PMA-induced THP-1 monocytes (the prototypic cell line for IL-1β production and inflammasome activation studies). We found that while *C. albicans* induced the release of IL-1β after 24 hours already at an MOI of 1:100 (*Candida:* THP-1), a 100-times higher dose of *C. parapsilosis* cells (MOI 1:1) was needed for the induction of IL-1β secretion. Moreover, using different isolates of *C. albicans* and *C. parapsilosis*, we proved that this difference was species rather than strain specific. Furthermore, our data strongly suggest that the difference in secreted IL-1β levels originates from the differential processing of IL-1β protein rather than from different transcriptional or translational regulation of IL-1β synthesis. Additionally, we found that the production of IL-1β in response to both *C. albicans* and *C. parapsilosis* is dependent on Caspase-1, Caspase-8 and Syk. In conclusion, our results show that there is a marked difference in the production of IL-1β induced by *C. albicans* and *C. parapsilosis*, possibly contributing to the differential Th-responses induced by the two species. Importantly, these results implicate that the activation of inflammasome, the protein complex responsible for IL-1β processing, may be less effective upon infection with *C. parapsilosis*. Our findings highlight the importance of studies focusing on different *Candida* species rather than *C. albicans* alone when investigating the immunity against these pathogens.

Keywords: candida, inflammasome, inflammation.

SUN-291
SAG-dependent UPS regulates macrophage death or survival and immune response

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Macrophages, at the frontline of immune defense, undergo apoptosis as an optimal strategy against a severe microbial infection. However, the mechanism on how the immune cell shifts between apoptosis and immune response is under-explored. Here, we show that ubiquitination by SAG-UPS (sensitive to apoptosis gene ubiquitin-proteasome system) confers survival advantage to the macrophages during early infection. We demonstrated that SAG plays a key regulatory role in balancing the ratio of pro- and anti-apoptotic factors in the infected macrophages. *SAG*-knockdown significantly reduced the ubiquitination of Bax and SARM (sterile alpha and HEAT/armadillo-motif-containing protein), stabilizing these pro-apoptotic factors and leading to intrinsic apoptosis. In contrast, under chronic infection-inflammation condition, macrophages overexpress *SAG*, leading to upregulation of pro-tumorigenic cytokines (IL-1β, IL-6 and TNF-α), and downregulation of anti-tumorigenic IL-12p40 and anti-inflammatory IL-10. Altogether, our findings identified SAG as a survival determinant for macrophages, as well as a modulator in manipulating timely immune response. This work provides a novel mechanistic perspective into how SAG-UPS acts as a functional link between immune defense and apoptosis or immune-overactivation and tumorigenesis. The potency of SAG-UPS suggests it to be a poten-

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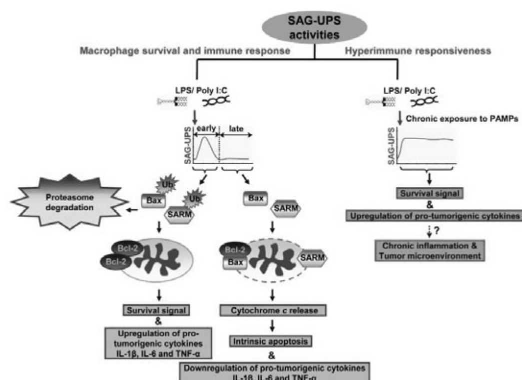

Fig. 1. SAG-UPS manipulates pro-tumorigenic cytokines, balances apoptosis and infection-inflammation in macrophages

tial target for developing immunomodulatory therapeutics against autoimmunity, immunodeficiency diseases and cancer.

Keywords: Apoptosis, Ubiquitination by SAG (Sensitive to apoptosis gene), Viral and bacterial infection conditions.

SUN-292
Search and identification of peptide biomarkers of colorectal cancer in sera

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Colorectal cancer (CRC) is a common and deadly disease in the world. The average lifetime risk to develop nonhereditary, sporadic CRC is approximately 5%. Each year more than one million people are diagnosed with CRC and about half of them die from this malignancy.Stage of the disease, is an important prognostic factor, with five year survival rates of more than 90% for localized CRC (stage I) and only about 10% for CRC that metastasized to distant organs(stage IV). The aim of the present work was a search and identification of peptide markers of CRC in sera using modern mass spectrometry techniques.

Blood sera obtained from 50 patients with CRC and 50 healthy donors (control) were used for isolation and identification of peptides.Serum samples of each analyzed groups were fractionated using magnetic beads with weak cation exchange surfaces, obtained eluates were analyzed by nanoLC-MS/MS using ABSciexTripleTOF 5600. All samples were analyzed by DDA (identification of serum peptides) and by SWATH (for label-free relative quantitative mass spectrometry analyses) approaches.

As a result of LC-MS/MS analysis of sera more than 6000 unique peptides originated from the almost 1000 unique proteins were identified. Among identified peptides 786 were unique for CRC samples, and 125 of those were originated from the proteins unidentified in the control samples. For the control group there were 1075 unique peptides, 259 of which were originated from the proteins unidentified in CRC samples. We believe that the presented data set contains valuable information which will enable interested researchers to identify of new potential biomarkers for colorectal cancer.

Keywords: Biomarkers.

SUN-293
Search for TRPA1 modulators

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TRPA1 receptors play significant role in neurogenic inflammatory pain. These receptors are expressed in mammalian somatic and visceral sensory neurons and they co-localize with TRPV1 in a subset of small diameter, unmyelinated, peptidergic neurons. Activators of TRPA1 are exogenous and endogenous irritants and mechanical stimuli. TRPA1 agonists from cigarette smoke and smog trigger eye irritation, cough and neurogenic inflammation of the airways. Endogenous agonists are compounds generated during tissue inflammation, asthma, chemical hypersensitivity, chronic cough, chronic obstructive pulmonary disease and stress. Thus, the TRPA1 is an important target for design of novel drugs.

The DNA encoding TRPA1 receptor was constructed on the base of cDNA from rat brain tissue using the PCR technique. The gene was cloned into the expression vector pcDNA4/TO, which allows provide inducible expression in mammalian cell lines. Sea anemone venoms were separated by HPLC method. Individual chromatography peaks were analyzed by MALDI-TOF (matrix-assisted laser desorption ionization, time-of-flight mass spectrometry). Activities of separated compounds were tested on cell line with inducible expression of TRPA1 by fluorescence spectroscopy method. Using genetic engineering approaches we produced recombinant analogues of individual compounds.

On the base of cells of the CHO line with inducible expression of TRPA1 was developed test-system for screening biological samples (natural venoms and individual compounds). From sea anemones venoms of *Urticina eques* and *Metridium senile* we isolated peptides modulating TRPA1 activity by HPLC separation. Recombinant peptides from *U. eques* and *M. senile* were produced in *E. coli* expression system.

As the result, we isolated novel peptide modulators of TRPA1 from sea anemone venoms. Peptides modulating TRPA1 activity are valuable biological tools in receptor research work.

Keywords: inflammatory pain, sea anemone venom, TRPA1 receptor.

SUN-294
Searching for novel peptide-based antimicrobials applicable in treatment of wounds

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Despite the significant achievements in the field of medicinal chemistry of antibiotics, the rising level of drug resistant pathogens emphasizes the need for novel compounds with an alternative mode of action. This problem becomes more and more evident in the medical treatment and managing of chronic wounds. This type of wounds are usually seen in diabetic feet and legs, radiation-induced skin damage, non-healing traumatic wounds etc [1]. In this case clinicians usually have to deal not only with the impaired mechanisms of natural skin regeneration, but also with bacterial and fungi infections on a wound site, causing either a delay in wound healing or its deterioration.

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Trying to find a solution to this particular problem, we designed, synthesized and evaluated biological properties of several simple bifunctional peptide conjugates, that are composed of two different peptides bind to each other through the PEG molecule (8-Amino-3,6-dioxaoctanoic acid). The first fragment of the conjugate responsible for its antimicrobial activity is formed by selected cationic antimicrobial peptide (AMP) [2,3], while the second one is represented by the peptides able to stimulate natural reparative processes in the skin, like fibroblasts and keratinocytes proliferation /migration [4] or antioxidant activity [5] .

All sequences of the native peptides used in the design of the conjugates were retrieved from the available literature data and synthesized by means of the solid-phase synthesis applying Fmoc chemistry. Their bactericidal and fungicidal activity was evaluated by means of well-established procedure of MIC (minimal inhibitory concentration) determination, while pro-proliferative activity was analyzed using standardized MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay using human keratinocyte cell line (HACAT) and fibroblasts obtained from human skin biopsies.

The preliminary results suggest that α-helical structure of tested conjugates as well as their native AMPs components is crucial to maintain their antimicrobial activity. In contrast to appreciable bactericidal and fungicidal properties of PEG-conjugates, their pro-proliferative effect on the keratinocyte and fibroblast cell line was not so pronounced.

Acknowledgement: This work was supported by the the Polish National Science Centre under the grant 2012/04/S/ST5/00074.

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Keywords: antimicrobial peptides, wound healing.

SUN-295
Sensitization of bacteria to antibiotics by combined antibiotic-photodynamic treatment

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The emergence of bacterial resistance to antibiotics raised the need to develop alternative technologies for eradication of pathogenic bacteria. One such technology is photodynamic antimicrobial chemotherapy (PACT), which is based on photosensitizers (dyes which are either non-toxic or with low toxicity) and visible light. Another technique, reported in this work, is based on the combined application of antibiotics and photosensitizers. Both methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) strains of *S. aureus* treated with sub-MIC (minimal inhibitory concentration) concentrations of Rose Bengal and sub-MIC concentrations of the antibiotics methicillin, kanamycin A and ampicillin exhibited sensitization to the antibiotic treatment and were inhibited by 3–15 times lower doses of the antibiotics. The MIC value of Rose Bengal was 5 times lower for MRSA than for MSSA. *P. aeruginosa* treated with Rose Bengal combined with methicillin or fucidin exhibited the same trend and the MIC values of the antibiotics were reduced 4–6 fold. Immobilization of Rose Bengal and ampicillin in polystyrene led to an increased inhibition zone relative to the activity of each component immobilized separately. Combined antibiotic-photosensitizer application showed good antibacterial activity against Gram-positive and Gram-negative bacteria at reduced antibiotic doses. It can be assumed that the antibiotics and Bengal Rose acted synergistically against both types of bacteria.

Keywords: antibiotic-photodynamic treatment, drug-resistant bacteria, methicillin-resistant S. aureus.

SUN-296
Serum cytokines and growth factors modulation on pituitary adenomas

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Background: Serum cytokine and growth factors levels have been investigated in relationship with invasiveness in pituitary adenomas in order to evaluate the involvement of these potential biomarkers in pituitary tumor cell proliferation.

The aim of this study was to evaluate the cytokine and growth factors profile in pituitary adenomas in order to establish a panel of biomarkers useful in early detection of this pathology.

Methods: We determined cytokine levels in sera from 22 patients (15 non-invasive; 7 invasive adenomas), angiogenic factors in sera from 66 patients (23 non-invasive; 43 invasive adenomas) and 33 healthy controls. Using MilliplexTM MAP Human Cytokine/Chemokine Panel (Millipore, MA, US) on a Luminex® 200TM system, we analyzed 12 analyte-specific bead sets: proinflammatory IL-1β, IL-2, IL-6, IL-8, IL-12, TNFα, GM-CSF, INFγ, anti-inflammatory IL-4, IL-10, and angiogenic factors VEGF and FGF-2. Multiplex data acquisition and analysis were performed using STarStation 2.3 (Applied Cytometry Systems, Sheffield, UK).

Results: Increased levels of IL-1β, IL-6 and TNFα were found in 27%, 32% and 41% of pituitary adenomas patients. Cytokines expression was significantly higher in invasive pituitary adenomas 86% compared to non-invasive ones 7%. IL-6 and IL-1β levels were 4, and respectively 4.7 fold higher than controls, while TNFα level was up to 1.8 fold higher. We have noticed a positive correlation between the cytokines level and the tumoral invasiveness. TNFα, IL-6 and IL-1β mean values were 2.2, 1.8 and respectively 2.8 fold higher in invasive adenomas compared to non-invasive ones.

Growth factors analysis also revealed an increase of their mean values in patients versus control: 1.6 fold higher for VEGF and 1.2 fold higher for bFGF. Growth factors values obtained by xMAP array were comparable with the outline obtained by ELISA tests.

Conclusions: Our findings demonstrate that cytokines and angiogenic factors levels are closely linked to pituitary tumoral behaviour. Expression of cytokines and angiogenic factors are strongly related to tumor invasion and in this way may act as supporting factors of pituitary tumour expansion. Luminex xMAP might be a suitable tool to evaluate tumoral development.

Acknowledgement: POSDRU 141531/2014.

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Keywords: Cytokines, growth factors, pituitary adenomas.

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SUN-297
Serum from patients with familial Mediterranean fever does not induce apoptosis

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Pyrin, the protein product of the familial Mediterranean fever (FMF) gene *MEFV*, plays regulatory role in inflammation and apoptosis. Pyrin mutation may lead to dysregulated apoptotic processes in FMF. However, the molecular mechanisms of neutrophil apoptosis have not been fully investigated. We have previously shown that spontaneous apoptosis of the neutrophils from FMF patients is accelerated. We aimed to determine if soluble factors in the serum of FMF patients are responsible for the accelerated neutrophil apoptosis and able to induce apoptosis in vitro. Isolated neutrophils from FMF patients and healthy subjects were analyzed immediately or incubated with 10% autologous or heterologous pooled serum for 18 h. Diseased and healthy neutrophils were investigated for apoptosis by flow cytometry with Annexin V/PI staining, as well as for Fas (CD95) expression with PE anti-human CD95 antibody at 0 time and after 18 h cultivation. FMF neutrophils displayed a higher rate of spontaneous apoptosis compared to healthy cells (1.8-fold, $p < 0.05$), while spontaneous Fas expression did not differ between the studied groups. Apoptotic rate and Fas expression of cultured FMF and healthy cells was not affected by autologous or heterologous sera, and was not significantly different between both studied groups ($p > 0.05$). Taken together, our results show that the serum from FMF patients does not directly modulate the rate of neutrophil apoptosis. Having observed no differences in Fas expression with increased apoptotic rate of diseased neutrophils at basal level and no differences at the values measured after the cultivation, we can assume that the elevated susceptibility of FMF neutrophils to undergo apoptosis spontaneously is due to in vitro manipulations. This is indicative of increased sensitivity of the cells towards stress conditions.

Keywords: apoptosis, familial Mediterranean fever, Fas.

SUN-298
Significance of serum 25-hydroxy vitamin D levels in Bulgarian patients with prostate cancer

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Aim: To investigate the relationship between the circulating vitamin D levels and various prognostic variables, associated with the severity and progression of prostate cancer (PCa) in Bulgarian patients.

Patients and Methods: 53 male patients (mean age 67.0 ± 7.1) with clinical suspicion for PCa (elevated prostate specific antigen, PSA and/or abnormal digital rectal examination) were enrolled in the study. All they were subjected to systemic transrectal ultrasound-guided tru-cut prostate biopsies (10 cores at least) and processed for standard histological and immunohistochemical examination. All malignant tumors detected were divided into two subgroups according to the tumor grade (Gleason score < 7 and 3 7). Serum 25-hydroxy vitamin D levels were assayed by HPLC tandem MS. PSA levels were evaluated immunochemically. Statistical analysis was performed by commercial software, using one-way ANOVA non-parametric and correlation analysis. Statistical significance was detected at $p < 0.05$.

Results: PCa was detected in 25 patients (mean age 68.0 ± 7.0), while in 28 patients (mean age 66.0 ± 7.2) benign prostate hyperplasia (BPH) was histologically proved. Significantly lower 25-hydroxy vitamin D levels were detected in PCa patients, compared to those with BPH ($p < 0.05$). There was a significant decrease in the 25-hydroxy vitamin D levels among patients with the most aggressive PCa – those with high grade tumors (Gleason score >7) ($p < 0.05$). With the increase of the Gleason score among patients, the 25-hydroxy vitamin D levels significantly decreased (Pearson $r = -0.39$). Surprisingly, higher 25-hydroxy vitamin D levels were found in PCa patients with a high (> 6) number of positive cores, but the difference was not statistically significant ($p < 0.089$). Other examined covariates, such as: PSA level, age and BMI, did not show any difference between the PCa and BPH patients. Significant seasonal variations in 25-hydroxy vitamin D levels, both for PCa and BPH patients, were detected ($p < 0.05$). During summer, the 25-hydroxy vitamin D levels for both groups reached values, close to the reference range (80–200 nmol/L), while during spring, the PCa group was 25-hydroxy vitamin D deficient (54.01 ± 3.331).

Conclusions: Our results suggest a potential beneficial role of vitamin D in PCa patients. Further studies are needed to strengthen the interrelationships between 25-hydroxy vitamin D levels and various laboratory and clinical parameters, used for diagnosis and prognosis of PCa.

Keywords: 25-hydroxy vitamin D3, prostate cancer.

SUN-299
Silencing of ARTD1 enhances RANKL-induced osteoclast formation by up-regulating IL1beta gene expression

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Bone homeostasis, remodeling and healing after fracture are governed by bone-forming osteoblasts and bone-resorbing osteoclasts. The differentiation of pre-osteoclasts during osteoclastogenesis is associated with the activation of the transcription factor nuclear factor-kappaB (NF-kappaB) by the receptor activator of NF-kappaB ligand (RANKL). Upon activation, NF-kappaB induces the expression of pro-inflammatory cytokines and osteoclastogenic factors, which all combined affect the differentiation process in an autocrine and paracrine fashion. ADP-ribosyltransferase Diphtheria toxin-like 1 (ARTD1), the most abundant poly(ADP-ribosyl) polymerase, is involved in the DNA damage response and in intracellular signaling, but has also been demonstrated to be a cofactor of NF-kappaB-dependent transcription. Using RAW 264.7 macrophages treated with RANKL, we show that ARTD1 silencing as well as inhibition of ADP-ribosylation enhance the formation of functionally active osteoclasts, as demonstrated by increased multinucleation, mineral matrix resorption activity, activity of tartrate-resistant acid phosphatase and expression of osteoclastogenic markers such as NFATc1, c-fos and cathepsin K. Interestingly, inhibition or knock-down of ARTD1 resulted in complete inhibition of IL6 transcription, but augmented transcription of IL1beta, two genes regulated by NF-kappaB and IL1beta was found to repress expression of IL6, indicating that IL1beta act as the main

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driver of ARTD1-regulated osteoclast maturation. These results provide strong evidence that ARTD1 is important for osteoclastogenesis and might alter bone formation and/or remodeling.

Keywords: inflammatory cytokines, osteoclast differentiation, transcriptional regulation.

SUN-300
Silent entry of mycobacteria into macrophages via Dectin-1?

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Innate immunity is the first line of host defense against invading microorganisms. It is based on the detection of invariant molecular signatures that are unique to microorganisms, referred to as pathogens-associated molecular patterns (PAMPs), by a limited number of pattern recognition receptors (PRRs). The latter include Toll-like receptors (TLRs), NOD-like receptors (NLRs) or C-type lectin receptors (CLRs).

Dectin-1 is a CLR that recognizes 1,3-β-glucans from yeast and fungi cell walls and that triggers innate immune responses through an NF-kB-dependent signaling pathway. Several reports indicate that Dectin-1 is involved in mycobacteria uptake by different cell types and in the subsequent cytokine production. However, the underlying mechanisms are still poorly understood and no mycobacterial ligands of this receptor have been identified so far.

Using a soluble form of human Dectin-1 (hDectin-1-Fc), we have shown that the receptor can indeed recognize mycobacterial cell wall components. However, we have found that mycobacteria do not induce the Dectin-1-mediated intracellular signaling pathway, suggesting that Dectin-1 may constitute for *Mycobacterium tuberculosis* a silent entry gate into phagocytic cells.

Keywords: Dectin-1, innate immunity, Mycobacteria.

SUN-301
Silica nanoparticles induce inflammatory response in human fibroblast cells

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The study of silica nanoparticles has attracted considerable attention in recent years, but there are some concerns about their toxicity.

Our objective was to assess the effect of silica nanoparticles to induce an inflammatory response in human lung fibroblasts (MRC-5 cell line) in order to expand the limited information known in this field.

The primary nanoparticle size distribution was a lognormal function, in the range 3–14 nm, most of them being of 5–8 nm. MTT and LDH tests were used in order to evaluate MRC5 cell viability after treatment with 6.3×10^5 particle SiO_2 for 24, 48 and 72 hours.We have also investigated the protein expression of interleukin 6 (IL-6) and interleukin 8 (IL-8), human prostaglandin E_2 (PGE_2), reactive oxigen species (ROS) and nitric oxide (NO) production.

The viability of the treated MRC5 cells decreased in a time dependent manner compared to control. Cell membrane damage was indicated by the increase in LDH by 3%, 14% and 22% after 24, 48 respectively 72 hours.

The level of ROS increased by 16%, 34 and 39%, whereas that of nitric oxide grew by 62%, 32%, 24% after 24 h, 48 h and 72 of exposure. A very significant increase of IL-6 and IL-8 expression time-dependent accompanied by a similar increase in PGE_2 productionin was registered.

All these data suggest that nanoparticles trigger an inflammatory response in MRC-5 cells.

Keywords: nanoparticles, inflammation, cytokines.

SUN-302
Silicon-based quantum dots induce inflammation in human lung cells and disrupt extracellular matrix homeostasis

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Quantum dots (QDs) are nanocrystalline semiconductor materials that have been recently tested for biological applications such as cancer therapy, cellular imaging and drug delivery, despite the serious lack of information of their effects on mammalian cells. Our aim was to evaluate the potential of Si/SiO_2 quantum dots to induce an inflammatory response in human lung fibroblasts (MRC-5 cell line).

The characterization of hydrodynamic size and zeta potential of Si/SiO_2 QDs dispersed in various solutions was performed using dynamic light scattering and laser Doppler velocimetry, respectively. MRC-5 cells were exposed to different concentrations of Si/SiO_2 QDs (25–200 μg/ml) for 24, 48, 72 and 120 hours. QDs internalization was observed due to their self-fluorescence and protein corona was established by SDS-PAGE. The QDs' effect on cell membrane integrity was evaluated by measuring the LDH release in the media. Lysosomes distribution was visualized after LysoTracker Green DND26 labeling. In order to assess inflammatory potential, enzymatic activity of matrix metalloproteases (MMPs) was investigated by gelatin and casein zymography. The protein expression level of MMPs and cytokines were quantified by Western blotting.

Our results showed that QDs uptake was dependent on biocorona formation and nanoparticles' stability in various biological media (Minimum Essential Medium without or with 10% FBS). The cell membrane damage indicated by the increase in LDH release after exposure to QDs was dose and time-dependent. The level of lysosomes increased proportionally with the concentration of QDs, while an accumulation of autophagosomes was also observed. Cellular morphology was affected, as shown by the disruption of actin filaments. The enhanced NO production and the increase in IL-6 and IL-8 protein expression suggested that nanoparticles triggered an inflammatory response in MRC-5 cells. QDs decreased the protein expression and enzymatic activity of gelatinases (MMP-2 and MMP-9) and MMP-1 caseinase activity, while the protein levels of MMP-1 and TIMP-1 increased.

This study reveals for the first time that silicon-based QDs are able to generate inflammation in lung cells and cause an imbalance in extracellular matrix turnover through a differential regulation of MMP and TIMP protein expression and enzymatic activity.

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Keywords: Autophagy, Inflammation, Quantum dots.

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SUN-303
Smad-dependent regulation of Cx43 expression during TGF-β1-induced activation of fibroblast-to-myofibroblast transition in primary human bronchial fibroblasts

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Fibroblast-to-myofibroblast transition (FMT) is induced in bronchial fibroblasts by TGF-β_1. It plays a pivotal role in the induction of airway wall remodeling during bronchial asthma [1]. FMT is regulated by the activation of Smad signaling and accompanied by up-regulation of α-SMA expression – a marker of myofibroblasts [2]. The involvement of connexin (Cx)43, a protein which constitutes gap junctional channels, in the regulation of FMT has been recently reported in cardiac tissue [3]. It prompted us to estimate the role of Cx43 in TGF-β_1-induced FMT undergone by primary human bronchial fibroblasts propagated from bronchial biopsies derived from asthmatic patients (AS HBFs). Up-regulation of Cx43 levels accompanied FMT and α-SMA accumulation upon prolonged exposure of AS HBFs to TGF-β_1 as was demonstrated by immunofluorescence, immunoblotting and flow cytometry analyses. This effect was correlated with the induction of Smad signaling, illustrated by nuclear translocation and accumulation of p-Smad2 in TGFβ_1-treated AS HBFs. Inhibition of gap junctional intercellular coupling by 18-α-glycyrrhetinic acid considerably reduced the efficiency of FMT. However, it had no effect on TGF-β_1-induced nuclear translocation of p-Smad2 and Cx43 expression levels in AS HBFs. On the other hand, transient inhibition of Cx43 expression by siRNA attenuated both the α-SMA transcript quantity and FMT, and Smad signaling activity in AS HBFs. These observations indicate the overlapping gap junctional channel-dependent/independent effects of Cx43 on the efficiency of FMT during airway wall remodeling. Gap junctional coupling may participate in FMT of AS HBFs through mediating by-stander effects in a Smad2-independent manner. On the other hand, Cx43 affects Smad activity in these cells in a channel-independent fashion.

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Keywords: Asthma, connexin 43, Smad pathway.

SUN-304
Smart inflammation sensitive self-reporting theragnosis

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We have designed and develop a novel class of nanocomposites for inflammation based hallmark functions using biocompatible metallic nano-objects (SPION, nanorod) assembled with a pH sensitive amphiphilic azide terminated block polymer, polystyrene-b-poly (acrylic acid) and temperature-responsive polymer Poly (N-isopropylacrylamide) (PNIPAAm) in a single nanoscopic platform. The nano-architecture is a uniform core-shell micellar assembly of polymer around the biocompatible metallic core. Doxorubicin and methotrexate are loaded within the architecture as the model therapeutic module. The drugs are linked through pH and enzyme sensitive bonds. The complete nano-architecture and linkages are characterized by electron microscopy, NMR and Photon Correlation Spectroscopy. The drug release response has been optimized with different cell line in vitro. The model suggest that change/increase in temperature, reduction of pH and the redox enzymatic activities are increased at the localized inflammatory sites, can be addressed by the developed module and the drug will be released at the inflammation sites only due to their specific linkage to the module. Again we have explored order–disorder micellar structures dependent T_1 & T_2 MRI properties of the module. This results indicate that the fabricated module can also be useful not only probing the inflammation site non invasively through MRI but also will give us idea on the extent of release of drugs at the inflammation sites. The outcomes of these results elucidate the potential of this fabricated nano-architecture for smart theranostics through physicochemical and microenvironment feature based drug delivery, site-specific therapy, real-time probing and non-invasive monitoring of the drug action course for personalized therapy.

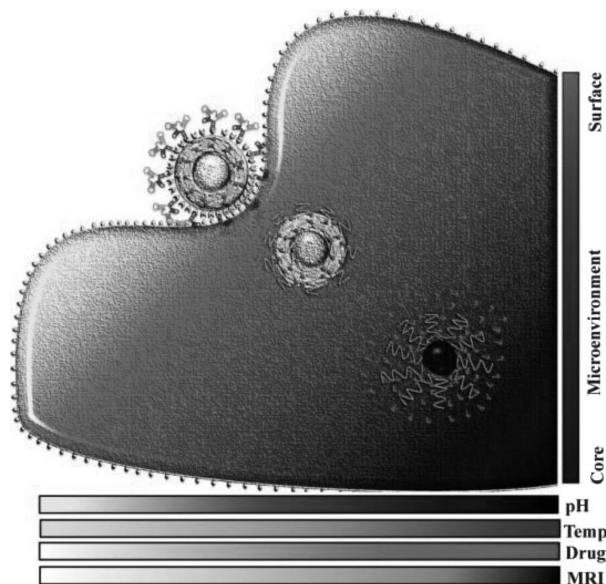

Fig. 1.

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Keywords: drug delivery, naonomedicine, nanoparticles, inflammation, cell death, Therapeutic target.

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SUN-306
Steroids inhibit VEGF expression via TLR4/Akt/NF-κB pathway in chronic rhinosinusitis with nasal polyp

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Vascular endothelial growth factor (VEGF) is elevated in chronic rhinosinusitis with nasal polyps. Steroids have anti-inflammatory properties and are ideal candidates for treating chronic inflammatory airways. The aims of this study were to identify the inhibitory effects and mechanisms of steroids on lipopolysaccharide (LPS)-induced VEGF expression in nasal polyps. Nasal polyp-derived fibroblasts (NPDFs) were stimulated with LPS alone or with both LPS and steroids were used to determine the expression levels of toll-like receptor (TLR)-4, myeloid differentiation primary response gene 88 (MyD88), and VEGF by using RT-PCR. VEGF protein level was analyzed by immunocytochemical staining and ELISA. Small interfering RNA (*si*RNA) for TLR4 was transfected to downregulate TLR4 expression. Activation of Akt and NF-κB pathway on VEGF expression were determined by western blot analysis, immunocytochemical staining and ELISA. Nasal polyp organ cultures were stimulated with LPS alone or in conjunction with steroids or LPS-RS (TLR4 inhibitor) and accessed the expression of VEGF. Steroids decreased the expressions of *TLR4*, *MyD88*, and *VEGF* mRNA and VEGF protein in LPS-stimulated NPDFs. Steroids inhibited LPS-induced VEGF expression levels in dose-dependent manner. The suppression of TLR4 transcription by *si*RNA treatment reduced LPS-induced expression of both TLR4 and VEGF in NPDFs. Furthermore, steroids inhibited the production of VEGF by blocking Akt and NF-κB activation and preventing with NF-κB translocation. Also, steroid and TLR4 inhibitor decreased VEGF expression in nasal polyp organ cultures. These results indicate that steroids inhibit LPS-induced VEGF expression through the TLR4/Akt/NFκB signaling pathway in chronic rhinosinusitis with nasal polyp.

Keywords: steroid, Toll-like receptor 4, vascular endothelial growth factor.

SUN-307
Structural bases for the antigenicity of peptide-MHC complexes

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We aim at understanding the structural bases for the recognition by T cell receptors of tumoral antigens presented by MHC molecules. We are investigating possible links between intermolecular interactions at the TCR-peptide-MHC interface characterized by crystallographic and surface plasmon resonance (SPR) techniques [1] and the ability of these peptide-MHC complexes to mount an efficient T cell immune response. Any advance in this direction would be extremely valuable for the development of anti-tumoral immunotherapy. By combining structural studies of several peptide-MHC complexes and analysis of the frequency of naive T cells that are specific for these peptide-MHC complexes [2], we have highlighted a few principles and have shown that several parameters should be taken into account. An optimum bulginess of the peptide out of the MHC groove seems to correspond to the highest frequency observed. The analysis of several different TCR specific for the same tumoral antigen, Melan-A [3], by SPR shows a good correlation between affinity at the molecular level and functional avidity at the cellular level. A in depth investigation of the contribution of both the α and the β chains will help us to understand the reasons underlying the biased usage of a specific TRAV encoded germline segment for the α chain and the structural rational supporting the difference in affinity and functional avidity for these TCR.

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Keywords: immunogenicity, major histocompatibility molecules, structural biology.

SUN-308
Structural similarity between the N-terminal domain of LonA proteases and the highly conserved RNA-binding PUA domains

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Homooligomeric LonA proteases are the key components of the protein quality control system in bacteria and eukaryotes. Proteolytic activity of LonA is coupled to ATP hydrolysis. Pioneering studies in the 1980s have shown that members of this family of proteases/ATPases are also nucleic acid-binding proteins, and their proteolytic and ATPase activities are stimulated by DNA binding. Such studies indicated that a number of different DNA species increased the rate of degradation of target proteins by the protease, and suggested that association with DNA might be involved in regulation of protein breakdown in cells. In particular, studies of the mitochondrial LonA demonstrated that this enzyme binds to DNA and RNA, and that the binding affinity is affected by the presence of a nucleotide and a protein substrate.

Structural data for the individual domains and/or their combinations have recently become available for several representative LonA proteins. Crystal structure of the N-terminal fragment of *E. coli* LonA comprising residues 1–117 revealed structural similarity to the PUA domain, a highly conserved RNA-binding motif found in a wide range of archaeal, bacterial, and eukaryotic proteins. Here we compare the structure of the N-terminal domain of LonA to the structures of the PUA domains from several protein complexes with RNA, with the aim to reveal possible epitopes for the interactions with nucleic acids.

Keywords: ATPase activity, Protein structure, Proteolysis.

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SUN-309

Structure based virtual screening of MDPI database: discovery of structurally diverse and novel DPP-IV inhibitors

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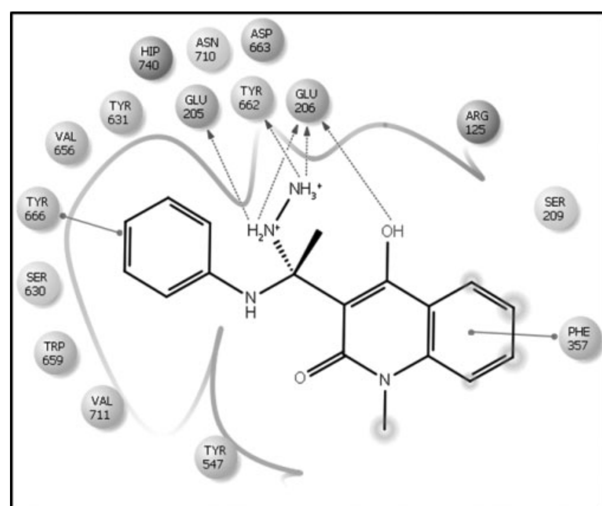
Inhibition of dipeptidyl peptidase IV (DPP-IV) has been emerged as a promising approach for the treatment of type 2 diabetes (T2D). Structure based virtual screening (SBVS) of Molecular Diversity Preservation International (MDPI) database was performed using Glide and Gold against DPP-IV enzyme. Six promising hits were identified and tested for DPP-IV inhibition. Three compounds were found to be active at low micromolar concentration. The 3-(1-hydrazinyl-1-(phenylamino)ethyl)-4-hydroxy-1-methylquinolin-2 (1H)-one (Compound A) was found to be the most potent hit with an IC_{50} of 0.73 μM. These three compounds (A, B and D) were then assessed for their glucose lowering effects in glucose fed hyperglycemic female wistar rats. The glucose lowering effects of compounds also confirms their potential as anti-diabetic agents. The present study demonstrates a successful utilization of *in-silico* SBVS tools in identification of novel and potential DPP-IV inhibitor



MDPI-12398 (A) IC_{50} 0.73 μM

Fig. 1.

Keywords: DPP-IV inhibitors, Type-2 diabetes, Glucagon-like peptide-1.

SUN-310

Studies on the immunogenicity of amyloid beta oligomers and their role in macrophage-mediated inflammation

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The central molecule in the pathogenesis of Alzheimer's disease (AD) is believed to be a small-sized polypeptide – beta amyloid (Aβ) which has an ability to assemble spontaneously into oligomers. Various studies concerning therapeutic and prophylactic approaches for AD are based on the immunotherapy using antibodies against Aβ which in some cases of clinical trials led to neuroinflammation. However, knowledge on the mechanisms of Aβ-induced immune responses is rather limited. Previous research on Aβ1-42 oligomers in rat brain cultures showed that the toxicity of these oligomers considerably depends on their size. In the current study, we evaluated the dependence of immunogenicity of Aβ1-42 oligomers on the size of oligomeric particles and identified the immunodominant epitopes of the oligomers. Moreover, we demonstrated that Aβ1-42 immune complexes increase the neurotoxicity of the Aβ1-42 oligomers.

Results: The analysis of mice serum antibodies revealed that 1–2 nm Aβ1-42 oligomers are highly immunogenic. In contrast, larger Aβ1-42 oligomers and monomers induced a weak IgG response in immunized mice. Monoclonal antibody against 1–2 nm Aβ1-42 oligomers was generated and used for the antigenic characterization of Aβ1-42 oligomers. Epitope mapping of both monoclonal and polyclonal antibodies demonstrated that the main immunodominant region of the 1–2 nm Aβ1-42 oligomers is located at its aminoterminus, between amino acids 1 and 19. This enabled us to use antibody in rat brain cultures (primary neurons with microglia) to investigate how antibodies can shape neurotoxicity of the Aβ1-42 oligomers. We have determined that not only antibody interacts with Aβ1-42 oligomers and forms immune complexes but also increases neurotoxicity of Aβ1-42 oligomers. We were able to confirm that this effect might be due interaction between antibodies and Fc receptors which are found on microglia cells.

Conclusions: Small Aβ1- 42 oligomers of size 1–2 nm induce the strongest immune response in mice. The amino-terminus of Aβ1-42 oligomers represents an immunodominant epitope which indicates its surface localization. The data demonstrate that Aβ1-42 oligomeric antigens in complex with specific antibodies can increase neurotoxic effects on primary neurons by Fc-dependent microglia activation. The results of the current study may be important for further development of Aβ-based vaccination and immunotherapy strategies.

Acknowledgments: This research was funded by a grant (No. LIG-04/2012) from the Research Council of Lithuania.
Keywords: antibodies, beta amyloid, neurotoxicity.

SUN-312

Study of catalytic antibodies and their implication in autoimmune disease

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Catalytic antibodies (or abzymes) are immunoglobulins capable of catalyzing an enzymatic reaction. In the late 1980s and the early 1990s, many abzymes were observed on the background of autoimmune disease (reviewed in [1]). This suggested the existence of a link between autoimmunity and the formation of natural catalytic antibodies. Abzymes have also been discovered in normal physiological states [2]. Despite advances in the study of catalytic antibodies, there are many questions yet to be answered about the nature of these molecules and their role in the immune system. To address these questions, a statistical analysis was performed on the genetic sequences of 40 abzymes [3]. We analyzed the gene subgroups, catalytic residues, and somatic mutations responsible for the catalytic function of these catalytic antibodies. It was shown that they display a high conservation degree with their germline counterparts, significant modification of the physico-chemical

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properties of their mutated amino acids, and a more frequent expression by rare gene subgroups. This suggests that there is a difference between the maturation process of abzymes and binding antibodies and can possibly explain their high occurrence in autoimmune disease. Following a new methodology [4] and using newly designed primers, we have constructed 4 phage displayed combinatorial libraries of single-chain antibody fragments (scFv) representing different immune repertoires: healthy or autoimmune, naive or immunized. The 4 libraries have been independently tagged and pooled together resulting in a single library of size 10^9, allowing us to perform a unique selection process. We select for catalytic antibodies in this pooled library by exploiting a suicide substrate used for immunization as the trapping agent. Here, we investigate the gene subgroup representation profiles among the 4 repertoires focusing on a window of 100 sequences per library. These results together will lead to a better understanding of the characteristics of catalytic antibodies and their link to autoimmune disease.

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Keywords: autoimmunity, catalytic antibodies, phage display.

SUN-315

The anti-inflammation effects of water extract of curcumae radix and cortex *Magnolia officinalis* for prevention of gastric ulcer in vitro and in vivo

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Tranditional Chinese medicines play an important role in the health care for Chinese people for several thousands years. Many herbs have been used on protecting gastric mucosa against chronic or acute inflammation. This study investigated the anti-inflammation effects of water extracts of Curcumae Radix and Cortex Magnoliae Officinalis, for prevention of gastric ulcer in vitro and in vivo. Our in vitro results showed that water extracts of Curcumae Radix and Cortex Magnoliae Officinalis specifically promoted the cell viability and decreased the production of reactive oxygen species (ROS) of the gastric epithelial cells in the acidic medium as compared with those of control cells. The results from the animal model of Shey's ulcer ligation suggested that the water extract of Cortex Magnoliae Officinalis decreased the productions of tumor necrosis factor-alpha (TNF-alpha) and interleukin 6 (IL-6) of gastric tissue and thus significantly decreased the gastric ulcer index in vivo. In conclusion, the water extract of Cortex Magnoliae Officinalis may protect the gastric mucosal tissue from the acidified injury by enhancing gastric epithelial cell survival rates and decreasing ROS, TNF-alpha and IL-6 productions in the injured gastric tissue.

Keywords: Anti-inflammation, Chinese herb, Gastric ulcer.

SUN-316

The determination of serum and urinary endothelial cell-specific molecul-1 (endocan, ESM-1) levels in patients with bladder cancer

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Bladder cancer is one of the most common malignancies in men. Endothelial cell-specific molecul-1 (endocan, ESM-1) is a proteoglycan and plays an important role in angiogenesis and inflammation. The aim of this study was to evaluate the diagnostic value of serum and urinary levels of ESM-1 in bladder cancer. The study included 50 bladder cancer patients, 50 with urinary tract infection (UTI) and 51 healthy volunteers. Serum and urinary ESM-1 levels were measured with enzyme linked immunosorbent assay (ELISA).

In bladder cancer group, serum and urinary ESM-1 levels were significantly higher than in the healthy subjects ($p = 0.003$ and $p < 0.0001$). Urinary ESM-1 levels in cases with UTI were also higher than in healthy volunteers ($p = 0.002$). There were no significant differences between bladder cancer and UTI groups in terms of serum and urinary ESM-1 concentrations.

In the three groups, urinary ESM-1 concentrations were higher than those of corresponding serum ESM-1 concentrations ($p < 0.0001$ for bladder cancer and UTI groups, $p = 0.002$ for healthy subjects). In bladder cancer group, there was a statistically positive correlation between serum ESM-1 and urinary ESM-1 concentrations ($r = 0.32$, $p = 0.002$). We determined the ability of serum ESM-1 levels to differentiate between bladder cancer patients and healthy subjects. The sensitivity and specificity were 50%, and 77%, respectively, with a 'cut off' point of 630 pg/mL. The sensitivity and specificity of urinary ESM-1 levels were 62%, and 71%, respectively, with a 'cut off' point of 1100 pg/mL.

When bladder cancer cases were divided according to pathological stages, there was no significant difference between invasive bladder cancer and non-invasive bladder cancer groups in terms of serum and urinary ESM-1 concentrations.

As a conclusion, serum and urinary ESM-1 concentrations increase in bladder cancer. This parameter also increases in serum and urine of cases with UTI. That urinary ESM-1 values were higher than serum ESM-1 values in all groups may be attributed to direct exfoliation of epithelial cells in bladder to urine.This condition must be taken into consideration when evaluating ESM-1 in bladder cancer.

Keywords: Endocan, Bladder cancer, urine.

SUN-317

The determination of the angiogenesis and apoptosis markers role on the prognosis of acute myocardial infarction

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Introduction: Acute coronary syndromes are a leading cause of mortality, morbidity, and health care cost. Innate and acquired immune responses influence the extent of atherothrombosis and the vulnerability of single lesions. Complement activation, interleukins and acute-phase proteins modulate the extent of myocardial damage, aggravating the prognosis. In this study, cells after ischemic injury in acute myocardial infarction (AMI), is developing angiogenesis or apoptosis, we aimed to learn, which way the cells prefer.

Materials and Methods: Our study was performed on 42 patients diagnosed with AMI (26 men, 16 women), 42 healthy controls (23 men, 19 women). We have tried tumor necrosis factor-α (TNF-α), Caspase-9, insulin-like growth factor 1 (IGF-1) and interleukin-8 (IL-8) in blood that collected by grouping. In our study, patients in the initial emergency contact, 24 per hour

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and a month later (30th day of) blood samples were taken during the controls.

Results: The differences were found for IL-8 between the value of the control-24.hours and control-30.day. Significant differences were found in IL-8 levels comparison in patients group. IGF-1 levels were not significantly different in comparison. TNF-α values for the intertemporal comparison with the control group; significant differences were found between the control-24.hours and the control-0.hours. There were differences in TNF-α between the 24.hours-30.day and 0.hours-30.day in patients according to the time value comparison. Value comparison of Caspase-9 did not differ significantly. Significant positive corelation was found between IL-8 and TNF-α.

Conclusion: After undergoing myocardial infarction, TNF-α levels were increased, Caspase-9, IGF-1 and IL-8 levels were decreased. One month later, measurement of TNF-α has also started to decline while other parameters have showed increase. These data will provide new meanings to myocardial infarction. For further studies of myocardial infarction patients should be monitored for a longer period.

Keywords: Acute Myocardial Infarction, Angiogenesis, Apoptosis.

SUN-318
The effect of dipeptidyl peptidase-IV inhibition on the immune functions in patients with type 2 diabetes

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Dipeptidyl peptidase-IV (DPP-IV) is a membrane bound multifunctional serine protease identical with the marker of activated lymphocytes CD26 and its soluble form can be found in blood plasma. Recently, specific DPP-IV inhibitors (the gliptins, e.g. sitagliptin) that improve insulin secretion by preventing the DPP-IV mediated degradation of incretins were introduced for the treatment of type 2 diabetes. In addition to the breakdown of incretins, DPP-IV however proteolytically modifies several other biologically active peptides such as neuropeptides and chemokines and its long-term inhibition could therefore lead to unfavorable effects including immune dysregulation.

The aim of this study is to assess the effects of DPP-IV inhibition by sitagliptin on the immune system in patients with type 2 diabetes.

Patients with type 2 diabetes were examined before the initiation of sitagliptin treatment and 4 weeks and 12 months thereafter, the results were compared intraindividually as well as with the control group of type 2 diabetic patients treated with other oral antidiabetic drugs. Immunophenotyping of Treg and T cells (CD4+, CD8+) was performed in freshly collected peripheral blood, the proportion of Th1 population and the presence and activation of NK cells were analyzed after in vitro stimulation with phytohemaglutinine, phorbol myristate 13-acetate and ionomycine in the presence of brefeldin A. DPP-IV enzymatic activity was determined in heparinized blood plasma and isolated blood mononuclear cell.

Compared to patients treated with other hypoglycemic drugs, plasmatic DPP-IV enzymatic activity was inhibited by 76.4 ± 15.5 and 60.5 ± 27.5% (mean ± SD) at 4 weeks and 12 months of sitagliptin treatment, suggesting long term systemic inhibition of DPP-IV in our patient cohort. After 4 weeks, the proportions of Treg and NK cells significantly decreased, whereas Th1 increased, but the changes normalized in the majority of patients after 12 months of treatment.

Our data show that sitagliptin treatment may lead to transient changes in the proportion of various lymphocyte populations in type 2 diabetes patients.

Grant support: IGA 12407-4/2011, PRVOUK-P27/LF1/1, SVV-260 032 and UNCE 204013.

Keywords: dipeptidyl peptidase, immune regulation, sitagliptin.

SUN-319
The effect of epigallocatechin-3–gallate on major peanut allergens' structure

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Epigallocatechin-3-gallate (EGCG) is the most abundant catechin in green tea. EGCG possesses anti-allergic properties due to its inhibitory effect on mast cells degranulation and histamine release. Peanuts are widely used for the preparation of a variety of foods, while being important elicitors of food allergy and the predominant source of ingested allergens worldwide. Homologs Ara h 2 and Ara h 6 are major peanut allergens known for their cross reactivity.

Covalent and non-covalent interactions of EGCG with proteins have been reported. However, the mechanisms of EGCG binding to allergens remain poorly understood and biological effects of these interactions have not been investigated so far. EGCG-allergen interaction could alter IgE epitopes and influence allergenic potential.

The main goal of this study was elucidation of EGCG effect on major peanut allergens', Ara h 2 and Ara h 6 structure by studying the interactions between EGCG and said proteins.

CD spectroscopy results revealed both Ara h 2 and Ara h 6 structure relaxation upon incubation with increasing amounts of EGCG. Increase in β-sheet content, at the expense of α-helix occurs at the EGCG concentrations around or above the K_d in case of both proteins.

Ara h 2 has a Trp-residue, a strong fluorophore whose fluorescence quenching (FQ) analysis could provide protein structural information. Ara h 6 only has Tyr-residues, so no such data could be obtained by FQ. In order to establish the strength and nature of the putative Ara h 2/EGCG interaction in solution, we employed various mathematical models – Stern-Volmer, Lehrer, Double logarithm and Langmoir analysis. Association constants were in the range of $2–4 \times 10^4$ M^{-1}, while the number of binding sites was determined to be 0.92 EGCG molecules/protein. Computational and docking analysis of binding affinity revealed most probable binding sites of EGCG on Ara h 6. The calculated free energy of binding of −7.9 kcal indicates stable complex formation between EGCG and Ara h 6.

Our results provide better understanding of physiologically significant effects which EGCG exhibits in presence of allergenic proteins. CD spectroscopy confirmed the same EGCG effect on both proteins' conformation. Ara h 2 FQ analysis results could indicate the strength and nature of EGCG interactions with Ara h 6 as well. Docking studies performed on Ara h 6 could also provide insight into EGCG binding sites of Ara h 2. These results show promise on revealing the potential contributing mechanism of anti-allergic properties of EGCG.

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Keywords: Epigallocatechin-3–gallate, Major peanut allergens, Protein polyphenol interactions.

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SUN-320

The effect of *Myrtus* L.(Myrtaceae) on the skin in thermal burn injury

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Thermal skin burn is one of the most common problems in the world. It induces the activation of an inflammatory process resulting in local and distant tissue damage. Leaves of the *Myrtus communis* subsp. *communis* (Myrtaceae) are used in the treatment of wounds and burns in traditional medicine. It was shown that the topical applications of the compound with free-radical-scavenging properties in patients significantly improved wound healing and protected tissues from oxidative damage. In the present study, we investigated the putative antioxidant effect of local *Myrtus* L.(Myrtaceae) treatment on burn-induced oxidative tissue injury. Under ether anaesthesia, Wistar albino rats (200–250 g) were exposed to a 90°C (burn) or 25°C (sham) water bath for 10 s. Leaves of *Myrtus communis* subsp. *communis* were collected from the Turgutlu region of Denizli and were dried in the shade at room temperature. Powdered leave samples (100 g) was extracted with ethanol in a Soxhlet aparatus, filtered and dried under vacuum and stored under refrigeration. *Myrtus* L.(Myrtaceae) was locally (100 mg/kg) applied on 4 cm^2 area after the burn and this was repeated twice a day. Rats were decapitated 48 h after burn injury. Skin tissue samples were taken from animals and homogenized in saline. Oxidant-antioxidant biochemical parameters were determined in homogenized skin samples. Results were evaluated statistically and discussed.

Keywords: Skin, Burn, *Myrtus* L., Antioxidant-Oxidant Parameters, Inflammation.

SUN-321

The effect of resistin and fractalkine on macrophage-smooth muscle cells cross-talk

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Resistin was found in human atherosclerotic lesions and was suggested to be an inflammatory marker of atherosclerosis and to promote atherogenesis. Fractalkine (FKN) has dual function acting as a cell adhesion molecule and chemokine mediating direct capture, firm adhesion and transmigration of leukocytes and its expression is enhanced in human atherosclerotic plaques. Both resistin and fractalkine were found at increased levels in the human atherosclerotic lesions, resistin being associated with CD68 monocytes/macrophages in carotid arteries and aortic aneurisms, and fractalkine predominantly associated with intimal smooth muscle cells (SMC) and with monocytes/macrophages but their role in this location is not very clear yet. Our recent data showed that resistin has pro-inflammatory effects on SMC and that fractalkine has proatherogenic effects in human monocytes/SMC cross-talk. The objective of this study was to explore the role of resistin and FKN in macrophage-SMC cross-talk and to uncover the molecular mechanisms involved. THP-1 cells (a monocytic cell line) were differentiated to macrophages with phorbol myristate acetate (PMA) and then interacted with SMC cultured on membrane inserts in the presence or absence of resistin or FKN. After 24 hours, the gene and protein expression of inflammatory mediators induced in macrophages by macrophages-SMC cross-talk was determined by Q-PCR and Proteome ProfilerTM Array (R&D Systems). Macrophages alone or macrophages activated with resistin or FKN were used as controls. Protein array experiments revealed that macrophages exhibit after their interaction with SMC an increased expression of chemokines (CCL1, CCL4, CCL5, CCL19, CXCL1, CXCL5, CXCL7) and cytokines (IFNγ, TNFα, IL-1α, IL-10, IL-2, IL-23, IL-27, IL-5, IL-6 and IL-8) and PAI-1 (plasminogen activator inhibitor-1). Moreover, resistin enhanced the inflammatory macrophage phenotype induced by their interaction with SMC, by increasing the level of the chemokines CCL1, CCL4, CCL5, CXCL1 and of the cytokines: IFNγ, IL-1α, IL-5, IL-6, IL-23, IL-27, IL-8 and PAI-1. In addition, resistin induced in macrophages interacted with SMC an increased expression of the chemokines: CCL10, CCL17, CCL3, CXCL11 and cytokines IL-13, IL-17, IL-17E, IL-ra, IL-1β and ICAM-1, MIF (Macrophage migration inhibitory factor), CD40L. Our preliminary data indicate that FKN also modulate macrophage-SMC interaction by increasing the expression of mediators involved in inflammation and fibrosis. Our results suggest that resistin and fractalkine may exert pro-inflammatory effects in macrophages-SMC cross-talk and the molecular mechanisms involved may reveal targets for novel anti-inflammatory therapies.

Keywords: fractalkine, macrophage-smc cross-talk, resistin.

SUN-322

The evaluation of serum amylase and lipase in the diabetic patients with chronic pancreatitis with a possible correlation with the pancreatic functions

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Diabetes mellitus is a chronic metabolic disorder which is associated with hyperglycaemia. It is caused by a derangement in the secretion or function of the endocrinal portion of the pancreas. There is a close anatomical and functional relationship between its exocrine and endocrine parts. The current study was designed to evaluate the blood glucose, insulin levels and the amylase, lipase activities of diabetic patients as representatives of the two parts of the pancreas respectively.

We were investigated exocrine function by assaying both amylase and lipase activity in 24 diabetic patients with chronic exacerbate and chronic pancreatitis (CEP, CP) and 50 healthy persons. Endocrine function in patients was analyzed based on the glucose and insulin concentrations in plasma. In addition, the analysis also age, BMI and smoking factor. Patients and healthy persons were separated into two groups according to whether smoked, or non-smoked cigarettes.

Significantly high serum amylase and lipase activities were found in the smoking diabetic patients with CEP (339.1 $\pm$ 38.9 [U/L]; 212.4 $\pm$ 41.7 [U/L]) and CP (52.5 $\pm$ 2.8 [U/L]; 79.7 $\pm$ 42.7 [U/L]) as compared to those in the non-smoking healthy controls (40.2 $\pm$ 13.4 [U/L]; 26.3 $\pm$ 10.7 [U/L]; $p < 0.001$ respectively). It is interesting that the enzymes activities were 8-fold higher in smoking patients with CEP compared to control. Also, the levels of fasting serum glucose were significantly higher in the smoking patients with CEP (177.6 $\pm$ 32.8 [mg/dL]) and CP (154.5 $\pm$ 28.6 [mg/dL] as compared to those in the non-smoking patients (142.6 $\pm$ 15.8 [mg/dL], $p < 0.001$; 136.2 $\pm$ 8.9 [mg/dL], $p < 0.05$, respectively) and controls (74.4 $\pm$ 15.7 [mg/dL], $p < 0.001$ and <0.01). The insulin level was found lower in the smoking diabetic

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patients (CEP: 1.4 ± 0.6 [μU/mL] and CP: 1.4 ± 0.8 [μU/mL] as compared to non-smoking patients and control (2.1 ± 0.4 [μU/mL]; 2.8 ± 0.6 [μU/mL] and 10.7 ± 2.9 [μU/mL], $p < 0.001$; respectively). In smoking patients with diabetes differed with regard to BMI as compared with non-smoking.

In patients with CP and diabetes, association of decreased pancreatic exocrine function with BMI and vascular disease suggests a role of pancreatic arteriopathy. The increase enzymes activities and a decrease in insulin level in patients with CEP and diabetes probably was associated with the exacerbation of pancreatitis due to smoking cigarettes.

Keywords: Diabetes, enzyme activity, pancreatitis.

SUN-323
The fatty acid composition of type 1 diabetic patients in Turkish population and its relation with glycosylated hemoglobin

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Background: Type 1 diabetes mellitus is a disease characterized by pancreatic B-cell destruction and insulin deficiency. The studies have long-standing continued on the relation between diabetes and atherosclerosis and there are also studies suggesting that HbA1c, glycosylated form of hemoglobin, may be associated with atherosclerosis in diabetic patients. The aim of this study is to evaluate the relation between fatty acid alteration and HbA1c levels of type 1 diabetic patients in Turkish population.

Material and Method: The study was performed with 59 type 1 diabetic patients, who were admitted to the Endocrinology and Metabolism outpatient clinic, and 42 healthy volunteers. The fatty acid composition, including was performed on gas chromatography and the fatty acid levels were calculated as % amounts by use of standard methyl ester mixtures. HbA1c levels were analyzed in an A1c HA-8160 analyzer by HPLC.

Results: The levels of stearic acid (C18:0) in type 1 diabetic group were significantly higher (0.45 ± 0.042 μg/μl) when compared to control (0.11 ± 0.027 μg/μl, $p = 0.000$). The results showed that the levels of unsaturated fatty acids, C18:1, C18:2, C18:3 and C22:4 were significantly lower in type 1 diabetic group, when compared to control ($p < 0.001$). The average HbA1c levels of type 1 diabetic group was 9.86% and the higher C18:0 levels were positively correlated with HbA1c levels ($r = 0.067$).

Conclusion: We found decreased unsaturated fatty acid composition and increased stearic acid levels in type 1 diabetic group, sustaining the hypothesis that type 1 diabetes is prone to atherosclerotic disease development. The positive correlation of HbA1c with C18:0 is also a supporting issue whereas need to be clarified with further studies.

Keywords: type 1 diabetes mellitus, fatty acid, HbA1c.

SUN-324
The impact of IL-33 in absence epilepsy

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In the recent studies have shown that glial cells are involved in the pathophysiology of epilepsy. Activated glial cells produce cytokines. It is known that the neuroinflammation process contribute to the pathogenesis of epilepsy. However there is an age-related early impairment of the neuron–glia interactions in rats with genetic absence epilepsy. IL-33 has immunomodulatory functions in the brain. It's role in absence epilepsy had never been studied before. In the preliminary findings of our study, 16 male rats, aged 2 months were used. Rats were divided into 2 groups as follows; WAG/Rij rat group and Wistar control rat group. All rats were decapited after that cortex and thalamus were dissected. Protein expression level was assessed with Western blot. Western blots were performed using standard techniques. IL-33 expression level was statistically increased in both cortex and thalamus as compared with control group. Our results suggest that IL-33 may play a role in pathogenesis of absence epilepsy. For further studies we aim to study IL-33 at 6 months age. In this respect the role of IL-33 in the pathophysiology of absence epilepsy are thought to be enlightened.

Keywords: Epilepsy, IL-33, WAG/Rij.

SUN-325
The impact of silymarin nano-formulation on experimental liver damage in Balbc mice

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Hepatoprotectivity, anti-oxidative, anti-inflammatory and anticancer effects are known to be showed by silymarin as flavonolignan with above mentioned characteristics. Clinically applied silymarin in high doses was found to be biologically safe. But, pharmacocinetic studies have shown the biopharmaceutical imitations of this agent as improper diffusion in the tissue, poor solubility in water, and decomposition in the intestine. For this reason in this study we aimed to formulate silymarin nanoparticles and to investigate the hepatoprotective effects of this particles on experimentally damaged liver tissue by TNF-alpha/D-Galactosamine application. For detecting the ultrastructural changes the liver tissues on silymarin administered and control animals were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 and and left in buffer overnight at +4°C. The tissue sample were post fixed in %2 osmium tetroxide and dehydrated in graded alcohol: 70, 90, 96 and 100%, then embedded in EPON 812 epoxy and sectioned on ultramicrotome (LEICA UC6). After staining the tissues were observed on transmission electron microscope (FEI Tecnai BioTWIN). Our results show that the ultrastructure of the liver cells of silymarin nano-particle administered animals are more similar to the control animal cells than the TNF-alpha/D-Galactosamine administered animal cells. Our results show the hepatoprotective effect of silymarin nanoparticles in experimental liver damage in Balbc mice.

Keywords: Balbc mice, Hepatoprotective, Silymarin.

SUN-326
The impact of thrombospondin-1 in pathophysiology of absence epilepsy

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Thrombospondins (TSPs) are glycoproteins. TSP-1 has identified as an astrocyte-secreted protein and regulate synaptogenesis. It's

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role in absence epilepsy had never been studied before. The aim of this study is to investigate the neural mechanism underlying absence epileptic seizure in WAG/Rij rats.1, 3 and 6-months-old female WAG/Rij (n = 21) rats were used in our study. All animals were decapitated using transcardiac perfusion. The cortex was dissected from brains of WAG/Rij rats in all group. TSP-1 protein expression levels were assessed with Western blot. Western blots were performed using standard techniques at 1 months old WAG/Rij rats, 3 and 6, respectively. p values <0.05 accepted as statistically significant. Compared with our preliminary results from WAG/Rij rats groups, 3 months age group showed significantly higher TSP-1 protein expression level. However 6 months age group also showed significantly lower TSP-1 protein expression level in cortex. TSP-1 protein expression levels in 1 months age group were found to be increased compared to 6 months age group. But this alteration in 1 months age group was not statistically significant. The differences of expression level and other TSP variants within groups and other brain region will be subjected to further analysis by us for confirmation. Our results suggest that TSP-1 is possibly involved in absence epileptic seizure maturation in cortex. This alteration should lead to maturation of synapses and contribute to the pathophysiology of absence epilepsy.

Keywords: Epilepsy, Thrombospondin-1, WAG/Rij.

SUN-327

The in vivo effects of chronic cadmium toxicity in the liver

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Accumulation of the widespread environmental toxin cadmium (Cd) in tissues results intoxicity. Liver is one of the most effected tissues. Cd exposure induces inflammation in effected tissues. Galectin-3 is inflammation related molecule, which takes part in cell survival, apoptosis, and migration.The present study was focused to evaluate role of TNF-α and IL-6 during Cd toxicity and their relationships with galectin-3 levels. Male Wistar rats were exposed to Cd at the dose of 15 parts per million (ppm) for 8 weeks. Inflammatory status in liver was evaluated with measurement of tissue TNF-α and IL-6 levels. Liver tissue caspase-3 level was used to identify apoptosis. Tissue galectin-3, TNF-α and IL-6 levels were evaluated by ELISA. A significant increase in galectin-3 tissue level was seen after Cd toxicity, this was accompanied with a significant increase in the TNF-α and IL-6 levels. Increased caspase-3 levels were measured after Cd toxicity. Chronic Cd toxicity induces inflammation and apoptosis in rat livers. Cd causes increased galectin-3 production in liver tissue. The formation of TNF-α due to Cd exposure may likely trigger this mechanism.

Keywords: cadmium, galectin-3, inflammation.

SUN-328

The inflammatory mediator tumor necrosis factor α (TNFα) has cell type-dependent effects on hypoxia-inducible factor 1 (HIF-1)

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Inflammation is often accompanied by hypoxia due to high cellular metabolic activity and accumulation of immune system cells at the sites of inflammation. The key mediators of the cellular response to hypoxia are the Hypoxia-Inducible Factors (HIFs) (1). HIF-1α, the regulatory subunit of HIF-1, can be induced by cytokines, mainly in cells of the immune system (2). In order to investigate the induction and function of HIF-1 under inflammatory conditions in cells of non-immune origin, we analyzed the effect of tumor necrosis factor α (TNFα) on human epithelial cancer H1299 and A549 cells, hepatocarcinoma Huh7 cells, as well as in primary bronchial airway smooth muscle cells (HBSMCs), under normoxia or hypoxia. Treatment of the cancer cells with TNFα did not affect the expression of HIF-1α, although mRNA expression levels of the inflammation markers RANTES, ICAM and VCAM were increased significantly. In contrast, TNFα up-regulated HIF-1α protein and mRNA levels, under both normoxia and hypoxia, in non-cancer primary HBSMCs, through an NF-kB-depended pathway. However, despite the induction of HIF-1α protein in HBSMCs, the transcriptional activity of HIF-1 was reduced by TNFα, due to inhibition of the interaction between HIF-1α and ARNT and subsequent blocking of HIF-1 binding to target gene DNA (3). Our results show that in contrast to cells of the immune system, TNFα is not an inducer of HIF-1α in lung and liver cancer cells and is a negative regulator of HIF-1 activity in primary smooth muscle cells. These suggest that cross talk between hypoxia and inflammation is highly complex and cell-type or tissue specific.

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Keywords: hypoxia-inducible factor-1 (HIF-1), inflammation.

SUN-329

The influence of tobacco smoke and occupational exposure in copper foundry on the antioxidants status of MT and PON in blood

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Metallotionein (MT) and paraoxonase (PON) are proteins both involved in protection against xenobiotics and free radicals. MT plays important role in protecting against a number of xenobiotics such as: heavy metals, carbon tetrachloride, chemiotherapy and UV radiation. MT also scavenges hydroxyl radical, superoxide anion radicals and hydrogen peroxide.

PON are a family of three enzymes called PON-1, PON-2 and PON-3. PON-1 is the most studied enzyme and hydrolyses the active metabolites of several organophosphorus insecticides (phosphotriesterase activity, PON-P) as well as protects against

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LDL oxidation. PON-1 has also antioxidant function and is involved in defense against hydrogen peroxide.

The aim of present study was to analyze MT concentration and PON-P activity in the blood of smoking and non-smoking smelters as well as investigate the relationship between these markers.

The concentration of MT in plasma was measured by enzyme-linked immunosorbent assay elaborated in our laboratory using a commercial antibody and own standard MT (isoforms MT-I and MT-II) isolated from human liver (1). PON-P activity in serum was measured spectrophotometrically at $\lambda = 405$ nm using paraoxon as a substrate (2).

The highest concentration of MT was observed in the plasma of smoking smelters (6.09 ± 3.76 μg/l), whereas in these group the lowest PON-P activity was observed (100.70 ± 54.24 U/l).

No statistically differences were found between the concentration of MT in the plasma of smoking (4.15 ± 1.84 μg/l) and non-smoking control group (4.07 ± 2.21 μg/l) as well as between non-smoking smelters (4.67 ± 2.61 μg/l) and smoking and non-smoking control groups.

The activity of PON-P was lower in serum of non-smoking smelters (110.72 ± 59.23 U/l) when compared to non-smoking (133.63 ± 66.21 U/l) and smoking control group (156.27 ± 57.62 U/l).

We have observed a negative correlation between MT concentration and PON-P activity in the blood of non-smoking smelters ($r = -0.84$, $p = 0.0024$) and non-smoking control group ($r = -0.47$, $p = 0.045$).

Tobacco smoke disturbs status of these antioxidants and their interacts, both in control and smelters groups.

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Keywords: metallothionein, Oxidative stress, paraoxonase.

SUN-330
The influence of β-glucans intake on activity of antioxidant enzymes

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β-glucans are polysaccharides, found in the cell walls of bacteria, fungi, and plants such as oat or barley. It is known that physicochemical properties of β-glucans vary between β-glucans of different source. Moreover, the physiological effects (i.e. antioxidant potential) of various β-glucan forms are correlated with their molecular weight, intrinsic viscosity, composition and chemical structure (Zhao et al., 2014). β-glucan possesses free radical scavenging activity and the ability to alleviate inflammatory conditions. What is more, β-glucan demonstrates significant protective properties against the severe oxidant induced lipid peroxidation in blood or plasma (Saluk-Juszczak, 2011). Inflammation is undoubtedly a major driving force for the stride of major chronic diseases (Poli et al., 2013). Therefore, the attention is concentrated on the role of natural antioxidants which are able to protect living organisms against the action of reactive oxygen species and in this way decrease the risk of various diseases.

The aim of the present study was to examine the antioxidant activity of two forms of oat β-glucans (low and high molecular weight) in laboratory animal model of enteritis.

The study was performed on mature Sprague Dawley male rats (n = 48), divided into 3 groups (fed control diet (K) and diet supplemented with small molecular wieght (G1) and large molecular wieght (G2) oat β-glucans. Within groups animals were divided into healthy individuals (K, G1, G2) and individuals with experimentally induced (intravenously injection of E. coli lipopolysaccharides) intestinal inflammation (Kz, G1z, G2z). The activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and reductase (GR) was determined using commercially available assays.

Enteritis was confirmed histologically. Injection with E. coli LPS resulted in a significant increase of activity of SOD, GPx and GR in Kz group. Addition of β-glucans to the feed in groups G1 and G2 significantly reduced SOD, GPx and GR activity. A decrease in GR, GPx and SOD activity was observed in G1z and G2z groups compared to Kz. The decrease in SOD and GR activity was larger in G2z than in G1z but the decrease in GPx activity was larger in G1z than in G2z.

In conclusion, B-glucan possesses antioxidant properties as manifested by a reduction in superoxide dismutase, glutathione peroxidase and reductase activity.

The research has been supported by grant NN312427440.
Keywords: β-glucan, rat, enteritis.

SUN-331
The interplay between MT1-MMP and ADAMs

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Membrane Type I-Matrix Metalloproteinase (MT1-MMP/MMP14), a membrane anchored enzyme, is essential for diverse physiological and pathological processes through extracellular matrix remodeling and pericellular proteolysis. Mutation in MT1-MMP causes Winchester Syndome characterized by the multicentric osteolysis and arthritis. Deficiency in MT1-MMP leads to severe growth retardation, craniofacial deformities, angiogenic defects and premature death in mouse.

Previously, most works involving MT1-MMP have solely related those developmental defects to the defective collagen remodeling, which cannot provide a precise molecular mechanistic explanation for those defects. We found that defective calvarial osteogenesis in *Mmp14*$^{-/-}$ mice is a consequence of compromised Fibroblast Growth Factor (FGF) signaling. In calvarial osteoblasts, the loss of MT1-MMP reduces cell surface presentation of FGFR2 in conjunction with formation of truncated FGFR2 fragments. The increased ectodomain shedding of FGFR2, mainly mediated by ADAM9, subsequently compromises FGF signal perception on cell surface and results in defective FGF-induced proliferation and downstream MAPK signaling. Furthermore, MT1-MMP can form a tertiary complex with FGFR2 and ADAM9, in which MT1-MMP proteolytically inactivates ADAM9 to protect FGFR2 from shedding mediated by ADAM9. Interestingly, targeted deletion of *Adam9* completely restores the defective FGF signaling and largely rescues the calvarial bone defects in *Mmp14*$^{-/-}$ embryos. These results suggest that MT1-MMP inactivates ADAM9 to maintain optimal FGF signaling for calvarial development, revealing a novel regulatory mechanism for FGF signaling (Figure A) (*Developmental Cell*, 2012). However, this ADAM9/MT1-MMP dependent regulatory loop for FGF signaling is tissue-specific as depletion of ADAM9 fails to rescue defective FGF-2 induced corneal angiogenesis in *Mmp14*$^{-/-}$ mice, suggesting that other proteins apart from ADAM9 may be involved in MT1-MMP dependent vascular development (*Cell Cycle*, 2012).

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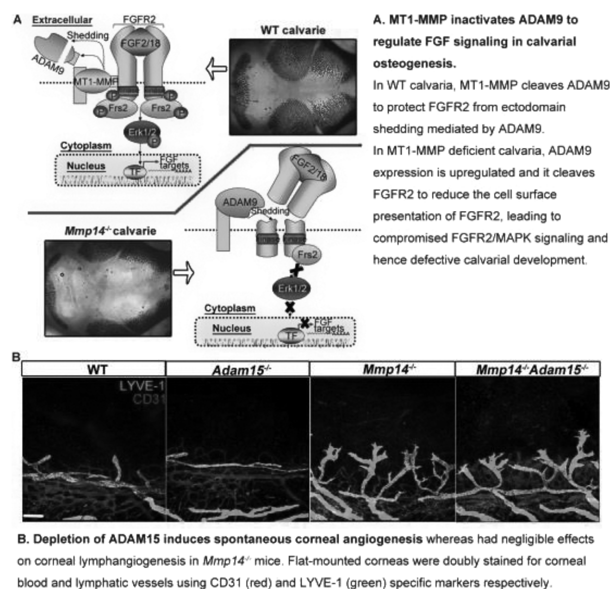

Fig. 1. (A) MT1-MMP inactivates ADAM9 to regulate FGF signaling in calvarial osteogenesis. In WT calvaria, MT1-MMP cleaves ADAM9 to protect FGFR2 from ectodomain shedding mediated by ADAM9. In MT1-MMP deficient calvaria, ADAM9 expression is upregulated and it cleaves FGFR2 to reduce the cell surface presentation of FGFR2, leading to compromised FGFR2/MAPK signaling and hence defective calvarial development. (B) Depletion of ADAM15 induces spontaneous corneal angiogenesis whereas had negligible effects on corneal lymphangiogenesis in *Mmp14*$^{-/-}$ mice. Flat-mounted corneas were doubly stained foi corneal blood and lymphatic vessels using CD31 (red) and LYVE-1 (green) specific markers respectively.

Besides the MT1-MMP and ADAM9 interaction, we also found that MT1-MMP also cleaves and inactivates ADAM15. Indeed, loss of ADAM15 leads to aberrant spontaneous angiogenesis in *Mmp14*$^{-/-}$ corneas (Figure B), indicating that the defects in corneal angiogenesis in *Mmp14*$^{-/-}$ mice are likely attributable to the deregulated activity of ADAM15. Therefore, the interplay between ADAM15 and MT1-MMP is essential for the maintenance of vascular homeostasis.

This work for the first time provides a new molecular explanation for the critical roles of MT1-MMP in the intramembranous ossification and vascular development. These findings raises a new research interest in which the interplay among MT1-MMP, ADAMs and FGFR2 signaling may be important for regulating multiple developmental processes.

Keywords: ADAM, Angiogenesis & Cranofacial Development, MT1-MMP.

SUN-332
The method of immunoassay for detection of antibodies against oxidized LDL using colloidal gold nanoparticles

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Atherosclerosis has increasing trend, so the sensitive and specific laboratory methods for early detection are of great importance. Atherosclerosis is *characterized* by the development of an arterial occlusion containing lipids and cellular deposits. In the recent years the autoimmune character of atherosclerosis was shown. The severity of this disease directly linked to activation of immune system. The cause of atherosclerosis is the oxidative modification of low density lipoproteins (LDL), which brings to the development of many pathological processes promoting both cellular and humoral immunity. Oxidized LDL (ox-LDL) appears in blood flow and binds to some plasma proteins: β_2-glycoprotein, C-reactiv protein. With these proteins ox-LDL initiates forming of highly immunogenic complexes. As a result antibodies against ox-LDL are developed in the blood of patients. The role of these antibodies is not clear. On the one hand, the detection of anti-ox-LDL antibodies in the serum of patients indicates atherosclerosis. On the other hand, there are several data reporting the presence of minor amounts of such antibodies in serum of healthy subjects too. Nevertheless, these antibodies can be accepted as diagnostic markers for detection of atherosclerotical processes and may be applied for evaluation of the severity of the disease.

The purpose of the presented work was the development of simple and accurate method for detection of anti-ox-LDL antibodies based on colloidal gold nanoparticles. The reason for the use of nanoparticles in immunoassays is those unique optical properties. Gold nanoparticles are colored in solution due to the surface plasmon resonance. In our study immunoassay using gold nanoparticles was developed to determine the anti-ox-LDL antibodies. 20 nm gold nanoparticles were prepared by the method of Frens. Ox-LDL was conjugated to these nanoparticles. Ox-LDL extracted from the atherosclerotic lesions has similar properties to ox-LDL induced in vitro by Cu^{2+}. The oxidation level of ox-LDL was determined by quantifying MDA (in the reaction with TBA) and protein carbonyl content (in the reaction with 2,4-DNPH).

The conjugation conditions were optimized at different pH values. The stabilization point of ox-LDL was determined. The conjugates were analyzed with serum of patients suffered with atherosclerosis, as well as with anti-human rabbit ox-LDL antibodies. The presented system will give a possibility to determine rapidly anti-ox-LDL antibodies both in serum of atherosclerosis patients and healthy donors. The results of our investigations in all probability will allow us to clarify the diagnostic role of anti-ox-LDL antibodies, about which currently there are different opinions.

Keywords: atherosclerosis, colloidal gold, low density lipoproteins.

SUN-333
The novel collagen peptide reduced the photo-aging induced by UVA irradiation

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Ultraviolet ray (UV) irradiation from the sun has deleterious effects on human skin, including sunburn, photo-aging. Skin photo-aging may occur due to the generation of reactive oxygen species (ROS) such as hydroxyl radical (HO·), superoxide anion radical ($O_2^{\cdot -}$), singlet oxygen ($^1O_2$) induced by UV irradiation. The chronic injury induced by ROS may damage fibroblasts in the dermal layer.

We have developed the pepsin-treated novel collagen peptide (P-NCP), which has a low allergenic potential over conventional products. Although P-NCP is used in cosmetics for excellent moisture retention and water-holding capacity, the purpose of using P-NCP except for these advantages has not been investigated. In the present study, we set out to examine the direct antioxidant effects of P-NCP on ROS generation using electron spin

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resonance (ESR), and to determine the effect of P-NCP on cell viability after UVA irradiation of human fibroblasts.

We investigated the antioxidant effects of P-NCP on ROS by ESR spin trapping with 5,5-dimethyl-1-pyrroline-*N*-oxide or 2,2,6,6-Tetramethyl-4-piperidinol and the effects on cell viability after UVA irradiation of human fibroblasts. P-NCP was fractionated into 19 fractions by gel filtration chromatography and the antioxidant effects of each fraction on $^1O_2$ were measured using ESR.

We confirmed that P-NCP directly inhibited $HO^•$ and $^1O_2$. Furthermore, addition of P-NCP to fibroblasts inhibited cell death induced by UVA irradiation in a dose-dependent manner. In addition, the antioxidant effect on $^1O_2$ was observed in the peptide fractions rich in Gly, Pro, Hyp, Glu, Ala, and Arg. Although Gly, Pro, Hyp, Glu, and Ala directly inhibited $^1O_2$, we found that together with these five kinds of amino acids, Gly-X-Y, which composed a repeating sequence of collagen, plays a key role in scavenging $^1O_2$. These results suggested that P-NCP can inhibit photo-aging related to ROS generation due to its antioxidant effects.

Keywords: Collagen peptide, ROS, UVA.

SUN-334
The nuclear receptor Nor1/Nr4a3, a novel regulator of beta-cell mass

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Background: The insulin-secreting pancreatic beta-cell plays a central role in the maintenance of glucose homeostasis and in the pathogenesis of both type 1 and type 2 diabetes mellitus. In both cases, production of pro-inflammatory cytokines causes beta-cell apoptosis, which results in the progressive deterioration of beta-cell mass and function. Thus, it is important to understand the molecular mechanisms underlying the regulation of beta-cell 'life and death' as this could lead to the development of new therapeutic options.

Our previous studies have identified *Nor1* as a potentially important gene in beta-cells. The *Nor1* gene encodes a nuclear receptor of the Nr4a family. Whereas Nor1 has been extensively studied in other tissues, its biological roles in the beta-cell remain relatively unexplored.

Aim: We therefore sought to investigate the expression, activity and potential role of Nor1 in the regulation of pancreatic beta-cell mass.

Results: Pro-inflammatory cytokines produced a significant up-regulation of Nor1 mRNA and protein levels in beta-cells. Interestingly, this effect seemed specific to Nor1, as other members of the *Nr4a* family were less affected by cytokines. Cytokines induced the translocation of Nor1 to the mitochondria, where it has been previously shown to exert pro-apoptotic actions. Over-expression of Nor1 induced beta-cell apoptosis but did not significantly impact proliferation. Conversely, siRNA-mediated silencing of *Nor1* abolished cytokine-induced apoptosis. Nor1-Knockout (KO) mice presented an increased beta-cell mass compared to wild type animals. Consistently, Nor1-KO animals displayed better glucose tolerance after injection of a glucose bolus.

Conclusion: Our study characterizes Nor1 as a mediator of cytokine-induced beta-cell death. We thereby demonstrate a critical role for Nor1 in the regulation of beta-cell mass and identify Nor1 as a new molecular target for the treatment of diabetes.

Keywords: Apoptosis, Cytokines, Diabetes.

SUN-335
The production of interleukins 8 and 10 are changed through modulation of NMDA receptor in lymphocytes

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Human T lymphocytes express ionotropic and metabotropic glutamate receptors which control immune responses, cell activation and death. Long-term circulation of T lymphocytes in blood may lead to downregulation/desensitization of glutamate receptors. This downregulation can change the intracellular signaling specificities and functional activities of lymphocytes. σ-ligands, like pentazocine and dextrorphan, have specificity and bind to NMDA glutamate receptors. Taking into account that during neuroinflammation, peripheral infiltrated leukocytes activate and release inflammatory molecules, including IL-8 and IL-10, we have examined the effects of NMDA and σ1-receptor ligands on IL-8 and IL-10 secretion by human lymphoid cells.

To evaluate the glutamate- and σ-mediated production of cytokines, we examined the action of noncompetitive NMDA receptor antagonist MK-801, ligand with certain σ-activities – dextrorphan and σ-agonist-pentazocine on the synthesis of IL-10, IL-8 in the Jurkat cell line. We have found that σ-ligands decreased the NMDA-stimulated synthesis of IL-8 and enhanced the production of IL-10. Since glutamate receptors are active in the control of T cell activation, we have studied the responses of peripheral blood lymphocytes (PBL) to various concentrations of glutamate and found that glutamate concentrations within normal plasma levels modulate the secretion of IL-10 positively, whereas the synthesis of IL-8 is not changed significantly. Since chronic activation of glutamate receptors by plasma glutamate in PBLs could induce receptor down-regulation, we examined the effect of high and low glutamate concentrations on the internalization of NMDA receptor subunits in PBLs. Our results have shown that long-term treatment of PBLs with glutamate at concentrations within normal plasma levels, in contrast to low concentrations, down-regulates the NR2A subunit. All together suggest the involvement of NMDA glutamate and σ1-receptors in the production of IL-8 and IL-10 by T lymphocytes.

Keywords: IL-8, IL-10, lymphocytes.

SUN-336
The protective role of autophagy in RAW 264.7 cells exposed to Si/SiO2 QDs is inhibited or augmented by oxidative stress?

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The persistent and intense fluorescent emission of quantum dots (QDs) makes them ideal for monitoring of biological processes at cellular level. However, QDs exposure is potentially cytotoxic and can induce cell death. There are several types of programmed cell death, one of them being autophagy. There is much debate whether nanoparticle induced autophagy is a toxic effect or a protective response to cell stress. For a better understanding of its function we tried to identify the cellular context accompanying autophagy.

The fluorescent Si/SiO_2 QDs were synthesized by laser ablation and had an average diameter of 5 nm, with a crystalline Si core. The 24 h half maximal inhibitory QDs concentration in

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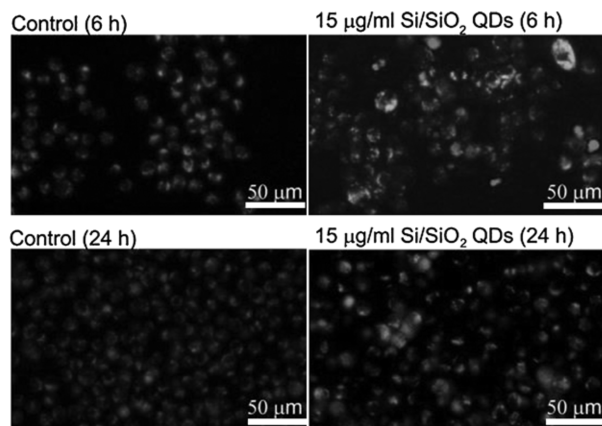

Fig. 1.

RAW 264.7 macrophages was 15.26 μg/ml and corresponded to a 45% decrease in live cell counts and a 38% decrease in viability by MTT assay. All further investigations were done on 15 μg/ml Si/SiO_2 QDs treated cells for 6, 12 and 24 h. Live cell numbers were reduced by 26% and 39% after 6 respectively 12 h, while MTT indicated 22% and 30% viability decreases.

Monodansylcadaverine stained authophagic vacuoles (light blue) and propidium iodide (red) stained dead cells observed by fluorescence microscopy (see figure) indicated that the autophagic process was most intense after 6 h. Microtubule-associated protein light chain 3 – II (LC3-II) immunobloting confirmed a maximal 4.6 fold expression increase after 6 h and a decrease thereafter. Longer QDs exposure increased dead cells number. Beclin 1 protein expression was decreased by 20 and 43% after 12 and 24 h, enforcing the autophagy inhibition.

Reactive nitrogen and oxygen species generation are central mechanisms of QDs toxicity. In moderate quantity, they are involved in cell signaling, being known inducers of autophagy. After the 24 h exposure, nitric oxide levels increased by 1.74 fold, while reactive oxygen species (ROS) rose by 1.8 fold. Lactate dehydrogenase levels in culture media were 1.6, 1.3 and 2.8 fold higher compared to control after 6, 12 respectively 24 h, emphasizing the process of necrotic cell death (also observed by bright field microscopy). Also, the levels of prostaglandin E2 in culture media of QDs exposed cells increased by 5.6, 4.3 and 4.7 fold after 6, 12 respectively 24 h.

Our data suggest an inverse relationship between autophagy and necrosis in the RAW 264.7 cells exposed to Si/SiO_2 QDs which is time dependent. Autophagy had a protective role in moderate oxidative stress, but at higher levels of nitric oxide and ROS it was inhibited and inflammation, followed by necrosis occurred.

Keywords: autophagy, macrophages, nanoparticles, inflammation, cell death.

SUN-337

The role of adiponectin in dexamethasone-induced intrauterin growth restricted rat placental development

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Placenta is an organ which acts as a barrier between maternal and fetal circulation during pregnancy and plays an important role in fetal development and growth. A healthy pregnancy is closely associated with normal placental development. Overexposure to maternal glucose and glucocorticoids causes several placental and fetal pathologies. Placenta tissue is a target for circulating adiponectin. It is not clear how adiponectin affects placenta and fetus in preeclampsia, diabetes and intrauterine growth restriction (IUGR). The aim of this study was to investigate the effect of adiponectin on placental growth in rats with intrauterine growth restriction.

IUGR was provided by injection of dexamethasone. Adiponectin and its receptors (AdipoR1 and AdipoR2) were detected in the control and IUGR rats placentas on day 14, 16, 18, and 20 of pregnancy using Western Blot and RT-PCR techniques. For adiponectin signal transduction, AMPK and eNOS proteins were measured by Western Blot technique. Serum concentrations of adiponectin were assessed by ELISA method.

There was a weight loss in dexamethasone injected rat placenta and embryo. We showed that adiponectin, AdipoR1 and AdipoR2 protein and mRNA expressions were increased in the IUGR group compared to the control group. We found that maternal dexamethasone treatment led to a decrease in AMPK and eNOS phosphorylation during rat placental development. Serum adiponectin levels were significantly lower in the IUGR group.

The reason of weight loss in dexamethasone injected rat placenta and embryo can be related to AdipoR1 and AdipoR2 proteins which are members of AMPK signal pathway. Our data indicated the increased AdipoR1, AdipoR2 protein and mRNA levels in the IUGR rat placentas on day 14, 16, 18, and 20 of pregnancy. We conclude that upregulation of adiponectin and its receptors provide normal placental growth in the IUGR placentas in contrast to glucocorticoids. Our study emphasizes the role of adiponectin to eradicate the glucocorticoids's adverse effects.

Keywords: Adiponectin, Intrauterine growth restriction, Placenta.

SUN-338

The role of ADP dependent glucokinase in the peroxide production of HL-60 cells

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ADP dependent glucokinase (ADPGK) catalyses glucose phosphorilation using ADP as a phophoryl donor thus saving ATP. ADPGK is highly expressed in leukocytes and has a possible role in the antibacterial defense mechanism. The glucose-6-phosphate produced by this enzyme metabolised through various pathways e.g. the pentose-phosphate pathway thus contributing to NADPH production. Among the effector functions of neutrophile granulocytes the reactive oxid species produced by NADPH oxidases has a large significance. We examined various aspects of ADPGK in undifferentiated and neutrophile-like, differentiated HL-60 cells. For the latter we used dimethyl-sulfoxide. Using qPCR we found that differentiated cells has a three-fold higher ADPGK expression than the undifferentiated cells. This difference was confirmed by Western-blot experiments as well. After transfection of both cell types with ADPGK siRNA, we measured peroxide production by Amplex Red method and found, that ADPGK silencing reduced peroxide production in the differentiated cells. These results indicate the ADPGK has a potential role in the bactericidal properties of neutrophil granulocytes by infulencing the peroxide production of these cells.

Keywords: None.

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SUN-339
The role of exogenous Hsp70 in ROS production in monocytes cell lines

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Reactive oxygen species (ROS) play an essential role in the organism functioning. One of the most important aspects of the ROS biological activity is associated with their damaging effect on tissue. And D. Harman hypothesis about an important role of the ROS damaging effects in the process of aging is well known. However, many aspects of ROS production regulation in cells remain unclear.

We have shown previously that one of the endogenous factors that cause suppression of the excessive ROS production by human phagocytes may be the heat shock protein 70 kDa (Hsp70) extracellular form.

To study the mechanisms of respiratory burst amplitude reducing in presence of Hsp70 we studied this process on THP-1, RAW 264.7 and J774 cell lines. ROS detection was carried out using the luminol-dependent chemiluminescence method and cells were activated by phorbol 12-myristate 13-acetate (PMA) in concentration 25 ng/ml to 2 μg/ml.

We have shown that PMA in concentration 2 μg/ml causes snowballing increasing of ROS level in THP-1 cells up to ten-fold comparing with control level. Activating cells by less PMA concentration resulted weak response. 30 min preincubating with 5 μg/ml Hsp70 resulted significant decrease of both background ROS production level and oxidative burst amplitude.

Snowballing ROS production was detected in RAW 264.7 and J774 cell lines after stimulation with PMA in concentration 500 and 100 ng/ml respectively. Significant PMA-induced ROS production decreasing effect of Hsp70 was also detected in this models. Additionally background ROS level suppressing effect of Hsp70 was confirmed on J774 cell line.

So, the antioxidant effect of exogenously supplied Hsp70 on spontaneous and induced ROS production was shown using different cell models.

Tris work is supported by Russian Foundation for Basic Research (RFBR) grant no. 14-04-32203-мол_а.

Keywords: monocytes activation, oxidative burst, reactive oxigen species.

SUN-340
The role of macrophage inositol requiring enzyme-1 in lipid-induced inflammation

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The epidemic increase in obesity constitutes a serious global health threat. Obesity increases the risk of many metabolic diseases such as insulin resistance, diabetes, fatty liver disease, and atherosclerosis. Metabolic overloading in obesity leads to a low grade, chronic inflammation in metabolically active tissues, known as metaflammation and causal to these pathologies that cluster around obesity. However, the underlying mechanism in the development of metaflammation remains largely unknown.

Metabolic overloading leads to stress in catabolic and anabolic organelles. Under metabolic stress conditions, such as excessive exposure to fatty acids and cholesterol, endoplasmic reticulum (ER) activates its unique membrane bound stress response system known as the unfolded protein response (UPR) to recover cellular homeostasis. ER has a crucial role in both metabolic stress-sensing and the initiation of inflammatory signaling pathways. However, the molecular mechanisms that link the ER stress response to inflammation under metabolic stress conditions is not well understood largely because specific tools to modulate the activities of the different UPR branches have been lacking. In this study, we specifically modulated the IRE1 branch to determine its contribution to lipid-induced inflammation in macrophages.

The classic UPR signaling is coordinated by three ER membrane-bound proteins; inositol-requiring enzyme 1 (IRE-1), PKR-like endoplasmic-reticulum kinase (PERK) and activating transcription factor 6 (ATF-6). The most evolutionary conserved arm of UPR involves the IRE1, which oligomerizes and autophosphorylates to activate its dual kinase and endoribonuclease activities under ER stress conditions. Here, by using specific IRE1 endoribonuclease inhibitors and RNAi-based knock-down we aimed to delineate the differential role of IRE1's kinase and endoribonuclease activities in translating metabolic stress into inflammatory response. We show that IRE1, in particular its endoribonuclease domain, regulates both the induction of the inflammasome and the production of pro-inflammatory cytokines in response to lipid stress in primary bone marrow derived mouse macrophages. Previous studies have shown mitochondrial reactive oxygen species (ROS) production plays a crucial role in bridging saturated fatty acids to inflammasome activation. Indeed, fatty acid induced mitochondrial ROS production is markedly suppressed in the IRE-1-deficient mouse embryonic fibroblasts. Taken together, our results demonstrate lipid-induced ER stress can activate inflammation through IRE1, in particular through its endoribonuclease domain. Specific endoribonuclease inhibitors for IRE-1 may present a novel potential therapeutic approach to metabolic disorders.

Keywords: None.

SUN-341
The role of Nox4 in oxidative stress underlying psoriasis pathology proved by in vitro fibroblasts-keratinocytes co-culture model

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The reductive-oxidative (redox) imbalance in the blood and skin of patients affected by psoriasis vulgaris was previously highlighted. In the present study, we established human primary fibroblasts cultures from the skin of 6 psoriatic patients and of 5 healthy subjects (controls). First, the redox profile of these fibroblasts was investigated by luminometric and fluorometric methods, flow cytometry, confocal microscopy and commercially available kits. We found significantly increased levels of total intra- and extra-cellular ROS production, lipoperoxidation, TBARS concentrations, and intracellular Ca^{2+}; decreased total antioxidant capacity in psoriatic fibroblasts with respect to control ones. The activation of MAPK pathways and increased expression of NFkB were also shown in psoriatic fibroblasts. Further, we showed highly increased superoxide production by mitochondrial electron transport chain and NADPH oxidase 4 (Nox4) expression in psoriatic fibroblasts. Second, to investigate whether the psoriatic fibroblasts can affect the redox profile of normal keratinocytes, in vitro fibroblasts-keratinocytes co-culture model was created. The main advantages of this in vitro model is its prevention of mixing keratinocytes with fibroblasts, allowing to study the effects of co-culture for each cell types separately. The co-incubation of human keratinocyte permanent cell line HACAT with psoriatic fibroblasts leaded to a significant increase in intra-cellular ROS production levels and to the activation of

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MAPK pathways in these cells with respect to the ones co-incubated with control fibroblasts. The knockdown of Nox4 with siRNA in psoriatic fibroblasts abolished these effects in keratinocytes co-incubated with silenced psoriatic fibroblasts.

Our results demonstrated the notable redox imbalance in dermal fibroblasts of psoriatic patients, which can be due to the increased activity of NADPH oxidase and mitochondrial electron transport chain. The proposed in vitro fibroblasts-keratinocytes co-culture turn to an easy-to-manage model for studying cellular pathology and interaction in the skin of psoriatic patients. Finally, the NADPH oxidase isoform 4 can be proposed as a potential target for adjuvant local therapy of psoriasis.

Keywords: Fibroblasts, NOX4, Psoriasis.

SUN-342
The role of oxidative stress and serum lipid levels in stable chronic obstructive pulmonary disease

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Objective: Chronic obstructive pulmonary disease (COPD) is has been associated with increased oxidative stress or reduced antioxidant resources. The main goal of this study was to evaluate serum ıschemia modified albumin (IMA), total oxidant status (TOS) levels and total antioxidant status (TAS) in stable COPD patients compared with the control group.
Methods: The study was performed on 51 stable COPD patients and 45 healthy controls. Serum lipids, IMA, TAS and TOS levels of the subjects were measured.
Results: Serum IMA levels and TOS levels of the COPD patients were significantly higher than those of control subjects. There was no difference between serum TAS, triglycerides, total cholesterol, LDL-C levels of the COPD patients and those of the control subjects. Serum HDL-C levels of the COPD patients were significantly lower than the corresponding levels of control subjects.
Conclusions: Our study indicates that serum IMA and TOS may play an important role in oxidative stress in COPD. The measurement of serum IMA and TOS levels may be useful for the evaluation of stable COPD.

Keywords: Chronic obstructive pulmonary disease, IMA, TOS and TAS, oxidative stress.

SUN-343
The role of PAI-2 in NLRP3 inflammasome activation and endotoxin-induced sepsis

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The NLRP3 inflammasome, a multiprotein complex, triggers caspase-1 activation and maturation of proinflammatory cytokines IL-1β and IL-18 upon sensing a wide range of pathogen- and damage-associated molecules. Dysregulation of NLRP3 inflammasome activity contributes to the pathogenesis of many diseases including cancer and metabolic disease, but its regulation remains poorly defined. Plasminogen activator inhibitor 2 (PAI-2), a member of a large group of proteins that inhibit serine proteases, is regulated by IKKβ-dependent activation of the transcription factor NF-κB. Previous studies demonstrated that mice with *Ikk*β deletion in myeloid cells are hypersusceptible to endotoxin (LPS)-induced septic shock, and increased LPS susceptibility is associated with elevated plasma IL-1β. Reconstitution of *Ikk*β-deficient macrophages with PAI-2 blocks IL-1β release after LPS stimulation. To further elucidate the physiological role of PAI-2 in LPS-induced septic shock, we generated transgenic mice that specifically express PAI-2 in myeloid cells, and then crossed this transgenic mice with myeloid-deleted *Ikk*β mice. PAI-2 expression decreased plasma IL-1β in myeloid-deleted *Ikk*β mic after LPS challenge. In addition, PAI-2 transgenic mice showed a decrease in circulating IL-1β level upon *Escherichia coli* infection. We also demonstrated that depletion of PAI-2 in macrophages resulted in NLRP3- and ASC-dependent caspase-1 activation and IL-1β secretion in macrophages in response to TLR activators and *E. coli* infection with no need of a second stimulus. Together, our data identify a new tier of TLR signaling in controlling NLRP3 inflammasome activation.

This work was supported by grants from National Science Council, Taiwan (NSC100-2320-B-002-096-MY3).
Keywords: Cytokines, inflammasome, Sepsis.

SUN-344
The role of PON1 and CC16 protein as antioxidant in young persons exposed to tobacco smoke

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Clara cells secretory protein (CC16) is produced by bronchiolar club cells. Its secretion can be modulate by cigarette smoking. Low concentration of CC16 in blood is typical for injury due to cigarette smoking. It is known that CC16 serum concentration is related to lipids system. Paraoxonase 1 (PON-1) is HDL-attached extracellular esterase, which contributes to protection of HDL against peroxidation. Both CC16 and PON-1 have antioxidative and anti-inflammatory properties against oxidative stress from tobacco smoke.

The aim of the study was to investigate a possible association between CC16 concentration and PON-1 activity in the individuals exposed to tobacco smoke.

The investigations were performed in the serum of 27 healthy persons. Data about smoking were obtained from direct personal interview and were verified by the determination of cotinine concentration – the metabolite of nicotine (ELISA method).The study population was in similar age (23.73 ± 4.83 years) and similar BMI (21.49 ± 2.87 kg/m^2). CC16 concentration was determined by ELISA kit. PON-1 activity was measured spectrophotometric method using paraoxon as substrate. BMI was calculated as weight/hight square [kg/m^2].

It was shown a significant decrease in PON1 activity in smokers (61.40 ± 32.12 IU/l) compared to non-smokers (117.54 ± 56.04 IU/l) ($p = 0.0071$). However, the difference in CC16 concentration between examined groups was not observed (28.99 ± 11.57 ng/ml for smokers and 28.11 ± 10.60 ng/ml for non-smokers). It was shown a correlation between CC16 concentration and PON1 activity ($r = 0.6573$, $p = 0.0202$) in non-smokers. The link between CC16 concentration and BMI in smokers was also shown ($r = 0.6703$, $p = 0.0122$).

PON1 is a very sensitive maker, which activity can be easy modulated by oxidative stress resulting from tobacco smoke. Decreased PON1 activity can induce the secretion of CC16 protein, which concentration is increased proportionally to BMI of

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persons exposed to tobacco smoke. Probably, both PON1 and CC16 protein play an important role as antioxidants against oxidative stress from tobacco smoke.

Keywords: CC16 protein, paraoxonase, tobacco smoke.

SUN-345
The role of TNF-α in the development and function of dendritic cells activated by collagen I

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Previous reports have demonstrated that dendritic cells (DCs) maturation and function can be regulated by collagen I, a major extracellular matrix (ECM) component. DCs interact with collagen present at the inflammatory sites and regulate the function of DCs. This interaction is known to enhance the expression of co-stimulatory molecules expression and secretion of cytokines such as interleukin (IL)-12 and tumor necrosis factor (TNF)-α. However, the understanding of the role of these cytokines in DCs maturation and function remains incomplete.

We studied the role of TNF-α in the development and function of DCs activated by collagen I. Upon inhibition of TNF-α (TNFn), collagen I-induced IL-12 secretion, expression of DCs maturation markers and genes associated with DCs activation pathway were suppressed. Additionally, we examined if TNFn exhibited any effect on expression of interferon regulatory factor 4 (*IRF4*), a transcription factor of IRF family which is expressed by DCs and is known to have a role in DCs development. Our data demonstrate that collagen I-activated DCs exhibited reduced *IRF4* expression whereas opposite effect was observed when TNF-α was neutralized.

To sum up, our data suggest that collagen I-induced DCs maturation and function is regulated by TNF-α production through down-regulation of *IRF4*. These data may implicate an importance in development of anti-TNF-α therapeutics for the various inflammatory disorders.

Keywords: anti-TNF-α, collagen I, dendritic cells.

SUN-346
The substrate length selectivity of aminopeptidases that generate antigenic peptides

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Endoplasmic Reticulum aminopeptidases (ERAP) 1 and 2, as well as the Insulin-Regulated Aminopeptidase (IRAP), are integral components of the adaptive immune system, due to their active participation in the antigen processing and presentation pathways. ERAP1 and ERAP2 reside in the lumen of the endoplasmic reticulum and trim antigenic peptide precursors to produce mature antigenic epitopes, which associate with Major Histocompatibility Complex class I (MHC-I) molecules that are then transferred to the cell surface for recognition by cytotoxic T-lymphocytes. That recognition allows the organism to constantly monitor for aberrant or infected cells and eradicate them. IRAP resides in specialized intracellular vesicles and participates in a different antigen presentation pathway, called cross-presentation. Previous studies have shown that ERAP1 exhibits a molecular ruler behavior, preferentially trimming longer peptides (9–16 residues), but sparing shorter ones (<8–9 residues), thus generating peptides with optimal length for MHC-I loading. It has also been shown that single nucleotide polymorphisms in these enzymes are associated with predisposition to autoimmune diseases such as Ankylosing Spondylitis, Behcet's disease and Psoriasis, and it has been hypothesized that this is due to altered antigenic peptide generation. In this study we investigated the substrate length specificity of ERAP2 and IRAP as well as a series of disease-linked ERAP1 alleles, using a model peptide-substrate series that carry a polyglycine motif of varying length, designed to probe the length selection preferences of each enzyme. Our results indicate that all ERAP1 alleles, are much more active in trimming peptides of 10 residues or larger, compared to smaller peptides. ERAP2 and IRAP were found to be more permissive in terms of substrate length, processing most effectively peptides of intermediate length. These results are in sharp contrast to the complete lack of length selection by Leucine aminopeptidase, a dominant cytosolic aminopeptidase that does not however participate in antigenic peptide generation. Detailed comparison of the ERAP1 alleles indicated that the polymorphisms have subtle but clearly distinct effects on ERAP1 activity and length specificity, suggesting that the polymorphisms employ at least two different mechanisms to affect enzyme function. Overall, these results indicate that each enzyme exhibits different substrate length specificity and this specificity can be influenced by allelic variation, something that may underlie the mechanisms behind disease predisposition.

Keywords: None.

SUN-348
TIMP-1 loaded nanoparticles: a therapeutic strategy for neuroprotection

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Introduction: There is a marked, deleterious increase in expression of Matrix Metalloproteinase-9 (MMP-9) during numerous pathologic conditions such as ischemic stroke, epilepsy and various excitotoxic/neuroinflammatory processes. Therefore, inhibition of MMP-9 is considered as a potential therapeutic target for neuroprotection. Currently available chemical inhibitors of MMP-9 are poorly specific and have many off-targets leading to unanticipated side effects. As development of specific inhibitors is always a challenging task therefore, we planned to evaluate neuroprotective effects of an endogenous inhibitor of MMP-9, Tissue Inhibitor of Matrix Metalloproteinase-1 (TIMP-1), which is a 28 KDa protein. However, the major obstacles of using TIMP-1 as a neuroprotective agent are its in vivo short half-life and low brain permeability. Hence, we planned to explore a nanotechnological approach for delivery of TIMP-1, by using poly lactic-co-glycolic acid (PLGA) based Nanoparticles (NPs), so in the future it can be developed as a neuroprotective agent.

Results: Here, we have developed TIMP-1 loaded PLGA NPs which can deliver TIMP-1 in a sustained release manner and can cross the blood brain barrier (BBB). These NPs were coated with polysorbate 80 (Ps80) to improve their BBB penetration. We evaluated these NPs for their in vitro and in vivo BBB penetration by using primary rat brain endothelial cell model and by tail vein injection in mice respectively. The in vitro and in vivo results have shown that NPs are non-toxic to endothelial cells and they have BBB penetration. Finally, we evaluated their neuroprotective effects on organotypic hippocampal slice culture using propidium iodide staining and LDH assay which have shown that TIMP-1 and TIMP-1 loaded have neuroprotective

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effects against Kainic Acid (KA) induced excitotoxicity. Moreover, we have shown through gelatinase assay that these effects are mediated through MMP-9 inhibition. Currently, we are exploring in vivo neuroprotective effects of TIMP-1 NPs.

Keywords: Matrix Metallo-Proteinase, Nanoparticles, Neuroprotection.

SUN-349

An in vitro study on the risk of non-allergic type-I like hypersensitivity to *Momordica charantia*

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Background: *Momordica charantia* (MC) is a tropical plant that is extensively used in folk medicine. However, the knowledge about side effects of this plant is relatively little according to knowledge about its therapeutic effects. The aim of this study is to reveal the effects of non-allergic type-I like hypersensitivity to MC by an experiment which was designed in vitro.
Methods: In the present study, the expression of CD63 and CD203c on peripheral blood basophils against different dilutions of MC extracts was measured using flow cytometry and compared with one another. In addition to this, intra-assay CV's of testing extracts were calculated for precision on reproducibility of test results.
Results: It was observed that the fruit extract of MC at 1/100 and 1/1000 dilutions significantly increased active basophils compared to same extract at 1/10 000 dilution.
Conclusion: In conclusion, *Momordica charantia* may elicit a non-allergic type-I like hypersensitivity reaction in especially susceptible individuals.

Keywords: None.

SUN-350

Toxic molecular mechanisms induced by magnetite nanoparticles in human pulmonary fibroblasts

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The control of biomolecules-nanoparticles interaction from molecular to cellular levels is crucial for potential risks of adverse effects on human health.

The aim of our study was to investigate the molecular mechanisms involved in the inflammatory and cell death processes in human pulmonary fibroblasts (MRC-5 cell line) after magnetite nanoparticles (MNP) exposure.

In order to accomplish these, MRC-5 cells were exposed to 12.5 μg/mL MNP for 24, 48 and 72 hours. As inflammatory markers, the level of prostaglandin-E2 (PGE_2), nitric oxide (NO) and the production of interleukin (IL)-6 and -8 were assessed using biochemical analyses. MNP potential to induce cell death in MRC-5 cells was evaluated by quantifying caspases activity and apoptotic cells through Annexin V/PI staining.

Results have shown a significant stimulation of NO and PGE_2 synthesis after 72 hours by 81% and 82% respectively, compared to control. A slight increase in IL-6 production occurred starting after 48 hours but no significant changes were observed in IL-8 levels. MNP caused no apoptotic events in pulmonary fibroblasts during the analyzed period but an increase of the caspase-1 activity by 35%, 21%, 25% after 24 h, 48 and 72 h compared to controls was noticed. It may be that activated caspase-1 regulated the release of pro-inflammatory cytokine as IL-1β generating the release of NO and PGE_2 into the extracellular milieu. The synthesis of PGE_2 could also be caused by IL-6.

Our findings suggest that human pulmonary fibroblasts exposed to MNP generates an inflammatory response through activation of caspase-1 and release of NO and PGE_2.

Keywords: nanoparticles, inflammation, cell death.

SUN-351

Toxicogenomic analysis of *Capparis ovata* water extract (MSCov)

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Capparis ovata water extract (*MSCov*) has been used for the treatment of multiple sclerosis and other immune diseases. However, its potential toxicity has not been completely investigated. The aim of the study was to determine the toxicology of *MSCov* in C57BL/6 mice and to investigate the underlying cellular mechanism further by microarray analysis. For this purpose, *MSCov* was prepared by using the plant's fruit, bud and flower parts (Turkish Patent Institute, PT 2012/04 093). Doses of *MSCov* at 500 mg/kg for 21 days were administered by oral gavage for subchronic toxicity in C57BL/6 mice. At the end of the experimental period, the animals were sacrificed, the liver tissues were isolated. Changes in the hepatic gene expression were identified with toxicology pathway PCR arrays to study the hepatotoxic mechanism of *MSCov*. A number of changes in the body weight and food consumption, absolute and relative liver weight, biochemical analysis were not observed after the subacute exposure to *MSCov*. A total 384 genes were screened which 14 genes were found to be significantly altered (2-fold, $P < 0.05$), including 14 up-regulated genes and no down-regulated genes. According to our biological pathway analysis, the *MSCov* resulted in aberrant gene expression in metabolic pathways such as phospholipids, cholesterol and asis and fatty acids. Real-time PCR analyses of several genes verified these results. Consequently, our gene expression microarray study will be useful for future *MSCov* toxicity studies. These results strongly suggest that *MSCov* has no or very very of low potenctial of toxicity and considered to be safe as an alternative or complementary therapeutics in MS treatment at the studied dose regime.

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Keywords: Toxicogenomic, Capparis ovata, multiple sclerosis.

SUN-352

Transcription factor hypoxia-inducible factor (HIF)-1alpha is relevant for necrosis of *Mycobacterium avium*-induced granulomas

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Mycobacterial infections are characterized by the formation of granulomas. Granulomas are well-organized aggregates of immune cells, namely infected macrophages. The granuloma's main function is to constrain and prevent dissemination of the mycobacteria while concentrating the immune response to a limited area. In some cases these granulomas undergo central necro-

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sis leading to their caseation although the underlying mechanisms are still poorly understood. It has been reported that reduced vascularization of granulomas may be one essential mechanism for caseation and some studies have demonstrated severely hypoxic regions at the center of the granuloma. The hypoxia-inducible factor – 1alpha (HIF-1α) has been shown to be important in some diseases, such as cancer and infections. HIF-1α is able under hypoxic conditions to transcriptionally regulate gene expression, allowing macrophage adaptation to hypoxia [1]. The Appelberg laboratory has developed a granuloma necrosis model that mimics the human pathology of *Mycobacterium tuberculosis,* using C57BL/6 (WT) mice infected intravenously with a low dose of a highly virulent strain of *Mycobacterium avium* (ATCC 25291). Such mice develop granulomas that, at 4 months of infection, exhibit central necrosis [2]. To determine the relevance of HIF-1α during *M. avium* infection we used a mouse strain deleted of HIF-1α under Cre-lox system in the myeloid cell lineage (HIF1αKO). Infected mice were euthanized at different times during the infection and the lungs, liver and spleen were aseptically collected. Bacterial loads were determined in the organs of infected animals. Morphometric analysis of granulomas was performed in haematoxylin-eosin stained liver sections. The localization of macrophages in the livers from infected mice was studied by immunohistochemistry by evaluating the expression of F4/80. The analysis of liver and spleen cell populations were determined by flow cytometric analysis. IFN-gamma and HIF-1α production has been evaluated *ex-vivo* by ELISA. The results obtained indicate that HIF1α KO mice are more susceptible to the infection and the onset of necrotic granulomas is faster.

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Keywords: Granuloma, Hypoxia, Mycobacteria.

SUN-353
Urinary hydroxyproline levels in prediabetic patients

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Objective: The metabolic stage between normal glucose homeostasis and diabetes mellitus (DM) is called prediabetes. Prediabetes is categorized as impaired glucose tolerance (IGT) and increased fasting glucose (IFG), both established risk factors for DM. Although the relationship between type 2 DM (T2DM) and bone metabolism has been widely investigated, study results have been contradictory. But it is clear that fracture risk is higher in these patients. Recently, it was reported that urinary hydroxyproline increased markedly in diabetic patients. We investigated the urinary hydroxyproline concentrations in patients with prediabetes and T2DM and compared the results to those of normoglycemic individuals at baseline and 2 hours after glucose loading.

Material and Methods: The ADA 2013 criteria were used to identify subjects. These persons were defined as having IFG (100–125 mg/dL) or IGT (2-h values in the oral glucose tolerance test (OGTT) of 140–199 mg/dL) as prediabetes. Based on a 75 g OGTT, subjects were divided into a group with normal glucose tolerance (NGT; n = 28), prediabetes (n = 29) or T2DM (n = 24). Urine concentrations of hydroxyproline were measured by spectrophotometric assay. The hydroxyproline results were expressed as mg/g creatinine. Creatinine in urine samples was determined by Jaffe′s method.

Results: Urinary hydroxyproline levels in patients with T2DM were found to be significantly higher compared with NGT ($p < 0.05$). Urinary hydroxyproline levels in prediabetic patients were higher than NGT group. But it was not found statistically differences ($p > 0.05$).

Conclusion: We suggest that urinary hydroxyproline could be an useful biochemical markers for monitoring possible bone mineral metabolism disorder in T2DM patients. Larger investigations are needed to understand the urinary hydroxyproline levels and bone metabolism in prediabetic patients.

Keywords: diabetes mellitus, hydroxyproline, prediabetes.

SUN-354
Variation within the CASP3 gene and the risk of Achilles teninopathy in a British case-control cohort

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Achilles tendon pathology (ATP) is a degenerative condition with known genetic risk factors[1]. Excessive tenocyte apoptosis has been observed in tendinopathy and components of the apoptosis pathway have previously been implicated in the aetiology of ATP[2]. Caspases play a key role in the execution and regulation of apoptosis, with caspase-3 being an important mediator of apoptosis[3]. Our aim was to determine whether a single nucleotide polymorphism (SNP) within the *CASP3* gene (rs1049253) was associated with ATP in a British cohort. We recruited 264 (133 ATP cases and 131 asymptomatic controls) British Caucasian participants for this genetic association study. ATP cases were clinically diagnosed with insertional tendinopathy, noninsertional tendinopathy, Achilles tendon rupture, or more than one pathology. TaqMan assay technology was used to genotype all participants using real-time PCR. A Pearson's chi-squared (χ^2) test was used to analyse for differences in genotype and allele frequency for the rs1049253 variant. We compared the collective ATP group against controls. We also conducted several sub-analyses taking into account the different types of tendinopathy. We found no significant difference in genotype ($p = 0.643$) or allele ($p = 0.635$) frequencies between the ATP group and controls. However, we did find a tentative genotypic association ($p = 0.025$) between male insertional tendinopathy cases and male controls. These data must be viewed with caution due to the relatively small sample size and replication in a larger cohort would be necessary to increase confidence. In conclusion, our preliminary data infer a possible role for the rs1049253 variant as a risk factor for insertional tendinopathy in British males. These results could further implicate the involvement of the apoptosis pathway in the development of ATP.

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Keywords: Apoptosis, Caspase-3, Tendinopathy.

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SUN-355
Vitamin D association with immune response in inflammatory bowel disease

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Inflammatory bowel disease is a group of chronic inflammatory conditions of the colon and small intestine. Crohn′s disease (CD) and ulcerative colitis (UC) are two main clinicopathological subtypes of IBD. Complex interactions between the immune system, environmental factors, gut microflora and host genotype are considered to underlie the pathogenic process. Vitamin D deficiency has emerged as one of the leading environmental candidates; its causal agency has yet to be fully demonstrated by an adequately powered study. Currently it is suggested that local activation of vitamin D co-regulates activity of the innate and adaptive immunity, mainly through inhibition of the action of pro-inflammatory transcription factor NF-kB and the production of different cytokines. Vitamin D receptor is highly expressed by various cells of the immune system. The panel of 18 genes for gene expression study linked to microbial response in IBD was designed and season-dependent differencies of expression levels were established. A set of 105 samples was analysed for immune profile determination. To investigate the role of genetic background considering vitamin D, we determined allele frequency distribution and genotypes of 4 VDR gene polymorphisms Apa-I, Taq-I, Fok-I and Bsm-I in UC, CD and healthy subjects and analysed their correlation to the immune panel parameters.

This work was supported by the Slovak Research and Development Agency grant APVV-0672-11, and is also result of the ‘Competence centre for R&D in molecular medicine’ (ITMS 26240220071) and . . .Research centre of molecular genetics (ITMS 26240220067) projects implementation supported by the Research and Development Operational Program funded by the ERDF.
Keywords: None.

SUN-356
WMJ-S-001, a novel aliphatic hydroxamate derivative, exhibits anti-inflammatory properties via MKP-1 in lipopolysaccharide-stimulated RAW264.7 macrophages

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Hydroxamate derivatives have been widely investigated and attracted considerable attention, due to their broad pharmacological properties. Recent studies reported their potential use in the treatment of cancer, cardiovascular diseases, arthritis or infectious diseases. However, the inhibitory mechanisms of hydroxamate derivatives in inflammation remain to be elucidated. In an effort to develop a novel pharmacological agent that could suppress abnormally activated macrophages, we investigated the anti-inflammatory effects and the underlying molecular mechanisms of WMJ-S-001, a novel aliphatic hydroxamate derivative, WMJ-S-001, in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages. The LPS-induced increases in *cox-2 mRNA* and protein levels and COX-2 promoter-luciferase activity were suppressed by WMJ-S-001. WMJ-S-001 inhibited p38MAPK, NF-κB subunit p65 and C/EBPβ phosphorylation in cells exposed to LPS. Treatment of cells with a p38MAPK inhibitor (p38MAPK inhibitor III) markedly inhibited LPS-induced p65 and C/EBPβ phosphorylation and COX-2 expression. LPS-increased p65 and C/EBPβ binding to the COX-2 promoter region was suppressed in the presence of WMJ-S-001. In addition, WMJ-S-001 suppression of p38MAPK and p65 phosphorylation, and subsequent COX-2 expression were restored in cells transfected with mitogen-activated protein kinase phosphatase-1 (MKP-1) dominant negative (DN) mutant. WMJ-S-001 also caused an increase in MKP-1 phosphatase activity in RAW264.7 macrophages. Furthermore, WMJ-S-001 increased survival rate in LPS-induced endotoxaemic mice. In conclusion, WMJ-S-001 may cause MKP-1 activation to dephosphorylate p38MAPK, resulting in the decrease in p65 and C/EBPβ binding to the COX-2 promoter region and COX-2 down-regulation in LPS-stimulated RAW264.7 macrophages. The present study therefore suggests that WMJ-S-001 may be a potential drug candidate in alleviating LPS-associated inflammatory diseases.

Keywords: hydroxamate, inflammation, mitogen-activated protein kinase phosphatase-1.

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CSI-03 – Membrane Organization & Super-Resolution

SUN-358
A facile in vitro assay using engineered yeast vacuoles for measuring membrane fusion driven by neuronal SNAREs

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Neurotransmission is mediated by membrane fusion between synaptic vesicles carrying neurotransmitters and the presynaptic plasma membrane. This fusion, known as synaptic vesicle fusion, is mediated by a variety of proteins, including SNARE proteins. Because of the critical role of synaptic vesicle fusion in neuronal communications, it has been thought that control of synaptic vesicle fusion can be used to regulate various neuronal activities and to develop cures to neuronal disorders. One such example is the clinical application of botulinum neurotoxins. Botulinum neurotoxins (BoNTs) are zinc-dependent metalloproteases that specifically cleave one or two of the neuronal SNARE proteins, blocking synaptic vesicle fusion, in a serotype-dependent manner. BoNTs are currently used to treat a wide variety of disorders, such as various neuromuscular diseases, myofascial pain and migraine, and multiple sclerosis. Thus, synaptic vesicle fusion and the synaptic SNARE proteins that mediate the process could be attractive therapeutic targets for various neuro-muscular diseases. To date, however, a facile assay, with high-throughput applicability, assessing synaptic vesicle fusion has been limited. Here, we took a synthetic biology approach and engineered the budding yeast *Saccharomyces cerevisiae* to express neuronal SNARE proteins in the vacuole, the yeast equivalent of human lysosomes. Using engineered yeast vacuoles, we established a facile in vitro assay for measuring yeast vacuole fusion driven by neuronal SNAREs.

Keywords: SNARE, synaptic vesicle fusion, yeast vacuole.

SUN-359
A high content screening microscopy approach to dissect the mechanism of Golgi-to-ER retrograde traffic

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In mammalian cells the biosynthetic pathway delivers newly synthesised molecules to intracellular compartments and the cell surface. At each transport step, forward traffic is counterbalanced by retrograde membrane flow, thereby ensuring organelle homeostasis. Although much is known about the machinery regulating the biosynthetic pathway, comparatively little is known about the retrograde route.

Brefeldin A (BFA) is a fungal metabolite that causes the disassembly of the Golgi complex and the redistribution of resident Golgi markers into the endoplasmic reticulum (ER). BFA treatment of cells results in the redistribution of resident Golgi constituents to the ER, via a process that can be visualised by microscopy in real time. This observation forms the basis of a very powerful morphological assay to assess Golgi-to-ER traffic. Using a cell line stably expressing a GFP-tagged Golgi enzyme, we have carried out two genome-wide gene depletion screens to systematically elucidate this pathway. These results provide the first systematic assessment of proteins associated with Golgi morphology and Golgi-to-endoplasmic reticulum (ER) transport in mammalian cells. Together our data reveal the high complexity and diversity of proteins associated with trafficking between these compartments, and also provides important insight into how the endomembrane system is organised in mammalian cells.

This work is significant on several levels. Firstly, our screens use a novel image analysis approach, incorporating texture feature analysis and machine learning, never before utilised on this scale. Secondly, the systems-wide information that has been gathered may be useful for the design of next generation drug delivery vehicles, allowing targeting of not only certain cell types with appropriate therapeutics, but also specific organelles within the early secretory pathway.

Keywords: high content screening microscopy, membrane trafficking, RNA interference.

SUN-360
A study of the supramolecular structure of K^+ channel clusters

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Multiple lines of experimental evidence highlight the lateral patchiness of bacterial and eukaryotic cellular surfaces, suggesting membranes in which proteins and lipids are organized in heterogeneous domains. This corroborates the notion that membrane signaling proteins like ion channels are assembled in supramolecular clusters, in which channel gating is coupled, possibly to achieve an optimal response to a single stimulus. How clustering occurs, the composition of clusters and how the stimulus may be transferred between channels remains largely unknown, let alone at atomic resolution. Here, using a combination of solid-state NMR experiments, biochemical methods and an extensive set of large-scale MD simulations, we report on the supramolecular structure of clusters of K^+ channel KcsA in complex *E. coli* membranes at unprecedented detail.

We present structural elements of the channel – channel interface, together with a comprehensive study of the membrane micro-environment of KcsA clusters that shows that clustering is accompanied by a stark enrichment of anionic lipids at the channel – channel interface. From the total of our data we infer influences of clustering on KcsA gating. Together with the pronounced influence of buried water[1,2] and specific lipids,[3,4] this study is another step towards the supramolecular structure of K^+ channels in complex physiological environments.[5]

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Keywords: coupled gating, ion channels, lipid – protein interactions.

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SUN-361
Antagonistic role of Eps15 and Eps15L1 in synaptic vesicle recycling

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Synaptic vesicle recycling (SVR) is a fundamental process for the recovery of proteins and lipids after neurotransmitter release. Different modes of SVR have been described, ranging from ultra-fast, fast and to slow. Their prevalence appears to be cell type and stimulus specific, adapted to the specific needs of a particular neuron. The molecular complexity of these different retrieval pathways just starts to be elucidated. Eps15 is an endocytic adaptor protein, which in *D. melanogaster* and *C. elegans* is involved in SVR.

In these animal models the reduction in synaptic efficacy is accompanied by a reduction at the protein level of the key endocytic players, making it difficult to judge whether Eps15 is primarily involved in SVR or merely in the stabilization of proteins relevant to SVR. In mammals two homologs exist, Eps15 and Eps15L1 and we investigated their role in SVR using knockout (KO) mice. Normal levels of endocytic proteins are maintained in brain extract from both Eps15- and Eps15L1-KO mice. Dye uptake experiments revealed that SVR is reduced in Eps15L1- but normal in Eps15-KO neurons. Additionally, synaptic vesicle number is reduced in Eps15L-KO neurons. By immunofluorescence analysis we detect increased clustering of the endocytic proteins AP2, CHC and Dynamin at synapses in Eps15L1-KO neurons compared to either wildtype or Eps15-KO neurons suggesting that clathrin/AP2 activity is impaired in Eps15L1-KO neurons. Consistently, upon stimulation we observed an increased number of large >80 nm vesicles in Eps15L1-KO neurons respect to wildtype controls suggesting increased formation of endosome like vesicles or a reduced resolution of such structures.

Surprisingly, in double-knockout neurons deficient for both Eps15 and Eps15L1 these phenotypes were reverted. This suggests that neither Eps15L1 nor Eps15 are strictly required for SVR. Rather, in the absence of Eps15L1, Eps15 serves as an 'inefficient' interaction partner that sequesters natural Eps15L1 interactors rendering them less active. In support of this hypothesis we found a shift in Eps15/Eps15L1 interactome and relocalization of the two proteins in the respective KO.

Keywords: endocytosis, synapse, synpatic vesicle recycling.

SUN-363
Assessing the conformation changes of pb5, the receptor binding protein of phage T5, upon binding to its *E. coli* membrane receptor FhuA

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Bacteriophages, viruses that infect bacteria, are the most abundant and diverse organisms on Earth. They play a crucial role in the ecology, evolution and pathogenicity of bacterial populations. Within tailed bacteriophages, interaction of the receptor binding protein (RBP) with the target cell triggers viral DNA ejection into the host cytoplasm. In the case of phage T5, the RBP pb5 and the receptor FhuA, an outer-membrane protein of *Escherichia coli*, have been identified. Here, we use small angle neutron scattering (SANS) and electron microscopy (EM) to investigate the FhuA-pb5 complex. Specific deuteration of one of the partners allows the complete masking in SANS of the surfactant and unlabelled proteins when the complex is solubilised in the fluorinated surfactant F_6-DigluM. Thus, individual structures within a membrane protein complex can be described. The solution structure of FhuA agrees with its crystal structure; that of pb5 shows an elongated shape. Neither display significant conformational changes upon interaction (Fig. 1). The mechanism of signal transduction within phage T5 thus appears different to that of phages binding cell wall saccharides for which structural information is available.

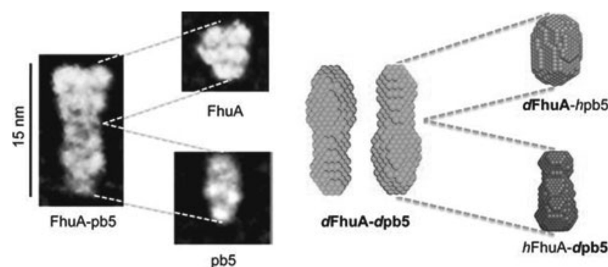

Fig. 1. Comparison of the EM and SANS projections. EM (left), SANS (right). Two SANS projections are presented, rotated by 180° since the relative position of FhuA and pb5 is not determined at this resolution. Projections are drawn to scale.

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Keywords: membrane proteins, phage, SANS.

SUN-364
Assimisome – ammonia assimilating machine of the wheat grain

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It is well known that wheat storage proteins are composed of more than 66% of glutamate. This suggests that there is a huge synthesis of glutamate in ripened wheat seeds. However, the biochemical mechanism of this phenomenon has not yet been studied. Therefore, we paid the attention to this mechanism. By gel chromatography on sepharose 4 B and by isopicnic ultracentrifugation we isolated from ripened wheat seeds the spherical structures in diameter 1.8 μm and buoyant density 1.1 g per cm^3. It was established that this structure has phosphatidylnositol (PI) to which are attached numerous molecular of NADPH- glutamate dehydrogenase (GDh) via own covalently bound Pi-anchors. We studied the kinetic properties of isolated structures. The value of Michael's constant to ammonia ions of the NADPH-Gdh speaks about an extremely high affinity of this enzyme to this substrate. This value is two orders better than with Km to ammonia ions of glutamine synthetase. All this results proved that our structures play a key role in ammonia assimilation in ripened wheat seeds. Thus, the discovered structure works as ammonia assimilating machine in the ripened wheat seeds. In this reason, we named this structure as *Assimisome*.

Keywords: Assimisome, phosphatidylnositol, NADPH- glutamate dehydrogenase.

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SUN-365
Boric acid supplementation can prevent oxidative damage caused by prenatal alcohol exposure in rat cerebral cortex

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In this study, the aim was to investigate the prenatal alcohol-induced oxidative stress on rat cerebral cortex of newborn pups and assess the protective and beneficial effects of Boric acid (BA) supplementation on rats with Fetal alcohol syndrome (FAS). Pregnant rats were divided into three groups as control group, alcohol group and alcohol + boric acid group. As markers of alcohol-induced oxidative stress in newborn pups' cerebral cortex, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) levels were measured. Although MDA levels in alcohol-administered group was significantly increased compared to control group ($p < 0.05$), its level in the alcohol + boric acid group was shown to significantly decrease compared to alcohol group ($p < 0.01$). CAT activity of alcohol + boric acid group was found statistically higher than the alcohol group ($p < 0.05$). GPx activity in the alcohol group was decreased compared to control group ($p < 0.05$). As a result, we found that alcohol was capable of triggering damage to membranes on cerebral cortex of rat pups and BA could be influential on antioxidant mechanisms resulting from prenatal alcohol.

Keywords: antioxidant activity, boric acid, fetal alcohol syndrome (FAS).

SUN-366
Caenorhabditis elegans P granule liquid phase separation resemble a collapse of the droplet phase at high ecological thermal ranges

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In contrast to endotherms, ectotherms are animals whose temperature varies with the environment. Soil nematodes like *Caenorhabditis elegans* do not regulate their body temperature through metabolic activity and depend upon of physiological strategies to bypass any ecological constraints. How organisms adapt to these conditions and what are the physico-chemical principles that shape the limits of evolution is a field largely unexplored. Here we study the temperature dependence of the cytoplasmic phase separation of liquid-like organelles called P granules that emerge as liquid condensates in the gonadal syncytium of the nematode that segregate to the embryonic cytoplasm of the P cell during embryogenesis. PGL-1 condensates are involved in the development of the germ line and nematode fertility together with GLH helicases and other RNA binding proteins. We discovered that the liquid droplet phase of PGL-1 dissolve as a function of temperature in a reversible manner with a critical point of 27°C and a volume fraction $\Phi = 0.002$ coinciding with the maximum fertile temperature of the nematode but not its maximum survival range. Our results suggest that P granule phase sepsaration process is tuned for mesothermic environments indicating that the volume fraction should be higher than $\Phi > 0.002$ with a P granule puncta count $N > 40$ in order to upkeep fertility at limiting temperatures.

Keywords: Liquid Phase Separation, Mesothermic, P granules.

SUN-367
Cancer cell tropism of glioblastoma-derived exosomes regulated by their lipid components

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Cell-to-cell communication is an important hallmark of multicellular organisms and expressed in the form of direct intercellular contact or transfer of secreted molecules. In the last two decades, extracellular vesicles (EVs) have been recognized as a novel intercellular communication tool. Exosomes with a diameter of 50–100 nm, are formed by inward budding into multivesicular bodies (MVBs). The fusion of these MVB with cell membrane causes the extracellular secretion of exosomes. Exosomes containing mRNA, microRNA, proteins, and lipids are transported into the target cells to be involved in various physiological/pathological phenomena. Despite of the accumulating evidence about biological functions of exosomes, 'exosomal principle', especially about their cellular incorporation, is not yet clear. We hypothesized that exosomes will be received a certain cellular tropism for effective intercellular communication, and the parent cells will regulate the exosomal direction by tuning their constituent molecules. In this study, we investigated whether cancer-derived exosomes were more taken into cancer cells than into normal ones. Exosomes derived from the human glioblastoma cells line U251-MG (U251) were highly purified by density gradient centrifugation. The exosomes ($U251_{exo}$) were hollow particles expressed CD63, and almost of them had a diameter of about 100 nm. $U251_{exo}$ were more taken up by U251 than by normal human astrocytes (Ast). In contrast, Ast-derived exosomes (Ast_{exo}) showed equal translocation into cancer/normal cells. Disrupting the ligand-receptor interaction on cells by enzymatic treatment against $U251_{exo}$ did not affected in their cancer-directional capability. To the end, we focused on the membrane lipid components as a crucial factor of the cellular tropism of the exosomes. In thin-layer chromatography analysis, lipid raft-related lipids (cholesterol and sphingomyelin; SM) accounted for a half of the total lipids of EVs, and the ratio of SM was different in their cell origins. Phosphatidylethanolamine was simultaneously enriched not only in cellular plasma membrane of U251 but also in U251-derived EVs, compared with Ast or Ast-derived EVs. The difference of exosomal lipid raft profiling and partial reflection of cellular lipid components to exosomal ones may alter the membrane affinity for cell-exosome interaction linking to exosomal interanalizing efficiency. These results could provide a novel cell targeting strategy for drug delivery as well as clues to understand the mechanisms of cell-to-cell communication via EVs in our body. Thus, the exosomal lipid components could be one of the important factors for their cell tropisms.

Keywords: cell tropism, exosome, lipid components.

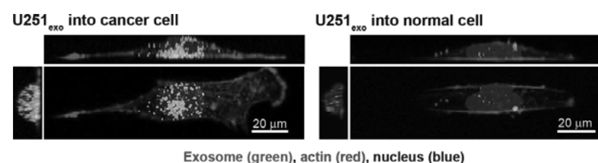

Fig. 1.

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SUN-368
Cell free expression, purification and solubilization of amyloid precursor protein's transmembrane domain with familial mutations V717I/G and L723P

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Alzheimer's disease (AD) is the most prevailing neurodegenerative disorder that affects elderly population all over the world. The hallmarks of AD are amyloid plaques and neurofibrillary tangles which were found in brains of patients. Plaques are made of beta-amyloid peptide – a product of sequential regulated intramembrane proteolysis of amyloid precursor protein (APP) by gamma-secretase complex. Many mutations found in APP transmembrane domain are associated with early onset of AD. Although these pathogenic (or familial) mutations are believed to influence the structure of APP, its dimerization capability and/or cleavage by gamma-secretase, the exact molecular mechanisms involved in these processes remain elusive. In this study we describe a highly efficient cell-free expression protocol for APP transmembrane domain with pathogenic mutations: V717I (also known as 'London' mutation), V717G and L723P (referred to as 'Australian' mutation). This method allows in short time to acquire milligram quantities of pure isotope-labeled peptides for high-resolution NMR spectroscopy.

Keywords: Alzheimer's disease, APP, familial mutations.

SUN-369
Cellular response of intestinal epithelial cells exposed to inorganic mercury and methylmercury

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The mercurial forms [Hg(II) and methylmercury, CH_3Hg] produce neurological and immune effects as well as hematological and renal alterations. The main route of exposure to these Hg species in humans is through the diet. Consequently, the gastrointestinal mucosa is exposed to these mercurial forms, though the potential toxic effects upon the mucosa are not clear. The present study evaluates the toxicity of Hg(II) and CH_3Hg (0.1–2 mg/L) in intestinal epithelial cells using the differentiated and undifferentiated human Caco-2 cell line.

The experiments made show the mercurial forms to reduce cell viability at high concentrations (≥1 mg/L), with cell death occurring mainly in the form of apoptosis. Exposure also produces the generation of reactive oxygen and/or nitrogen species and a significant decrease in glutathione content at the highest concentrations (≥1 mg/L). This redox imbalance could be the cause of the lipid peroxidation observed after short exposure times (30 min). Such conditions of stress lead to modulation of stress proteins and of tumor necrosis factor-alpha expression. In general, toxicity does not appear to be dependent upon the cell differentiation state. The abovementioned effects may be the cause of the increase in permeability observed in the differentiated cell monolayers, where redistribution of F-actin and ZO-1 protein and dowregulations of proteins of the intercellular junctions are observed after the treatments with mercury.

Keywords: Mercury; intestinal epithelium; intercellular junctions.

SUN-370
Clathrin- and dynamin-independent internalization of PDGF via Rho GTPases

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Platelet-derived growth factors (PDGFs) belong to the family of disulphide-linked dimer ligands which act via transmembrane receptors with tyrosine kinase activity. Activation of the PDGF receptor (PDGFR) regulates major cellular processes like proliferation and migration. In parallel to evoking signaling responses, PDGF binding to the PDGFR induces endocytosis of ligand-receptor complexes which decreases a number of available PDGFR on the plasma membrane. We recently showed that in human fibroblasts PDGF can be internalized in the presence of dynamin inhibitors, arguing that both dynamin-dependent and dynamin-independent pathways can mediate PDGF uptake.

In light of these findings we are currently investigating dynamin-independent internalization of PDGF, which requires the activity of proteins from the Rho family of GTPases. We analyzed endocytic transport of PDGF-PDGFR complexes under overexpression of active or inactive mutants of Rac1, RhoA and Cdc42. We also employed chemical inhibitors directly blocking Rho GTPases or interfering with downstream components of their signaling pathways. Overexpression of Rac1 mutants or acute block of its function with Rac1 inhibitor (NSC 23766) did not affect internalization of PDGFR. The overexpression of active Cdc42 caused formation of enlarged PDGF-positive endosomes. Such accumulation of PDGFR on endosomes may in turn lead to its prolonged signaling. Moreover, overexpression of inactive Cdc42 or blocking its function with a chemical inhibitor (ML 141) decreased the internalization rate of PDGFR. Finally, in cells overexpressing an active form of RhoA, PDGF-containing endosomes were trapped near the plasma membrane, whereas inactive RhoA mutant reduced uptake of PDGFR. This effect was mimicked by blocking RhoA activity using C3 transferase from Clostridium botulinum. Furthermore, interfering with Rho-associated protein kinase (ROCK), which is a major downstream effector of the RhoA, also led to a partial inhibition of PDGFR endocytosis. Currently, we are testing how the above described changes affect signaling induced by PDGF, as we previously showed that acute inhibition of dynamin activity only moderately affected PDGF endocytosis, but it specifically decreased downstream signaling of PDGF via Stat3 transcription factor.

More generally, in this project we expect to verify a hypothesis that the components governing endocytic trafficking, such as dynamin or Rho GTPases, may selectively regulate certain signaling effectors activated by a growth factor, like PDGF.

Keywords: Clathrin- and dynamin-independent endocytosis, PDGF receptor, Rho GTPases.

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SUN-371
Coagulation factors X and Xa bind to phospholipid membranes in a hysteresis manner which allows them to be retained in thrombi under flow conditions

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Introduction: Many regulatory processes in biology involve reversible association of proteins with membranes. Proteins that bind to phosphatidylserine are implicated in diverse processes such as intracellular signal transduction cascades, plasma membrane-cytoskeleton interactions, phagocytosis of apoptotic cells, and blood clotting.

Binding to phosphatidylserine-containing membranes is the first step in the all membrane-dependent reactions and its understanding is a prerequisite for the analysis of their mechanisms. Clotting proteins bind to phosphatidylserine-containing membranes, but clear picture of this interaction has yet to emerge.

Therefore, we show the binding of factors X and Xa to negatively charged phospholipid membranes.

Methods: The binding of coagulation factors to negatively-charged phospholipid membranes was studied with an Accuri C6 cytometer (BD Biosciences, USA) and an Axio Observer Z1 confocal microscope (Carl Zeiss, Jena, Germany) using fluorescein-labeled FX or FXa. Fluorescence intensity was converted to a mean number of molecules per platelet or per phospholipid vesicle using special calibration beads. Computer simulations of fXa binding to a single platelet or to platelet aggregate were carried out using the Virtual Cell environment (http://vcell.org).

Results: Apparent equilibrium binding of fX and fXa to synthetic PS:PC (25:75) was saturable and specific, with 7991 ± 847 binding sites per vesicle and apparent k_d of 435 ± 75 nM for FX, 11 920 ± 1882 binding sites per vesicle with an apparent k_d of 747 ± 57 nM for FXa.

The association data were fitted to a exponential decay model with rates of $k_+ = 0.022 \pm 0.007\ nM^{-1}s^{-1}$ and $0.578 \pm 0.038\ nM^{-1}s^{-1}$ for fX and fXa respectively. However, data from dissociation experiments showed that the binding was not actually reversible: after a 20-fold dilution, the concentration of the bound factor Xa decreases to a value much higher than the equilibrium one. The initial apparent dissociation constant, k_- was of $2.29 \pm 0.26\ s^{-1}$ and $2.41 \pm 0.08\ s^{-1}$ for fX and fXa respectively.

Thus the process of binding and dissociation has been a hysteresis-like one, posing a kind of 'memory'. By mathematical modeling, we have shown that this behavior can only be explained by trimerization of factors.

Our model predicted that the phenomenon of oligomerization may be important for to keep factors on the surface thrombi. Our experiments confirmed the prediction model. Thus, we can assume that the trimerization factors ensures their fixing to the surface of a thrombus under the flow.

The study was supported by the Russian Foundation for Basic Research grants 13-04-00401.

Keywords: Coagulation factors X and Xa, Membrane-dependent reactions, Platelets.

SUN-372
Comparative analysis of growth, UV- resistance and cell wall structure of *Salmonella enterica* serovar Derby ('*S. derby*') cells

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The association between the form and function of microorganisms is one of the frequently discussed questions in biological investigations.

The *aim* of current investigations was the comparative analysis of growth, UV-resistance and cell wall structure of S. *derby* strain and its plasmid curried derivative.

The following *S. derby* strains were used in this study: *i.* wild conditional pathogenic strain of *K89*, having a R-factor (100 thousand bpn) and giving to bacterial cells multiple resistance to following antibiotics: chloramphenicol, penicillin and tetracycline; *ii. K82,* the plasmid-curried derivative of *K89*; and *iii. K134*, the UV- sustainable mutant of *K89*.

Bacteria were grown in LB medium, pH 7.5, under aerobic conditions. The duration of lag phase and specific growth rate were determined as described previously (1–3).

X-ray diffraction method under the small and large angles was used to investigate the cell walls' structure and its intramembrane organization. Samples were prepared according to investigations described previously (4).

The differences in growth of *K89* cells and its plasmid curried variants were described, and the role of intercellular interactions on growth of K82 UV- resistant cells were shown by us. The changes in the form and size of the *S. derby* cells after the plasmid elimination were detected. It was established the liquid-crystal structure of cell walls for all of investigated bacterial strains. The cell walls from the curried from plasmid K82 strains are more hydrophobic, than the cell walls of K89 and K134 strains. At the same time the association between UV- resistance and qualitative and quantitative composition of phospholipids in *S. derby* cell walls was established. In particular, the increase of contents of acidic phospholipids in K82 strains was shown.

We assume that the structural changes in cell walls of *S. derby* play a key role in bacterial growth and UV- resistance.

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Keywords: cell wall, *S. derby*, UV-resistance.

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SUN-373
Comparison of the effects of some antipsychotics on lipid bilayer

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Antipsychotics, which are used in therapy of schizophrenia, are compounds classified as the ligands of membrane G protein-coupled receptors (GPCRs). Especially most of them are considered to be the antagonists of dopamine receptors. However, the accurate mechanism of action of these drugs, which is responsible for their therapeutic effects, is not explained sufficiently. Besides the interaction with receptors (specific activity), neuroleptics exhibit also a nonspecific activity – i.e. the interaction with cell membranes.

The present study were designed to evaluate interactions of some antipsychotic drugs with lipid bilayers. The study was performed for: amisulpride (AMS), sulpiride (SU), thioridazine (TH) and olanzapine (OLP). The monolayer liposomes made of POPC (neutral charge) as well as of POPC/POPG (3:1 molar ratio) (negative charge) have been used as the model of biological membranes. The isothermal titration calorimetry (ITC) has been employed to obtain thermodynamic parameters of the interactions. Additiolnally, the influence of neuroleptics on the permeability of a bilayer in terms of water (dynamic light scattering measurement DLS) was studied as well as the influence on a surface charge of liposomes (zeta potential measurement).

The obtained results indicate the strongest effect of interaction with lipid bilayer for thioridazine and olanzapine. Amisulpride seems to exhibit a weak interaction. No effect for sulpiride was observed.

These results show differences in effects induced by antipsychotic drugs, what indicates that these drugs are not neutral for cell membranes. In this context the obtained results point to the necessity of further research of the influence of neuroleptics on biological membranes with the use of more complicated liposomal systems or membrane fraction of cell lines.

Acknowledgements: We acknowledge the financial support from the project Interdisciplinary PhD Studies ‘Molecular sciences for medicine’ (co-financed by the European Social Fund within the Human Capital Operational Programme)
Keywords: Drug-lipid interactions, Neuroleptics.

Abbreviations: POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol).

SUN-374
Conformational changes in an aquaporin-nanodisc system modeled with SAXS/SANS

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Structural information about macromolecular bio-molecules is crucial for understanding their function. Small angle X-ray/neutron scattering (SAXS/SANS) can be used as either a complementary method to X-ray crystallography and NMR, or as a main tool when the preferred methods are unavailable. SAXS/SANS requires homogeneous samples for proper modeling but for membrane proteins the detergent micelles are heterogeneous, thus making the method problematic. The problem can be circumvented by incorporating the target membrane protein into nanodiscs. They consist of a disc formed bilayer enveloped by two copies of a modified apolipoprotein. The nanodiscs intrinsic homogeneity and reproducibility makes them ideal for SAXS/SANS.

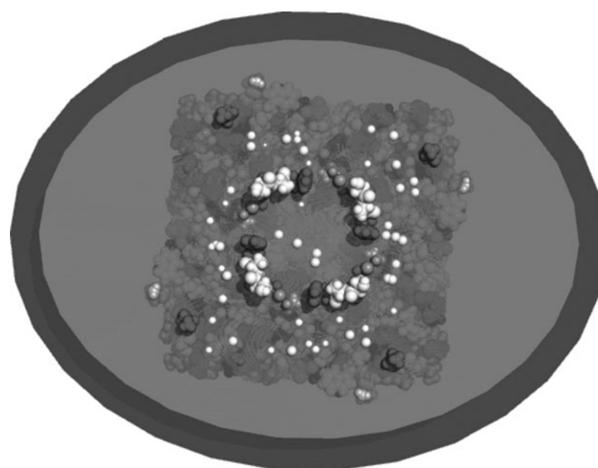

Fig. 1.

Here we have used an water channel as a model system to test the limitations of this method. The spinach leaf aquaporin SoPIP2;1 was reconstituted into nanodiscs and with data from SAXS measurements and subsequent SANS measurements we could model the aquaporin into a nanodisc.

In good agreement with previous studies, SoPIP2;1 had to be modeled as a tetramer. The intracellular loops had to be included in the model to fit the data and the lateral location of these loops was just as important. Furthermore, the number of lipids in the model correlates well with the amount measured in the phosphate assay.

Since SoPIP2;1 is a gated protein and a crystal structure exists of both the open and closed state, a comparison of fits between the two states was made. A slight preference towards the open conformation was seen. The results strongly indicates that the method described here is viable for probing conformational properties in membrane proteins in an environment that is both more native and less heterogeneous than detergent micelles.

Keywords: Aquaporin, Nanodisc, SAXS.

SUN-375
Congenital tufting enteropathy, a new model for studying intestinal villous morphogenesis

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The intestinal villous epithelial layer undergoes permanent renewal, suggesting the existence of mechanisms regulating the maintenance of the polarized epithelial monolayer. While intensive research is focusing on crypt cells and cell fate determination, mechanisms underlying the maintenance of the villous homeostasis are largely unknown.

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Congenital Tufting Enteropathy (CTE) (MIM #613217) constitutes an interesting working model. This rare human disorder, responsible for intestinal failure, is characterized by abnormal morphology of the intestinal villi, with aberrant focal stacks of multiple layers of enterocytes, named 'tufts'. Mutations in the *EPCAM* and *SPINT2* genes have recently been correlated with CTE in 94% of cases. Here we aimed at understanding the cellular functions of EpCAM and Spint2 in enterocytes, and characterizing the impact of their mutations on villous morphogenesis. We addressed these questions by developing integrated approaches at the cell biological, biophysical, and physiopathological levels.

We show that endogenous EPCAM and SPINT2 are located at the lateral membranes and the terminal web. We analyzed either duodenal biopsies of CTE patients, or stable Caco2 clones depleted for EPCAM or SPINT2. Ultrastructural analyses were performed using transmission electron microscopy, and immunohistological analyses for cell organization, cell differentiation and polarity markers. Our results clearly show specific defects in enterocyte cell polarity and adhesion in the mutant enterocytes: microvillus atrophy, mislocalization of brush border components, and weakened cell-cell junctions. Although Caco2-depleted cells display a large panel of cell organization abnormalities, they still formed epithelial monolayers in 2D cultures. We thus generated 3D culture supports that recapitulate villus topology and constraints. Placing mutant human enterocytes on synthetic villus cultures revealed strong perturbations in the maintenance and dynamics of the epitelial monolayer, mimicking the tufts observed in CTE patient mucosa.

We demonstrated that the CTE disorder is characterized by the loss of enterocyte polarity and differentiation. As a consequence, correct cell dynamics along the villous architecture is impaired in mutant enterocytes.

Keywords: Intestinal morphogenesis, EpCAM, SPINT2.

SUN-376
Detachment of key adhesive proteins from the membrane skeleton is observed in phosphatidylserine-positive platelets

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Background: The attachment of membrane glycoproteins to the cytoskeleton in activated platelet subpopulations has been poorly studied. In the resting platelets, adhesive membrane proteins are attached to the cytoskeleton with special proteins, and their degradation is supposedly controlled by calpain, a calcium-dependent protease. Upon strong physiological activation, two platelet subpopulations (PS (phosphatidylserine)-positive and PS-negative) are formed, which have different functions in hemostasis and thrombosis. We developed a new flow cytometry-based single-cell approach to investigate the attachment of membrane glycoproteins to the membrane skeleton in platelet subpopulations.

Methods: Platelets were isolated from whole blood by centrifugation and gel-filtration. Then they were activated with physiological agonists such as thrombin and collagen-related peptide. Subpopulations of PS-positive and PS-negative platelets, were separated by flow cytometry due to their different PAC-1 binding. The attachment of membrane glycoproteins (integrin $\alpha_{IIb}\beta_3$, GpIb and P-selectin) to the cytoskeleton was estimated by fixing the platelets with paraformaldehyde after activation in the presence of fluorescently-labeled antibody (CD61, CD42b or CD62P) to surface glycoproteins and treating fixed platelets with detergent. Additionally the status of cytoskeletal proteins was evaluated by electrophoresis.

Results: We observed a release of membrane surface glycoproteins in only the PS-positive platelets (fixed platelets after detergent treatment), but not in the PS-negative activated platelets and resting platelets. This effect was prevented by calpain inhibitors (calpeptin and MDL28170). These results suggest that the degradation of cytoskeletal proteins that provide attachment of the membrane surface glycoproteins to the platelet cytoskeleton occurred only in PS-positive platelets. This effect correlated with the degradation of talin and filamin as detected by protein electrophoresis.

Conclusions: The inhibition of calpain resulted in a significant decrease in the proteolytic degradation of cytoskeletal proteins, suggesting that calpain is involved in the regulation of the attachment of integrin $\alpha_{IIb}\beta_3$, GpIb and P-selectin to the cytoskeleton of platelets. Our data suggest that this detachment of membrane glycoproteins may play a role in the regulation of the adhesive properties of platelet subpopulations.

Financial support: This work was supported by the Russian Foundation for Basic Research (RFBR) grant 13-04-00401.
Keywords: adhesive proteins, cytoskeleton, platelets.

SUN-377
Development of live cell PALM imaging approaches to studying GM-CSF receptor endocytosis and JAK2 activation on the cell surface

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JAK2 kinase plays a very important role in transducing proliferative and survival signals for many cytokines and is involved in regulating the vital function of numerous special tissues. Recently the importance of JAK2 was further emphasized since specific JAK2 mutations were identified in many tumorous and non-tumorous hematopoietic diseases. Various pharmacological inhibitors of JAK2 kinase are also currently under clinical trials, underscoring the importance of JAK2 function in human diseases. We here report the controlling mechanism of JAK2 activation by cytokine GM-CSF and oncogenic JAK2 V617F mutation and proposes a comprehensive hypothetical model for JAK2 kinase activation and oncogenesis based upon biochemical and molecular biology analyses and fixed cell-superresolution microscopic images. In this model endocytotic clathrin-coated pits are proposed to serve as a signaling platform for GM-CSF receptor and JAK2 is activated by the clathrin-coated pit-localized CK2 after JAK2 acquired a liganded-receptor-induced conformational change. Furthermore, we aimed to establish the live cell-Forster resonance energy transfer (FRET) and -photoactivated localization microscopy (PALM) technologies to visualize and analyze the JAK2 activation by cytokines and the interaction of GM-CSF receptor with endocytotic machinery components. To achieve this goal, we used the complementary metal oxide semiconductor (CMOS) camera to achieve the frame rate of 556 frames per second with the pixel number of 256 x 256 and high sensitivity, and used the switchable and bright fluorescence probe such as reversible photoactivatable fluorescence protein, dronpa, (in PALM) and photoswitchable labeling dye (in STORM) to reduce the acquisition time with the larger numbers of photon. By improving the acquisition time of live cell super-resolution fluorescence localization imaging, the behavior of clathrin-coated pit and JAK2 at the plasma membrane in live cell was recorded and studied with temporal resolution of 0.5 second and spatial

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resolution of 30–40 nm. Detailed results will be presented at this meeting.

Keywords: Clathrin-dependent endocytosis, cytokine signal transduction, super-resolution microscopy.

SUN-378

Development of the cellular model to study on TrkC- dependent neuritogenesis

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PC12 cell line has become a widely used research tool for many aspects of neurobiology. By means of genetic engineering a subset of PC12 cells was engineered to drive doxycycline (Dox) -induced expression of gene encoding TrkC (receptor of neurotrophin 3, NT- 3) in the Tet-On system. In our study we have used second- generation Tet promoter (P_{tight}) which contains both shortened minimal CMV promoter sequences and tetO elements positioned in an optimized manner. P_{tight} promoter provides low basal expression in the absence of Dox. We have developed the cellular model to study on TrkC- dependent neuritogenesis, where *trkC* expression is controlled by tetracycline promoter. In this particular model *trkc* expression is regulated by Dox in a concentration- dependent manner. NT-3 treatment and subsequent activation of TrkC results in ERK1/2 kinases phosphorylation leading to neurite outgrowth.

Keywords: neuritogenesis, PC12-Tet-On, TrkC.

SUN-379

Different regulation of store-operated calcium channels by calcium sensors Stim1 and Stim2 proteins

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Store-operated channels are the major calcium entry pathway in nonexitable cells. Stim proteins are Ca^{2+} sensors of endoplasmic reticulum. Stim proteins are essential for activation of store-operated Ca^{2+} entry. After store depletion Stim proteins change their conformation and activate store operated calcium channels of plasma membrane. On one hand different store-operated channels could coexists in one cell, on the other hand in mammals there are two homologues of Stim proteins.

The goal of this study was to compare regulation of different store-operated channel types by Stim1 and Stim2 proteins.

To evaluate the role of Stim calcium sensors in activating endogenous store-operated channels we use HEK293 cell line. Electrophysiological properties of calcium channels are well described in this cell line, what make it useful model to study store-operated channels. There are three types of Ca^{2+} channels in cell-attached patches from HEK293 cells: I_{max}, I_{NS} and I_{min} (Bugai et al., 2005, JBC).

To separate Stim1- and Stim2-operated channels we used knockdown – overexpression strategy and partial depletion of calcium stores for selective activation of Stim2.

In single-channel patch–clamp experiments with Stim1 or Stim2 overexpression (knockdown) we discovered that I_{NS} channels are activated by Stim1, I_{min} channels are activated by Stim2 and I_{max} channels are regulated by both Stim homologues upon store-depletion.

There is big difference in calcium sensitivity between Stim1 and Stim2 proteins. Stim1 proteins are activated only by strong store depletion, which is evoked by micromolar concentrations of thapsigargin. Stim2 proteins are activated even by small changes in Ca^{2+} store, which do not activate Stim1. In further experiments we induce partial store depletion for specific activation of Stim2 proteins. Partial store depletion lead to Imin channel activation.

We hypothesized that difference in regulation of various store-operated channels by Stim1 and Stim2 proteins define intracellular calcium signaling.

The study was supported by the program of 'Molecular and Cellular Biology' RAS, research grants from the Russian Basic Research Foundation, Dynasty Foundation and the Russian Scientific Foundation 14-14-00720.

Keywords: Patch-clamp, Stim proteins, Store-operated calcium entry.

SUN-380

Dimeric spatial structure of HER2 transmembrane domain in junction to the juxtamembrane regions corresponding to the receptor inactive state

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Receptor tyrosine kinases, such as the members of human epidermal growth factor receptor (HER) family, play critical role in regulating cell metabolism, growth and differentiation. It was shown, that HER members are active in dimeric state and the ligand binding causes rearrangement of transmembrane and intracellular domains of the receptor. Activation of the catalytic domain of the HER receptors is controlled primarily by an allosteric interaction between two protein kinase domains in an asymmetric dimer. Several studies of HER activation led to the conclusion that cytoplasmic juxtamembrane regions of these receptors are essentially important for the HER signaling. Upon ligand binding, juxtamembrane regions in preformed dimer of the HER on the cell surface are supposed to change their conformation from parallel, corresponding to inactive state, to antiparallel, which is proposed to be active. It is also assumed that different functional states of the receptor correspond to different dimerization interfaces on the transmembrane domain. Thus, understanding the principles, lying in the basis of intermolecular interactions of transmembrane and juxtamembrane regions in such receptors is essential for detailed description of the activation mechanism and is necessary for rational design of new drugs, targeting directly the transmembrane and juxtamembrane domains.

Here we investigate the spatial structure and backbone mobility of the HER2 transmembrane domain homodimer in junction to the cytoplasmic juxtamembrane region in membrane-like environment using solution NMR spectroscopy. Our data reveal the parallel orientation of the juxtamembrane regions in the dimer that allows to assign the obtained structure to the inactive state of the full-size receptor. The transmembrane domains interact via a non-standard motif located at the N-terminal part of the transmembrane helix. The interaction is driven mainly by apolar contacts of bulky side chains. The measured parameters of NMR relaxation reveal that the mobility of transmembrane and juxtamembrane regions is quite similar, suggesting that the juxtamembrane regions are buried in the micelle. This conclusion is confirmed by the

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observed intermolecular contacts between amino acid residues and lipid molecules of the micelle. The obtained spatial structure is the evidence in favor of the 'ligand-induced rotation' mechanism, suggested for HER receptors, and provides an insight into the structural basis of signal transduction by HERs.

This work is supported by Russian Academy of Sciences (program 'Molecular and Cellular Biology').

Keywords: activation mechanisms of transmembrane receptors, human epidermal growth factor receptors, spatial structure of HER2 transmembrane domain.

SUN-381

Dissecting the retrograde membrane trafficking pathway using bacterial toxins and nanoparticles: A tale of two genome-wide RNAi screens

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Newly synthesised molecules are delivered to the plasma membrane, the extracellular space and other intracellular compartments by the biosynthetic pathway. At each transport step, organelle homeostasis is maintained by a retrograde membrane flow which counterbalances forward traffic.

Despite our considerable knowledge of the molecular machinery associated with forward trafficking pathways, much less is known about retrograde transport pathways linking the cell surface and endosomes with early secretory pathway organelles such as the Golgi complex and endoplasmic reticulum. Nevertheless, these routes are important, as they are known to be exploited by a number of viruses and protein toxins as part of their mechanism of action. This retrograde route also holds promise for the delivery of therapeutic agents, and as such it is essential that we understand its regulatory machinery.

In order to identify the proteins that regulate internalisation and retrograde transport pathways two independent genome-wide RNAi screens coupled to high-content image analysis were performed in HeLa cells. Nanoparticles were used to study endocytosis and endosomal-lysosomal delivery pathways, while Shiga-like toxin was the probe to understand transport from the cell surface to the Golgi complex. These studies revealed roles for members of the Rab family of GTPases and components of the cytoskeleton not previously described to be involved in either process, in addition to various other classes of molecule, as key regulators of these pathways.

The results from these screens not only contribute to our general knowledge of the mechanisms of retrograde traffic, but are also valuable in the development of more efficient drug delivery strategies to cells.

Keywords: Genome-ride RNA screening, Membrane traffic, Retrograde trafficking.

SUN-383

Effect of gender and chronological age on erythrocyte membrane quality in blood bank storage condition

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Despite the use of various storage solutions, a series of changes in erythrocytes have been occurring and number of functional red blood cells has been decreased. It is known that both in vivo and in vitro, storage lesions caused membrane dysfunction by interfering redox balance and transport function. In our study, the erythrocytes with different ages in Citrate phosphate dextrose-adenine-1 (CPDA-1) storage solution were used to investigate the contribution of gender difference on both radical and non-radical mediated damage. For this purpose, whole bloods from both genders were fractionated with 'Percoll density gradient' method. Fractions were stored in CPDA-1 solution which used as preservative and Oxidative biomarkers in membranes such as ferric reducing antioxidant power (FRAP), pro-oxidant-antioxidant balance (PAB), and advance glycation end products (AGE) were analysed at the end of 7 day storage period. Middle age erythrocytes of male rats showed a significant increase in FRAP activities ($p < 0.05$ versus respective female group), whereas PAB levels were increased in aged fraction of male rats ($p < 0.01$ versus young and middle age). Additionally, aged fraction of male rats was found to be increased in PAB levels compared to aged fraction of female rats ($p < 0.01$). Lastly, middle age fraction of female rats was found to be increased in AGE levels compared to respective male group ($p < 0.01$). This study reveals that changes in erythrocyte membrane redox status during blood bank storage depends unequivocally on gender dependent redox homeostasis. During storage period, male erythrocyte is exposed to free radical mediated oxidative stress but female erythrocyte is under non-radical glycative stress. However male donors could be better choice. The gender-dependent action in storage period needs further investigations.

Keywords: aging, blood storage, erythrocyte.

SUN-384

Effect of low molecular antioxidants on functionality RLIP76 and MRP1 in human erythrocytes

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RLIP76 was found in almost all tissues, including human erythrocytes. Protein's nature determines its active participation in the cell signaling processes and membrane regulation. To date, cell detoxification mechanisms by RLIP76 when appeared of redox imbalance is almost unknown. In this context, aim of this study was to evaluate expression level and functional activity of RLIP76 in human red blood cells (RBC) under the influence of α-tocopherol, ascorbic acid (AA) and N-acetylcysteine (NAC).

It was found that fluorescence intensity of RLIP76-positive red blood cells (RBC) after 24 h loading therapeutic concentrations of NAC and α-tocopherol increased on average by 25–35% relative to control, for AA statistically significant differences

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compared with control (control was a fluorescence intensity RLIP76-positive cells untreated with investigated antioxidants) had not been established.

After treatment of RBC by NAC export 2,4-dinitrophenyl-S-glutathione (DNP-SG) – conjugates increased on average by 20% compared to untreated cells. At the same time, 24 h (and 1 h) effects of α-tocopherol and AA on human RBC had no effect on transport DNP-SG-conjugates from the cells. It is known that besides RLIP76 in glutathione-mediated detoxification of RBC also involved MRP1 protein.

To clarify the contribution of MRP1 in this process it was determined the residual retention of its substrate calcein under the action of the studied antioxidants. We found that for α-tocopherol and NAC fluorescence intensity of calcein didn't change relative to untreated cells, whereas effect of the AA was reduced on average by 20%, which indicates about an increase in functional activity of MRP1.

Thus, intracellular imbalance of 'antioxidant-prooxidant' in favor of the antioxidants can alternately result in both MRP1 activation (in the case with AA), and changing functionality RLIP76 (under the NAC action) involved in maintaining the redox homeostasis in human erythrocytes.

Keywords: Erythrocytes, transporters, antioxidants.

SUN-385

Expression and purification of the human endothelin receptor B for structural studies

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Human endothelin receptor B (EDNRB) is a member of class A (rhodopsin-like) G protein-coupled receptor (GPCR) family. Its dysfunction leads to many severe diseases such as hypertension, atherosclerosis, heart failure, renal disease and various forms of cancer. Several drug candidates targeting endothelin receptors have failed clinical trials, while only few that have been approved carry undesirable side-effects. Despite the high importance of these receptors, no structural information for them is available, mainly because of challenges related to heterologous expression, purification and crystallization of human membrane proteins. We have engineered a stabilized version of ENDRB by fusing a small soluble protein apocytochrome b562 in the intracellular loop 3, expressed the modified construct in baculovirus infected sf9 insect cells, and purified it to homogeneity by metal affinity chromatography. The purified protein displays an increase in thermostability upon addition of different antagonists, and shows promising behavior in pre-crystallization LCP-FRAP diffusion assays in lipidic cubic phase (LCP). LCP crystallization trials with this ENDRB construct bound to several different antagonists are currently underway.

Acknowledgements: The work was supported by the 5top100-program of the Ministry for science and education of Russia and Optec Company grant for young scientists.

Keywords: G Protein Coupled Receptors, membrane proteins, protein expression and purification.

SUN-386

Expression of the human HCN channels – promising targets for nervous system and heart diseases treatment

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Among wide range of human membrane proteins (MPs) the hyperpolarization-activated cyclic nucleotide-gated channels (HCNs) mediate functioning of heart and nervous system. Although these channels have crucial importance for pharmacology and medicine, their exact structure and molecular mechanisms of functioning still remain unknown.

The studies of HCNs are hampered by low expression level in host-based systems. To solve this problem we used cell-free expression (CFE) system based on *E. coli* extract. Such CFE systems have shown their efficacy for the production of MPs of different classes. The main advantage of these systems over host-based systems is that the yield of target protein achieves several mg/ml and allows to carry out further structural and functional studies. Also the so-called open nature of CFE systems makes it possible to add ligands and membrane-mimicking environments (micelles, bicelles, etc) directly into a reaction mixture and stabilize the target MP in a soluble and properly folded state.

In this work we used the CFE systems based on S30 extract from *E. coli* for the production of several variants of the transmembrane (TM) and cyclic nucleotide-binding (CNB) domains of the human HCN1 channel. We developed protocols for TM-HCN1 and CNBD-HCN1 expression either in a form of a precipitate or in a soluble form. Also the refolding protocols were developed. Yields of the TM-HCN1 and CNBD-HCN1 in optimal conditions were 1.2 –1.5 mg per 1 ml of the reaction mixture.

This work is crucially important for pharmacology and medicine and opens new horizons in structural and functional studies of human HCN1.

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Keywords: HCN channels, membrane proteins, structure study.

SUN-387

Expression, purification and functional analyses of human CysLT1 and GPR17 proteins

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Leukotrienes are important inflammatory mediators that are produced from arachidonic acid through its oxidation. Their release in organism leads to bronchial smooth muscle constriction, arise blood vessel permeability, stimulate mucus secretion and attract immune cells. Leukotriene effects are realized via interaction with specific receptors, belonging to a superfamily of G-protein coupled receptors.

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CysLT1 is a cysteinyl leukotriene receptor activated mostly by leukotriene D4. It is shown that this protein is involved in inflammatory disorders such as asthma, allergic rhinitis, urticaria, rheumatoid arthritis at al. CysLT1 antagonists zafirlukast, montelukast etc are popular antiasthmatic medicaments but those drugs have considerable shortcomings.

GPR17 is an orphan receptor although its interaction with LTC4, LTD4 as well as purines was proved. This protein is involved in multiple sclerosis, ischemia and leukemia diseases. Thus CysLT1 and GPR17 are important drug targets.

The knowledge of CysLT1 and GPR17 structures could become a key to a specific drug-design. This can be achieved by means of X-ray crystallography method. To obtain protein crystals one needs high amounts of pure and stable protein with crystal contacts.

In this work for CysLT1 and GPR17 mutant constructions with fusion partner BRIL were created, expressed by means of baculovirus expression system, purified, solubilized from membranes. Purity, stability and aggregation level were analyzed by western-blotting, size-exclusion chromatography and thermal shift assay methods. The yield of proteins after purification was 1 mg from 1 liter of cell culture.

This work was supported by the 5top100-program of the Ministry for science and education of Russia.

Keywords: Baculovirus expression, Cysteinyl leukotriene, G-protein-coupled receptors.

SUN-388
FERM and PDZ domain containing protein 2 regulates epithelial cell polariZation

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Epithelial cell polarization is characterized by an asymmetric distribution of proteins and lipids between apical and basolateral membranes. Establishment and maintenance of cell polarization involves many biological processes including membrane trafficking, signal transduction and cytoskeletal dynamics. FRMPD2 is a novel multi-PDZ domain protein implicated in cell polarization. FRMPD2 consists of an N-terminal KIND-domain, followed by a FERM domain and three PDZ domains. Thus FRMPD2 likely serves as a scaffolding protein for a larger protein complex. We have shown that FRMPD2 localizes at the basolateral membrane of polarized epithelial cells colocalizing with adherens- and tight junction markers. Decreased expression level of FRMPD2 lead to impairment of tight junction formation accompanied with loss of cell polarity. We find that basolateral targeting of FRMPD2 depends on its FERM domain and is mediated by PtdIns(3,4)P2. Interactions with p0071, ARVCF and δ-catenin – members of the catenin family suggest that FRMPD2 localizes at the basolateral membrane in an E-cadherin dependent manner. Recent studies have shown that FRMPD2 interacts with NOD2 and acts as a scaffold protein that localizes NOD2 to the basolateral membrane of epithelial cells and provides spatial specificity to NOD2-mediated immune responses. NOD2 is an important part of the innate immune system of intestinal epithelia and its recruitment to the plasma membrane is essential for NF-κB activation. In addition, genetic studies have revealed that mutations of NOD2 are closely associated with Crohn's disease. Using the yeast two hybrid system we have identified several new interaction partners for FRMPD2, among these interaction partners are candidates associated with immune-mediated diseases like systemic lupus erythematosus (SLE) and psoriasis (also associated with Crohn's disease). Our results highlight novel insights into molecular function of FRMPD2 in epithelial cell polarization and a potential role of FRMPD2 in immune-mediated inflammatory diseases.

TRANSPOL.

Keywords: Cell polarity, epithelial cells, PDZ domain.

SUN-389
Functionality of integral membrane proteins MRP-family under changing of zinc homeostasis in human erythrocytes

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Nowadays there is a hypothesis that Zn^{2+} may be used in cancer therapy jointly with anticancer drugs because have both DNA-protective for normal and DNA-destabilizing effects for tumor cells.

So, the goal of work – to clear the influence of zinc homeostasis in human erythrocytes on the functionality of the integral membrane protein-transporters ABCC (MRP)-family, which are involved in multidrug resistance phenotype and play a key role in the pharmokinetics a wide number of the anticancer drugs.

Results: Incubation erythrocytes with $ZnSO_4$ in vitro in pharmacological or toxic concentrations (50–500 μM) results in rise of intracellular level of labile Zn^{2+} in nanomolar range and accumulation of ROS but doesn't change GSH level. It is established MRP-proteins functionality activation in these conditions. We show that important role in such changing plays modification of phospholipids physical state and membrane cholesterol lateral distribution. Further we proved that one of the possible mechanisms of Zn-induced MRP-proteins activation in erythrocyte membranes is the derangement of structure and function of lipid rafts which have a central role in cell signaling.

Conclusion: The results testify about existence of link between rise of cytosolic concentration of labile Zn^{2+} and functioning of protein-transporters MRP-family in human erythrocytes where modification of lipid rafts structure and function play a key role. Moreover one of the trigger of such changes is disbalance of 'prooxidant/antioxidant' in cells.

This work was partly supported by grant of the Cariplo Foundation organized by the Landau Network-Centro Volta.

Keywords: zinc homeostasis, MRP proteins, red blood cells.

SUN-390
Gene-engineering and expression of the human endothelin A receptor' modifications

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Endothelin system is generally responsible for regulation of vascular tissue tension, also it is responsible for cell proliferation stimulation, regulation of cytokines and growth factors ejection into the cardio-vascular system with the participation of endothelins – group of paracrine local peptide (approximately 21 a.r.) hormones. Two (ET-1 and ET-2) of them (ET-1, 2, 3, 4) cooperate with ETA-receptors. ET-1 is prevailing type in cardio-vascular system. Its overexpression in blood plasma and tissues is a marker of

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different diseases. Also, cancer cells express endothelins which, in turn, stimulate cancer cell growth.

ETA-receptor is the one of two types G protein-coupled receptor (GPCR) family in human endothelin system. It's present in smooth muscle tissues, vascular fibroblasts, cardiomyocytes, neurons, osteoblasts, adipocytes, reproductive system, melanocytes.

The blockade of the ETA-receptor has a huge therapeutical potential. Some clinic research has led to the success in the treatment of atherosclerosis, heart attacks, hypertension, pulmonary dysfunction, nephritic injuries. Endothelins are the reasons of several types of inflammation and autoimmune reactions. So, the ability of this receptors activity regulation leads to important achievements in fundamental science and medicine.

This report describes the results of gene-engineering of different human ETA-receptor modifications' and their expression with the help of the baculovirus expression system in sf9 insect cells. Further we plan to prepare (purify and stabilize) this protein for the crystallization and further structure determination.

Acknowledgements: The work was supported by the 5top100-program of the Ministry for science and education of Russia.
Keywords: G Protein Coupled Receptors, membrane proteins, protein expression and purification.

SUN-391
Glycocalyx alterations at apoptosis and its role in immune response and host-pathogen interactions: sweet taste of cell death

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Current report describes the role of two types of apoptotic cell-derived membranous vesicles (ACMV), each bearing distinct glycosylation patterns, in the immune response and host-pathogen interactions. Dying cells possess two active pathways of modification of glycocalyx: a) caspase-dependent activation of plasma membrane-assosiated neuraminidases leads to the formation of desialylated glycoepitopes on ACMV originating from plasma membrane (PM); b) with the aim to compensate membrane surface loss due to apoptotic blebbing dying cells expose on their surface immature membranes of endoplasmic reticulum (ER), bearing a moiety of oligomannosidic glycans. PM-derived ACMV are usually big (>3 μm) and contain nuclear material (histone and DNA), which actively translocates into the ACMV at the late stages of formation. ER-derived ACMV possess oligomannnosidic glycans, attributable to ER, that represent immunologically novel epitopes rapidly cleared by macrophages. Exposure of ACMV-contained nuclear material may support the formation of anti-nuclear (anti-histone and anti-DNA) antibodies in disorders, associated with impaired clearance, like SLE. At the same time adherent-invasive *Escherichia coli* (AIEC) cells, causing uropthatogenic infections and Crohn's disease, are known to utilize oligomannose-specific lectin FimH at the tip of their fimbriae to adhere to the host cells. We demonstrated that AIEC induce formation of ER-derived ACMV and attach to them. This process fosters bacterial infection and host cell colonization.

Keywords: apoptosis, Glycotargeting, plasma membrane.

SUN-392
Glycosphingolipid synthesis to study fluctuation-driven aggregation of nanoparticles on lipid membranes

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Shiga toxin entry into cells occurs by a clathrin-independent pathway in which the first step is the aggregation of initially-separated membrane-bound toxin particles. The molecular driving force of this aggregation is not yet understood, but it is known that toxin particles bind to the membrane via specific interactions with glycosphingolipid Gb3 headgroups. We demonstrate here using computer simulations that when rigid planar nanoparticles adsorb to a lipid bilayer, there is a regime in which they perturb the thermally-excited membrane shape fluctuations sufficiently to drive them together. We derive a theory of this fluctuation-induced aggregation as a form of Casimir force. The simulations predict that the nanoparticles will not aggregate if they are bound to the membrane by flexible linkers that remove the particles from direct contact with the membrane. To test this prediction, we have synthesized Gb3 analogues whose sugar headgroups are separated from their ceramide backbone by short PEG linkers, and we find that Shiga toxin particles do not aggregate although they bind normally to the membrane surface. We conclude that the non-specific interactions of the Shiga toxin particle with the membrane surface are necessary for their aggregation.

Keywords: None.

SUN-393
Golgi-to-ER recycling regulates the transport rate of membrane cargoes along the secretory pathway

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The Endoplasmic Reticulum (ER) and the Golgi complex are dynamically connected by both anterograde and retrograde vesicular transport pathways. Although the molecular mechanisms underlying the retrograde pathway have been extensively characterized, whether and to what extent plasma membrane-directed cargoes escape from this recycling has been poorly analyzed. To investigate this problem, we compared the transport of a set of membrane cargoes at the ER-Golgi interface by a combination of live cell imaging and temperature blocks. When the transport between the *trans*-Golgi network (TGN) and the plasma membrane is blocked by incubation at 20°C, VSVG (Vesicular Stomatitis Virus Glycoprotein) is exported from the ER and accumulates in the Golgi; in contrast, a mutant form of VSVG lacking its di-acidic-based ER export signal (VSVG AxA) attains a steady state distribution between the ER and the Golgi, suggesting that a Golgi-to-ER retrograde pathway, in combination with a slow rate of exit from the ER, determines this intermediate localization. iFRAP analysis, as well as treatment with H89, which blocks COPII-dependent export, revealed indeed that VSVG AxA, at variance with the wild-type protein, is recycled

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from the Golgi to the ER. Our analysis was then extended to other outward-directed membrane cargoes that lack any known export signal: a tail-anchored construct (FP-22) and the EGF Receptor (EGFR). We found that both these cargoes are included into an energy-dependent Golgi-to-ER recycling pathway with similar first order rate constants of Golgi-to-ER transport. To explore which retrograde route is involved in the recycling phenomenon, we used dominant negative mutants of the two Golgi-to-ER pathways (Arf1/COPI- and Rab6-dependent). In cells overexpressing inactive Rab6, VSVG AxA was more concentrated in the Golgi apparatus at 20°C, and the rate of its retrograde transport from the Golgi to the ER was slowed. The weight of Rab6-dependent recycling in delaying transport of membrane cargoes to their final destination was evaluated by comparing the effect of dominant negative Rab6 on the transport of wt and AxA-VSVG to the cell surface; at variance with wt-VSVG, we found that dominant negative Rab6 stimulated VSVG-AxA's transport to the cell surface. In conclusion, our results identify recycling as a novel limiting step in the transport of membrane cargoes along the secretory pathway, and indicate that a positive signal is required to avoid cargo recruitment into the retrograde Golgi-to-ER pathway.

Keywords: Live cell imaging, Retrograde transport, Secretory pathway.

SUN-394
The Crystal Structure of the Phosphatidyl Inositol 4- kinase IIα

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Phosphoinositides are a class of phospholipids generated by the action of phosphoinositide kinases with key regulatory functions in eukaryotic cells. Here we present the first atomic structure of a phosphatidylinositol 4-kinase type IIα (PI4K IIα), in complex with ATP solved by X-ray crystallography at 2.8 Å resolution. The structure revealed a rather non-typical kinase fold that could be divided into N- and C-lobes with the ATP binding groove located in between. Surprisingly, a second ATP was found in a lateral hydrophobic pocket of the C-lobe. Monte Carlo simulations revealed two membrane binding modes and the putative function of the hydrophobic pocket. Taken together our results suggest a mechanism of PI4K IIα recruitment, regulation and function on the membrane.

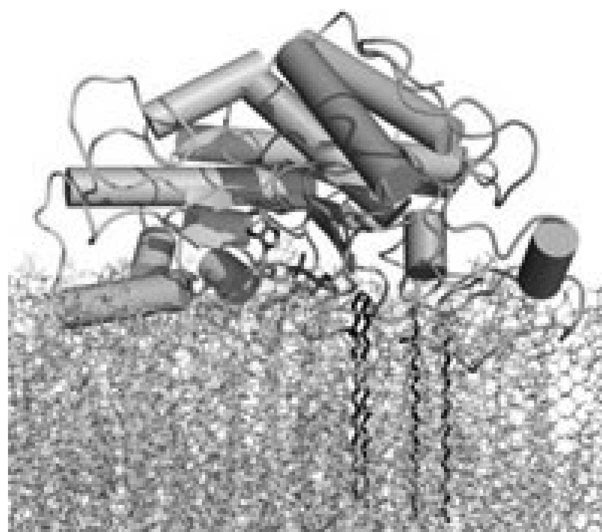

Fig. 1.

Keywords: Crystal structure, Lipid kinase, phosphatidylinositol 4-phosphate.

SUN-395
Human guanylate-binding protein 1 tethers giant unilamellar vesicles in a nucleotide-dependent manner

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Human guanylate-binding protein 1 (hGBP1) is the most studied protein within the family of guanylate binding proteins (GBPs). GBPs belong to the dynamin superfamily of large GTPases and have seven isoforms in humans. Different dynamins are shown to be involved in the processes of membrane deformation, but so far for hGBP1 this feature was not reported. HGBP1 is expressed to high levels after treatment of the cells with interferons with the highest expression for interferon γ and it was reported to be involved in different antipathogenic responses. However, the molecular mechanism of antipathogenic activity of hGBP1 is poorly understood.

HGBP1 shares common features with other members of dynamin superfamily and shows nucleotide-dependent self-activation and oligomerisation of the protein. In contrast to dynamins, the protein established a different way for membrane association. In the course of the posttranslational modification, the protein is coupled to a lipid anchor (isoprenoid). Although hGBP1 was intensively investigated, almost all studies were performed using the soluble protein without farnesyl anchor.

Previous studies indicated the requirement of GTPase activity of the protein for binding of farnesylated hGBP1 to the liposomes. In contrast, we show that GTP-binding is sufficient for association of the protein to the surface of liposomes and GTPase activity is not necessary required. Moreover, GTP turnover of the enzyme orchestrates dissociation of farnesylated hGBP1 from the surface of the liposomes. By video recording, we demonstrate the farnesylation-dependent ability of hGBP1 to mediate tethering of liposomes in a nucleotide-dependent manner, which is reversible for GTP and non-reversible for its non-hydrolysable analog.

Keywords: GTPase, membrane tethering.

SUN-396
Identification of bacterial sphingophospholipids and their activation of murine macrophages via Toll-like receptor 4

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Sphingobacterium spiritivorum has five unusual sphingophospholipids (SPLs). We determined the complete chemical structures of these SPLs. The compositions of the long-chain bases/fatty acids in the ceramide portion, isoheptadecasphingosine/isopentadecanoate or isoheptadecasphingosine/2-hydroxy isopentadecanoate, are characteristic. It is reported that several bacterial sphingoli-

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pids composed of ceramide are recognized by CD1-restricted T and NKT cells and that a non-peptide antigen is recognized by γδT cells. The mechanisms of host immune responses against these SPLs remain unknown. The immune response against bacterial lipid components is considered to play important roles in microbial infections. We demonstrated that these bacterial SPLs activated murine bone marrow macrophages (BMMs) via Toll-like receptor (TLR) 4 but not TLR2, although they slightly activated CD1d-restricted NKT and γδT cells. Interestingly, this TLR 4-recognition pathway of bacterial SPLs involves the fatty acid composition of ceramide in addition to the sugar moiety. A non-hydroxy fatty acid composed of ceramide was necessary to activate murine BMMs. The bacterial survival was significantly higher in TLR4-/- mice than in TLR2-/- and wild-type mice. The results indicate that activation of the TLR4-dependent pathway of BMMs by SPLs induced an innate immune response and contributed to bacterial clearance. In this study, we clarified the immune recognition of bacterial SPLs, identified structure/function relationships in this recognition, and assess the impact of SPLs on bacterial burdens.

Keywords: Sphingobacterium, sphingolipid, Toll-like receptor 4.

SUN-397

In vivo studies of aquaporin 0 interaction with calmodulin using bimolecular fluorescence complementation

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Protein-protein interactions (PPIs) are important in close to all vital processes in the cell. Ranging from stable protein complexes to transient interactions the studies of PPIs is not only critical in the understanding of protein functions but also difficult to monitor. Bimolecular fluorescence complementation (BiFC) allows PPIs to be studied by analyzing fluorescence as a result of an interaction. Non fluorescent fragments of YFP is fused to the protein targets and upon assembly into a complex fluorescence is emitted.

We show that the BiFC method in *S. cerevisiae* can be used to confirm the tetramerization of human aquaporin0 (hAQP0) which was used as a positive control for the assay. Also, no tetramerization is observed between hAQP0 and hAQP2, acting as a negative control.

The method was then applied to the the well-known interaction between aquaporin 0 and calmodulin (CaM). Whereas previous studies have an in vitro approach, we can show the complex formation of hAQP0-CaM in vivo. By truncating the C-terminal of hAQP0 just before the putative CaM interaction site the fluorescence is drastically decreased as expected when the interaction site is removed.

S. cerevisiae is an excellent system for PPI studies and in the case of hAQP0-CaM it has been shown to also work for interactions between a membrane protein and a soluble protein. This particular complex can now be studied more easily compared to the mammalian cell systems used previously and the consequence of minor changes in either protein can be quantitated and related to differences in the affinity of the complex.

Keywords: bimolecular fluorescence complementation, membrane proteins, protein-protein interactions.

SUN-398

Intramembrane helix–helix interactions of receptor tirosine kinases: structural biology and implications for signaling and human pathologies

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Cell membrane is important part of living cell. Receptor tyrosine kinases play a key role in biological processes occurring within the membrane. However detailed mechanism of their functioning has not been completely understood yet as there are no structures of full-length receptor tyrosine kinases. Fibroblast growth factor receptor 3 (FGFR3) transduces biochemical signals via lateral dimerization in the plasma membrane, and plays an important role in human development and disease. The most frequent pathogenic mutations G380R and A391E in the transmembrane (TM) region of FGFR3 are associated both with cancer and with disorders in skeletal development. Relatively small size of complexes of TM fragments of FGFR3 (with membrane adjacent regions, tmFGFR3) with detergents or lipids allows one to study their detailed spatial structure using three-dimensional heteronuclear high-resolution NMR spectroscopy. An effective expression system and purification procedure for preparative-scale production of tmFGFR3 in norma and with point mutations for structural and functional studies were developed. The purified peptides were reconstituted in lipid/detergent DPC/SDS (9/1) micelles and characterized using dynamic light scattering, CD and NMR spectroscopy. In the solved NMR structure, the two transmembrane helices pack into a symmetric left-handed dimer, with intermolecular stacking interactions occurring in the dimer central region. Some pathogenic mutations fall within the helix-helix interface, whereas others are located within a putative alternative interface. This implies that although the observed dimer structure is important for FGFR3 signaling, the mechanism of FGFR3-mediated transduction across the membrane is complex. We propose an FGFR3 signaling mechanism that is based on the solved structure, available structures of isolated soluble FGFR domains, and published biochemical and biophysical data.

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Keywords: membrane protein receptor, NMR Spectroscopy, structural biology.

SUN-399

Isolation of mycobacterial mutants that disrupted the phospholipid synthetase gene, and their properties

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In pathogenic bacteria, cell envelopes are essential in evading various attacks on the host defense system. The membranes of the gram-positive bacteria are overlaid cell walls consisting of a thick layer of peptidoglycans. Phospholipids are one of the indispensable components for formation of bacterial membranes. The synthesis of phospholipids is thus important in mycobacteria.

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However, little is known about the effects of various phospholipid compositions on bacterial growth or survival in macrophages. We constructed mutants that disrupted a phosphatidylserine synthetase (PSS) gene and investigated their properties. PSS gene knockout mutants (KO strains) were constructed from *Mycobacterium smegmatis* mc^2 155 and *Mycobacterium bovis* BCG. Also, the compensated strains (Comp) were prepared from KO strains by transformation with the plasmid DNA including the lost gene. The phospholipid compositions, growth rates, and drug susceptibilities were compared among wild type (WT), KO, and Comp strains and the survival rates of the WT strain and mutants in bone-marrow macrophages (BMM) were determined. The growth rates of the WT, KO, and Comp strains were similar to each other. PSS generally produces phosphatidylserine (PS) from cytidine diphosphate diacylglycerol (CDP-diacylglycerol) and serine in bacteria. And phosphatidylethanolamine (PE) is synthesized from PS by phospatidylserine decarboxylase. The composition of phospholipids in these strains on thin layer chromatography (TLC) revealed that the amounts of PS and PE were low, but detectable in WT and Comp strains of both *M. smegmatis* and *M. bovis* BCG, whereas in the PSS-KO strain, neither PS nor PE were detected on TLC. The survival rate of the KO strain in BMM was lower than those of the WT and Comp strains. We found that PSS was not an essential enzyme in mycobacteria. PS and PE were dispensable for their growth. However, composition of bacterial phospholipids had an effect on survival of mycobacteria in macrophages. Drug susceptibility of PSS-KO mutants was also altered.

Keywords: phospholipids, gene, knock-out.

SUN-400
LAPTM4B regulates ceramide clearance from late endosomes

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The late endosomal organelles (LE) have a central role in the degradation and recycling of sphingolipids, but the mechanisms by which sphingolipid catabolites exit LE are not well understood. In order to identify novel regulators of sphingolipid transport, we applied a siRNA screen of lysosomal transmembrane proteins and identified the Lysosome Associated Protein TransMembrane 4B (LAPTM4B) as a facilitator of ceramide exit from LE.

LAPTM4B interacts directly with ceramide based on its high affinity for a cross-linkable ceramide, and cells depleted of LAPTM4B display markedly increased ceramide levels. Knockdown of acid ceramidase (ASAH1) in LAPTM4B-depleted cells aggravates the ceramide storage observed by LAPTM4B silencing alone. Conversely, LAPTM4B overexpression suppresses the ceramide accumulation of ASAH1-deficient cells, suggesting that LAPTM4B and ASAH1 provide alternative means for decreasing LE ceramide. Moreover, LAPTM4B-depleted cells display increased amounts of active cathepsin D, and are sensitized to lysosomal membrane permeabilization as a consequence of the sphingolipid accumulation. Together, these data reveal a novel route for ceramide exit from LE facilitated by LAPTM4B, and pinpoint a role for LAPTM4B in regulating sphingolipid- and lysosome-mediated cell death. This identifies sphingolipid metabolism as a potential target for treating LAPTM4B overexpressing tumors.

Keywords: endosomal protein, lipid - protein interactions, Sphingolipid metabolism.

SUN-401
Lipidomic profiling of anandamide effects on decidual metabolic pathways

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In rodents, in response to blastocyst implantation, endometrial stromal cells proliferate and differentiate. Later, decidual cells undergo a cycle of regression, which occurs mainly by apoptosis [1]. This process is essential to support placental development and conceptus growth. In humans, abnormal decidualization has been linked with unexplained infertility, miscarriage and endometrial pathologies. However, the exact mechanisms controlling decidual regression are not fully understood. Recently, the role of endocannabinoid system in reproductive health has evolved [2]. We have previously shown that endocannabinoid machinery operates on decidual cells and that anandamide (AEA), the main endocannabinoid, induced apoptosis of decidual cells through cannabinoid receptor 1 (CB1) [3]. Also, disruption of endocannabinoid signaling lead to deferred implantation/decidualization and compromises pregnancy outcome [2]. In this study, we intended to analyze by lipidomic analysis possible changes in phospholid (PL) membrane profile of rat primary decidual cells triggered by AEA-induced apoptosis. In brief, PLs were extracted and classes separated by thin layer chromatography with subsequent analysis by mass spectrometry and by direct analysis of the total lipid extract by liquid chromatography-mass spectrometry. Fatty acid quantification was studied by gas chromatography with flame induction detection. Relevant data obtained were: an increase in phosphatidylserine and a decrease of cardiolipin and phophatidylinositol content, as well as an increase in hydroperoxides radicals in treated cells. It was also observed an increase in the content of a particular class of PL, plasmalogens, and in the content of long chain fatty acids specially with high degree of unsaturation. These data will be useful as a starting point to disclose metabolic pathways within decidualization process disruption.

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Keywords: Endocannabinoid System, Lipidomics, Reproduction.

SUN-402
Lipid–protein nanodiscs open new possibilities for NMR study of the interactions between water-soluble peptides and lipid membranes

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Membrane-active peptides (MP) play important role in the life of various organisms and their communities. Membrane-lytic antimicrobial peptides of eukaryotic origin act as a natural antibiotic component in the “innate” immune system. Some peptide neurotransmitters and hormones, as well as neurotoxins acting on the

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membrane receptors, have intrinsic membrane activity. The function of such peptides could involve two steps: the initial binding to the membrane surrounding the receptor, and subsequent formation of the ligand-receptor complex. The biological membrane or suitable membrane mimicking environment is required for the structure-functional studies of membrane-active peptides. Lipid-protein nanodiscs (LPNs) represent one of the most versatile membrane mimicking system. Each nanodisc encloses nanoscaled fragment of flat lipid bilayer stabilized in solution by the apolipoprotein or special membrane scaffold protein (MSP).

The applicability of LPNs for investigation of water-soluble MPs had not been studied previously. This question is not trivial, because LPNs involve additional anionic protein component (MSP, charge -6), while majority of antimicrobial peptides and membrane-active neurotoxins are positively charged. Here we investigated this question on the example of the three cationic water-soluble MPs of different origin. It was shown that pore-forming antimicrobial peptide arenicin-2 from marine lugworm (charge +6) disintegrates LPNs containing zwitterionic phosphatidylcholine (PC) or anionic phosphatidylglycerol (PG) lipids. In contrast to that spider toxin VSTx1 (charge +3), a modifier of Kv channel gating, weakly interacts with the MSP, effectively binds to the LPNs containing anionic lipids (POPC/DOPG 3:1), and has lower affinity to the LPNs composed from zwitterionic lipids (POPC). Snake neurotoxin II (NTII, charge +4), an inhibitor of nicotinic acetylcholine receptor, possesses affinity to the LPNs containing anionic lipids (POPC/DOPG 3:1, or POPC/DOPS 4:1) and does not bind to the LPNs/POPC. NMR study revealed that NTII interacts with the LPN/POPC/DOPS surface in several orientations. One of possible NTII orientations permits the early proposed specific interaction between toxin and the polar head group of phosphatidylserine from the receptor environment. Thus, LPNs could be used for structural studies of water-soluble membrane-active neurotoxins and their interaction with a membrane.

This work was supported by the Russian Foundation for Basic Research (14-04-01270).
Keywords: None.

SUN-403
Localization and dynamics study of mouse Klrb1 protein isoforms in mammalian cells

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Although natural killer (NK) cells have been known for almost forty years, characterization of their surface receptors still uncovers novel functional issues. Proteins Klrb1A and C are members of a type II family of transmembrane lectin-like receptors on the surface of NK cells important for their activation. They are involved in the protection of the organism against tumors and virally-infected cells. Recently, a new isoform of mouse protein Klrb1A which is missing a transmembrane domain was found as mRNA transcript. The examination of its expression and localization in the cell is a subject of our research. Since there are only limited molecular data on the rest of the family, it is necessary to study these proteins as well and correlate our results to proteins having transmembrane region.

A recent development in microscopic techniques allows a detailed study of Klrb1 proteins in their physiological environment – living cells. Klrb1x-GFP/mCherry fusion proteins were generated to investigate their stoichiometry and dynamics and verified by transient transfection, confocal microscopy and immunoblotting. Also, all proteins were expressed heterologously in *Escherichia coli* and using biochemical methods, they were structurally characterized. For identification of monomer and dimer forms in living cells, Klrb1 receptors were expressed in COS-7 cells, separated on SDS-PAGE under reducing and non-reducing conditions, and immunoblotted using anti-GFP antibody. The results showed the existence of monomer (A, C2) and dimer (C1) isoforms of Klrb1 receptors in living cells. Also the live cell imaging of Klrb1 activating receptors is presented and the data are correlated with these from the structural characterization. Our outcomes shed a light on molecular dynamics of each isoform in cell membrane and demonstrate the subcellular localization of the truncated Klrb1A isoform which is mainly retained in the cell cytoplasm with significant diffusion character.

This work was funded by Institutional Research Concept of the Institute of Microbiology RVO61388971; Ministry of Education Youth and Sports of the Czech Republic and European Regional Development Funds (CZ.1.07/2.3.00/20.0055, CZ.1.07/2.3.00/30.0003 and CZ.1.05/1.1.00/02.0109); Research Project of Charles University (UNCE 204025/2012); Grant Agency of Charles University (800413) and Operational Program Prague – Competitiveness project CZ.2.16/3.1.00/24023.
Keywords: Klrb1 protein isoforms, Live cell imaging, Structural characterization.

SUN-404
Loss of association of REEP2 with membranes is responsible for a hereditary motor neuron disease

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Hereditary spastic paraplegias (HSP) are clinically and genetically heterogeneous neurological conditions affecting cortical motor neurons. Their main pathogenic mechanisms are thought to involve alterations in endomembrane trafficking, mitochondrial function and lipid metabolism. With a combination of whole genome mapping and exome sequencing, we identified three mutations in *REEP2* in two families with HSP: a missense variant (c.107T>A, p.Val36Glu) that segregated in the heterozygous state in a family with autosomal dominant inheritance and a missense change (c.215T>A, p.Phe72Tyr) that segregated *in trans* with a splice site mutation (c.105 + 3G>T) in a family with autosomal recessive transmission. REEP2 belongs to a family of proteins that shape the endoplasmic reticulum, a continuous network of membranous sheets and tubules. *In vitro,* the p.Val36Glu variant in the autosomal dominant family had a dominant negative effect; it inhibited the normal binding of wild-type REEP2 to membranes. The missense substitution p.Phe72Tyr, in the recessive family, decreased the affinity of the mutant protein for membranes that, together with the splice site mutation, is expected to cause complete loss of REEP2 function. REEP2 binds directly to membranes, probably by the insertion of its hydrophobic domains into the phospholipid bilayer. As shown for other members of this family of proteins, this property might regulate the curvature of endoplasmic reticulum membranes where the protein resides. Loss of this function would lead to decrease in endoplasmic reticulum membrane curvature, explaining the expansion of endoplasmic reticulum sheets and endoplasmic reticulum swelling observed in fibroblasts from an affected individual. The altered

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morphology of endoplasmic reticulum caused by the loss of REEP2 function also altered the interaction between endoplasmic reticulum and mitochondria. The consequences of mutations in *REEP2* on the mitochondrial functions currently are under investigations.

Keywords: Endoplasmic reticulum, membrane shaping, mitochondria.

SUN-405
MAIDEN: model quality assessment for intramembrane domains using an energy criterion

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Membrane proteins represent 25% of all human proteins and nearly 50% of them are drug targets. Knowing the structure of a membrane protein is helpful to characterize its function and mechanism at the molecular level. Despite major advances in solving structures experimentally, most membrane protein structures remain unknown. This lack of available structures, along with the physical constraints imposed by the anisotropic environment of the lipid bilayer, constitutes a difficulty for membrane proteins modelling. Assessing the quality of membrane protein model is therefore critical.

In this study, we have developed a knowledge-based scoring function to distinguish between native (or near-native) structures and non-native ones, using a non-redundant set of 66 membrane proteins sharing no more than 30% of sequence identity. This distance-dependent statistical potential called MAIDEN is specific of the location in the lipid bilayer of each interacting residue. Deriving an accurate statistical potential from such a small data sets is challenging. To overcome that difficulty, we have based the construction of our potential on a kernel density estimation of distances distributions. By a jackknife cross-validation procedure, we show that our potential outperforms a potential optimized on globular proteins (DOPE) in discriminating native membrane protein structures from random sequence decoys. MAIDEN scoring function is also more efficient than DOPE in separating accurate membrane protein models from inaccurate ones. These results suggest that MAIDEN statistical potential will be useful for the modeling of membrane protein structure.

Keywords: Bioinformatics, membrane proteins, structural biology.

SUN-406
Membrane trans fatty acids in human testicular cancer cell membranes: the signal transduction pathways and effects of chemotherapeutics and antioxidants

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Testicular germ cell tumors (TGCTs) represent the most frequent malignancy in young males. The number of diagnosed cases has gradually increased, and the current frequency of tumor incidence is 50% higher than it was 30 years ago. However, the causes of this increase remain unclear. Bleomycin is used in chemotherapy regimens in the treatment of patients having TGCT. Mitogen activated protein kinases (MAPKs) are important mediators involved in the intracellular network of interacting proteins that transduce extracellular signals to intracellular responses. There is no study in the literature investigating the effects of Bleomycin, N-Acetyl-L-Cysteine (NAC), and Curcumin on membrane fatty acid changes and the underlying mechanisms related to MAPK pathway in the testicular cancer NTera-2 cells. To establish a causative relation between the cell membrane fatty acid changes and the signal transduction pathways among MAPK kinase family, we incubated Ntera-2 cells for 24 h with Bleomycin, NAC, Curcumin, Bleomycin+NAC or Bleomycin+Curcumin. Membrane fatty acids in the NTera-2 cells were isolated, derivatized and analysed by gas chromatography. We determined the levels of MAPK pathway members; p44/42-MAPK, p38-MAPK, MEK, p-MEK, JNK and pJNK. Bleomycin and curcumin increased the saturated fatty acid percentage of membrane lipids, whereas decreased the percentage of monounsaturated and polyunsaturated fatty acid. Bleomycin and curcumin led to a significant increase in trans lipid isomers of oleic and arachidonic acids, while N-Acetyl-L-Cysteine had no such an effects. 24 h incubation of NTera-2 cells with Bleomycin, Curcumin or their combination resulted in a strong activation of two MAPKs (pJNK, and p38-MAPK) and inactivation of p-MEK and p-ERK. Incubation with NAC alone did not cause any change in p38-MAPK and pJNK levels. Coincubation of NAC with Bleomycin attenuated the activation of p38-MAPK and pJNK which were upregulated by Bleomycin and caused an enhancement in p-MEK and p-ERK levels. Bleomycin, curcumin or their combination reduced the level of EGFR. Our results indicate that Bleomycin has an essential role in the regulation of membrane fatty acid profile alterations in human testicular cancer cell line. These results highlight the role of the membrane asset for fatty acid remodeling and suggest the potential of lipid-based strategies for influencing cell response and fate in testicular germ cell tumors.

Keywords: Bleomycin, Curcumin, N-Acetyl-L-Cysteine.

SUN-407
Modulation of cholesterol level and glutamate transport in brain nerve terminals by polymers of cyclodextrines

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Introduction: Recently, we demonstrated that a decrease in the level of membrane cholesterol may be used for neuroprotection under pathological conditions including stroke, cerebral hypoxia/ischemia, traumatic brain injury that were associated with an increase in glutamate uptake reversal. Visa versa in norm, a decrease in the concentration of membrane cholesterol may cause neurotoxic consequences resulted from the enhancement of the extracellular glutamate level because of a decrease in glutamate uptake. Also, novel acute effects of cholesterol acceptor methyl-beta-cyclodextrin (MCD) on glutamate transport in rat brain nerve terminals was demonstrated. However, these effects may be associated with ability of MCD to penetrate the plasma membrane of nerve terminals.

Methods: Planar Lipid *Bilayer* technique, spectrofluorimetry, radiolabeled assay.

Results: Several polymers of MCD was synthesized in Institute of Macromolecular Chemistry NAS of Ukraine. Comparative analysis of the effects of MCD and MCD polymers on cholesterol level, as well as uptake and ambient level L-[^{14}C]glutamate in nerve terminals and synaptic vesicle acidification was performed It was shown that MCD polymers in comparison with single molecule of MCD are able to decrease uptake and increase

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the ambient level of L-[^{14}C]glutamate in a narrow concentration range. Also, MCD polymers similarly with MCD molecule are able to dissipate the proton gradient of synaptic vesicles.

Discussion: This data create background for usage of MCD polymers in nanoneurotechnology.

Conclusion: Synthesized MCD polymers demonstrated similar effects with MCD monomer in modulation of transport of neurotransmitter and cholesterol level in nerve terminals underlying their high potential for usage in nanoneurotechnology.

Keywords: glutamate uptake, methyl-beta-cyclodextrin polymers, synaptic vesicle acidification.

SUN-408
Molecular dynamics study of human erythrocyte cell membrane

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We have performed 100 ns molecular dynamics (MD) simulation of human erythrocyte asymmetric membrane and investigated its molecular and structural properties.

We have created a phospholipid bilayer, using the phospholipid composition of human red blood erythrocyte membrane. The model also consists of cholesterol molecules and the transmembrane part of Glycophorin A (GpA) protein. The final system was solvated in water with 33 molecules per head group to reach fully hydrated crystalline state of the system. At the starting point of MD simulation, the phospholipid bilayer size was 10.5 x 9 x 9 nm^3 with 57640 atoms. Further, the system was minimized and subjected to a short MD simulation in NVT ensemble to avoid bad Van der Waals contacts. Finally, 100 ns MD simulation was carried out in NPT ensemble. The pressure and the temperature of the system were maintained to 1 atm and 310 K correspondingly. Particle Mesh Ewalds (PME) method was used for electrostatic interactions and for Van der Waals interactions cutoff radius was 14 Å. MD simulation was carried out by NAMD code using CHARMM27 force field, parallel on a Linux cluster with 64 processors.

The thickness of the membrane in equilibrium state is 51–52 Å. It is shown that some phospholipid molecules are arranged tighter in the inner layer of the membrane than in the outer layer but at the same time the area per phospholipid molecule head group is almost the same in both layers of erythrocyte membrane model. Detailed investigation of cholesterol molecules orientation in the phospholipid bilayer and the membrane surface roughness was performed. The cholesterol molecules density was calculated and found out that they are mainly localized in the hydrophobic part of the membrane, more precisely, below the polar heads of phospholipid molecules. This kind of cholesterol molecules localization, contribute to more rigid packing of phospholipid molecules in the membrane. The membrane surface molecular structure investigation shows that, in spite of asymmetric character of the membrane, the undulations of its inner and outer layers are similar to each other. The surrounding phospholipids' influence on GpA protein structural properties were also investigated. The Gly79, which plays a vital role in protein dimerization, stabilizes the GpA dimer by hydrogen bonds between Gly79 and Val80, Gly83 amino acids. In this regards, we have measured the distance between Gly79 and Val80, which is found to be about 7Å and close hydrogen- hydrogen contact between two amino acids is about 4.5Å as clearly shown in figure.

Keywords: erythrocyte membrane, Molecular dynamics simulation.

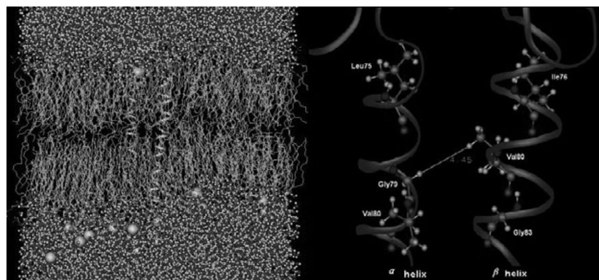

Fig. 1.

SUN-409
Molecular organization and membrane interactions of the cavin coat complex

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First observed in the 1950s, caveola membrane invaginations are a striking feature of many vertebrate cell types, and are critical for cell signaling, endocytosis and mechanotransduction. Caveolae formation depends on at least two protein families, the caveolin membrane embedded proteins (Cav1, Cav2 and Cav3) and the cavin peripheral membrane proteins (cavin1, cavin2, cavin3 and cavin4). Their importance in normal cell physiology is highlighted by the identification of caveolin and cavin mutations leading to different diseases including muscular and lipodystrophies. Despite this, there is currently no atomic level information addressing the mechanisms that underpin caveola assembly. Here we show for the first time that a minimal N-terminal domain of the cavin proteins (the HR1 fragment) is required and sufficient for their homo and hetero-oligomerisation. The crystal structures of mouse cavin1 and zebrafish cavin4 HR1 domains reveal highly conserved trimeric coiled-coil architectures, with unique intra-subunit interactions that determine the specificity of coiled-coil formation by the cavin proteins. A conspicuous feature of the HR1 domain is a highly basic surface patch, conserved among all cavins and across all species, which we show is able to mediate interaction with negatively-charged membrane lipids displaying the highest affinity for phosphoinositides (PIs). Mutations in this domain prevent membrane association and perturb caveolae formation in vivo. Interestingly the cavin proteins possess intrinsic membrane remodeling properties in vitro, that we propose is important for the formation of caveolae. Finally, we show that full-length cavin proteins possess characteristic rod-shape structures that reflect the coiled-coil architecture of the HR1 assembly domain. These rod-like structures can assemble through lateral and end-to-end interactions and have dimensions corresponding closely to the striations observed on the surface of caveolae *in vivo*. We therefore propose the striations forming the common coat of caveola are composed of polymerised cavin trimers, with membrane invagination requiring the presence of both caveolin and negatively charged lipid headgroups.

Keywords: caveolae, cavin, X-ray crystallography.

SUN-410
Neuronal SNAREs are sufficient to induce lysis-free endomembrane fusion

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Intracellular membrane fusion requires not only SNARE proteins but also other regulatory proteins such as the Rab and Sec1/

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Munc18 (SM) family proteins. Although neuronal SNARE proteins alone can drive the fusion between synthetic liposomes, it remains unclear whether they are also sufficient to induce the fusion of biological membranes. Here, through the use of engineered yeast vacuoles bearing neuronal SNARE proteins, we show that neuronal SNAREs can induce membrane fusion between yeast vacuoles and that this fusion does not require the function of the Rab protein Ypt7p or the SM family protein Vps33p, both of which are essential for normal yeast vacuole fusion. Although excess vacuolar SNARE proteins were also shown to mediate Rab-bypass fusion, this fusion was accompanied by massive leakage of luminal content. By contrast, neuronal SNARE-driven vacuole fusion occurred without impairing membrane integrity. Taken together, these results suggest that neuronal SNARE proteins suffice to induce lysis-free endomembrane fusion independently of Rab and SM protein functions.

Keywords: SNARE, Synaptic vesicle fusion, yeast vacuole.

SUN-411
New complex topology (knots and slipknots) in membrane proteins

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Currently more than 1000 structures of membrane proteins are known, whereas alpha-helical transmebrane proteins are represented by 76 families. Recently, using so-called matrix model to detect complex topology in proteins, we found that more than 2% of those known membrane proteins possess non-trivial topology! These proteins are secondary transporters that represent 8 out of 76 classified families. These are fascinating and surprising new results, which can help to understand membrane organization. Moreover, our analysis shows that all those proteins represent just three types of topological complexity: slipknots motifs (so called shoelaces motif, when pulled by termini will untie) with trefoil knot or figure-eight knot, and knot motif (pulling by terminal will tight knot inside membrane). Moreover the active site is located inside a knotted core (for knotted proteins). Those results directly indicate that complex topology is strongly conserved in those proteins across different species and should play some important role.

Keywords: free energy landscape, membrane protein, topology.

SUN-412
New N-terminal fusion tags for milligram-scale cell-free production of GPCRs

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G-protein-coupled receptors (GPCR) belong to one of the biggest families of membrane proteins. In spite of the fact that they are highly relevant to pharmacy, they have remained poorly explored. One of the main bottlenecks encountered in structural-functional studies of GPCRs is the difficulty to produce sufficient amounts of the proteins. Cell-free systems based on bacterial extracts from *E. coli* cells attract much attention as an effective tool for recombinant production of membrane proteins. GPCR production in bacterial cell-free expression systems is often inefficient because of the problems associated with the low efficiency of the translation initiation process. This problem could be resolved if GPCRs were expressed in the form of hybrid proteins with *N*-terminal polypeptide fusion tags. In the present work, three new *N*-terminal fusion tags are proposed for cell-free production of the human β2-adrenergic receptor, human M1 muscarinic acetylcholine receptor, and human somatostatin receptor type 5. It is demonstrated that the application of an *N*-terminal fragment (6 a.a.) of bacteriorhodopsin from *Exiguobacterium sibiricum* (ESR-tag), *N*-terminal fragment (16 a.o.) of RNAse A (S-tag), and Mistic protein from *B. subtilis* allows to increase the cell-free synthesis of the target GPCRs by 5–38 times, resulting in yields of 0.6–3.8 mg from 1 ml of the reaction mixture, which is sufficient for structural-functional studies.

Keywords: cell-free, fusion tag, GPCR.

SUN-413
Overcoming antibiotic resistance in *Staphylococcus aureus*

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Oligomers of acylated lysyls (OAKs) are chemical mimics of host defense peptides presenting broad spectrum antimicrobial activities. Previous studies have documented the ability of a miniature OAK, $C_{12(\omega7)}KKC_{12}K_{amide}$, to affect growth of gram-positive bacteria (GPB) and to act synergistically with certain antibiotics on gram-negative bacteria. Here, we investigated the OAK's ability to affect antibiotic resistance in *S. aureus*.

Despite its activity over a wide range of GPB, the OAK displayed synergistic activity with antibiotics only against *S. aureus* strains. Thus, chemo-sensitization of MRSA clinical isolates to oxacillin was achieved by up to 1000 folds. At the MIC, the OAK exerted a bacteriostatic effect and delayed emergence of resistance to oxacillin even at sub-MIC values. Membrane permeability was mildly compromised, resulting in membrane depolarization and inhibition of the induction and/or export of resistance factors such as β-lactamase and PBP2a. Furthermore, data collected using the mouse peritonitis-sepsis model support the likelihood for synergy to occur under *in-vivo* conditions as well, upon co-administration of the drugs. Based on the current data, we propose a mechanism suggesting that re-sensitization of MDR bacteria to multiple antibiotics is triggered by OAK-induced transient membrane depolarization which, simultaneously hinders multiple drug resistance mechanisms

Collectively, the findings support the view that targeting bacterial membrane potential could represent an efficient means to enhance antibacterial therapy, particularly since depolarization can simultaneously alter a wide range of crucial processes that derive their energy from the proton-motive force, rendering bacterial antibiotic resistance a more difficult task to accomplish.

Keywords: host defense peptides mimicry, membrane potential, synergy.

SUN-415
Prenatal alcohol–induced neuroapoptosis in rat brain: protection by folic acid and betaine

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When alcohol is consumed during the prenatal period, it may result in Fetal Alcohol Syndrome (FAS) in the offspring. The aim of this study is to investigate neuroapoptosis on the cerebral cortex of rat pups caused by prenatal alcohol exposure with modified liquid diet and to illuminate the protective effects of betaine and folic acid

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supplementation. Being alcohol-induced apoptosis indicators on the cerebral cortex of rat pups, cytochrome c, caspase 3 and calpain levels were checked. Cytochrome c level in ethanol+folic acid group ($p < 0.05$), caspase 3 level in ethanol+betaine+folic acid group ($p < 0.01$), calpain level in both ethanol+folic acid ($p < 0.01$) and ethanol+betaine+folic acid groups ($p < 0.01$) were found to be significantly lower than ethanol group. Cerebral cortex tissues were examined histologically in terms of congestion, edema, necrosis and chromatolysis. While ethanol increased the number of apoptotic cells, it was decreased in ethanol+betaine ($p < 0.05$) and ethanol+betaine+folic acid groups ($p < 0.05$). Morphometric examination showed that while the diameter of apoptotic cells increased with ethanol administration ($p < 0.01$), they were lowered at ethanol+betaine ($p < 0.001$) and ethanol+betaine+folic acid groups ($p < 0.001$). As a result, we found that ethanol is capable of triggering cell death in the ethanol-administered rat pups and folic acid and betaine may reduce apoptosis either alone or together.

Keywords: Betaine, Folic acid, Neuroapoptosis.

SUN-416
Probing membrane protein structure with small angle neutron scattering and molecular modeling

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Probing the solution structure of membrane proteins represents a formidable challenge, notably using small-angle scattering due to residual scattering contributions of detergent molecules. Using SANS measurements, we studied the conformation of the Bordetella pertussis outer-membrane transporter FhaC. By using molecular modeling and starting from six distinct conformations of FhaC embedded in lipid bilayers, we generated ensembles of protein-detergent arrangement models. The computational methodology allows for the generation of a large number of protein-detergent arrangements. The chi square fits of back-calculated versus experimental curves are decidedly discriminative and allow for the elimination of both protein conformation and detergent organization. Good fits were obtained for relatively compact, connected detergent belts, that, however, display small detergent-free patches on the outer surface of the beta-barrel. The combination of SANS and modeling clearly enabled us to infer the solution structure of FhaC. We believe that our strategy that combines explicit atomic detergent modeling with SANS measurements holds significant potential for structural studies of other detergent-solubilized membrane proteins.

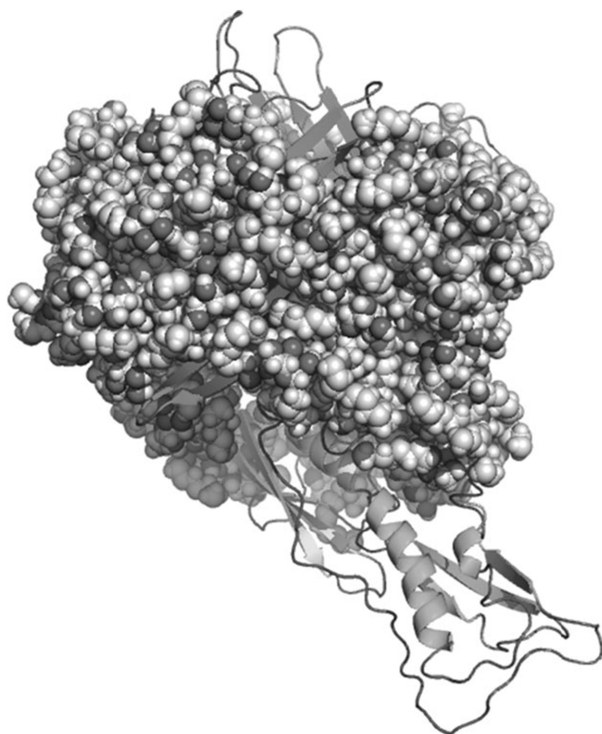

Fig. 1.

Keywords: membrane proteins, Molecular modeling, SANS.

SUN-417
Purification and functional characterization of *Saccharomyces cerevisiae* Gdt1p, a novel Golgi-localised Ca^{2+}/H^{+} antiporter

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Calcium (Ca^{2+}) is a ubiquitous and versatile intracellular messenger responsible for controlling numerous cellular processes. In yeast, the cytosolic concentration of Ca^{2+} (50–200 nM) is tightly regulated through diverse Ca^{2+} pumps and exchangers located in different compartment of the cell. In a recent study, we showed that the Ca^{2+}:H^{+} Antiporter-2 (CaCA2) family member of yeast, Gcr1-dependent translation factor-1 protein (Gdt1p) contributes to Ca^{2+} homeostasis [1]. Gdt1p is a Golgi-localised membrane protein of 280 amino acids composed of 6 transmembrane domains with an antiparallel topology [1]. Notably, the *gdt1Δ* yeast strain was sensitive to high concentration of external calcium. The WT phenotype is restored by the expression of the human ortholog TMEM165 which is involved in Congenital Disorders of Glycosylation (CDG) in human [2].

The aim of our study is to elucidate how Gdt1p regulates Ca^{2+} homeostasis in the Golgi complex and to demonstrate its primary transport function. Here we have used the nisin-inducible expression system of *Lactococcus lactis* for the production of Gdt1p. The recombinant Gdt1p expressed in the cell membrane of *L. lactis* has been solubilised and purified in the presence of Foscholine. Gel-filtration analysis suggested that the purified recombinant Gdt1 forms a tetramer. We are currently reconstituting the purified recombinant Gdt1 into proteoliposomes in order to measure direct ion transport. Our study will provide us mechanistic insights into the molecular function of Gdt1p in the budding yeast.

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Keywords: Ca^{2+} homeostasis, Gdt1 protein, Saccharomyces cerevisiae, Reconstitution and proteoliposome.

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SUN-418

Redox regulation of photo-fermentative hydrogen production by *Rhodobacter sphaeroides*

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Bacterial anaerobic growth has been shown to be coupled with decrease of external redox potential (E_h) from positive down to low negative value, which result transfer of electrons within bacterial membrane and formation of proton motive force [1]. *Rhodobacter sphaeroides* performs a photo-fermentation of organic compounds with hydrogen (H_2) production [2] which can be a redox regulated one. In this work regulation of H_2 photoproduction through the change of E_h during anaerobic growth of *R. sphaeroides* MDC6521 from Armenian mineral springs was represented. Membrane-permeating reducer, DL-dithiothreitol (DTT), maintaining negative values of E_h, was determined to suppress the specific growth rate. Membrane-non(poorly)-permeating oxidizer, ferricyanide, maintaining positive values of E_h, also decreased the bacterial growth rate. However, DTT enhanced H_2 yield by *R. sphaeroides*; whereas ferricyanide inhibited H_2 production. In the presence of 0.5–1 mM DTT H_2 yield was increased during 48–96 h: after 72 h H_2 yield was ~1.3-fold higher compared to control. But in medium with 2 mM DTT H_2 production was not observed during 48–72 h growth. In contrast to DTT, H_2 yield disappeared in the presence of oxidizer: by addition of 0.5–2 mM ferricyanide H_2 yield decreased ~2–5-folds during 72 h growth. Thus, the reduced medium is preferred for H_2 production by *R. sphaeroides*. The relationship between H_2 production and E_h changes has been suggested: H_2 production by these bacteria is observed under strong reducing conditions. This might result redox state of responsible enzymes. The external reagents, which modify the redox environment and affect bacterial photo-fermentation, can be used for understanding of regulatory pathways of bacterial metabolism and for optimization the conditions for efficient H_2 production.

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Keywords: H2 photoproduction, Redox homeostasis, Rhodobacter sphaeroides.

SUN-419

Rim21 senses alteration in plasma membrane lipid asymmetry and elicits the signal at the plasma membrane in a ubiquitination-dependent manner

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In the eukaryotic plasma membrane (PM), lipid composition differs between the inner (cytosolic) and outer (extracellular) leaflets, which is called lipid asymmetry. For example, phosphatidylserine and phosphatidylethanolamine are mostly confined to the inner leaflet while phosphatidylcholine and sphingolipids are enriched in the outer leaflet. Lipid asymmetry is generated and maintained by inward (flip) and outward (flop) movement of lipids between the leaflets. We previously reported, in yeast, that alteration in PM lipid asymmetry is sensed by the Rim101 pathway[1], which was originally reported to detect external alkalization.

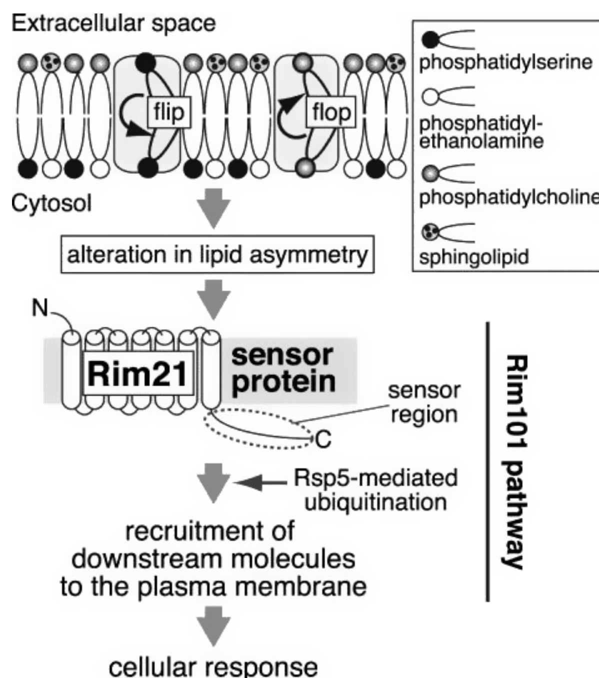

Fig. 1.

In the present work, we have investigated how the Rim101 pathway senses altered lipid asymmetry and ambient pH, and how the signal is transduced. We found that a PM protein Rim21, one of the most upstream molecules in the Rim101 pathway, acts as the sensor molecule detecting altered lipid asymmetry and ambient pH[2]. Biochemical analyses revealed that Rim21 forms a sensor complex with two PM membrane proteins Dfg16 and Rim9. Dfg16 and Rim9 were shown to be required for delivery of Rim21 to the PM. The C-terminal cytosolic region of Rim21 (Rim21C) contains clusters of charged amino acid residues. Rim21C fused with GFP was localized to the PM, although Rim21C is a hydrophilic region. In cells with disturbed lipid asymmetry due to inactivation of plasma membrane flippases, Rim21C dissociated from the PM and dispersed in the cytosol. These observations clearly indicate that Rim21C alone can detect alterations in lipid asymmetry, in another word, sensor motif is included in Rim21C. By mutational analysis of Rim21C, we identified the region playing a critical role in sensing of lipid asymmetry. Together with other results, we will discuss the mechanism of lipid asymmetry sensing.

We also investigated how the signal elicited by Rim21 is transduced. When Rim21 was stimulated by alteration in lipid asymmetry or external alkalization, downstream molecules including the arrestin-related protein Rim8, calpain-like protein Rim13, ESCRT III subunit Snf7, and scaffold protein Rim20 accumulated at the PM. Activity of the ubiquitin ligase Rsp5 was required for recruiting the downstream molecules to the PM, suggesting that ubiquitination mediates Rim101 signaling at the PM.

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Keywords: lipid, membrane, ubiquitin.

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SUN-420
Role of neutral sphingomyelinase in dopamine transporter trafficking

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Dopaminergic neurotransmission in the brain plays a central role in the control of movement, hormone release, and many complex behaviors. Dopamine (DA) uptake into presynaptic terminals is thought to be a key step in neurotransmission termination. Dopamine transporter (DAT) is Specific transport protein tightly control the availability of DA, in the synaptic cleft by mediating rapid transmitter reuptake at presynaptic terminals. DAT undergo highly regulated trafficking between the cell surface and endosomal compartments (Rao et al., 2011; Sager and Torres, 2011). Here, we suggest that neutral sphingomyelinase 2 (nSMase2) may regulate DA uptake and DAT trafficking. Previously we found that nSMase2 is a mediator of DA uptake (Kim et al., 2010). Interestingly, transfection of nSMase2 siRNA or pretreatment with the nSMase2-specific inhibitor GW4869 resulted in decreased DA uptake. Reciprocally, exposure of PC12 cells to cell-permeable C_6-ceramide induced a concentration-dependent increase in DA uptake. In mouse striatum and PC12 cells, we performed immunoflorescence staining to find out the localization of nSMas2 and DAT. In the dorsolateral mouse striatum, we observed colocalization of DAT and nSMase2. In PC12 cells, DAT are colocalized with nSMase2 in plasma membrane. We examined the physiological relevance of the DAT–nSMase2 interaction by performing coimmunoprecipitation experiments in synaptosomal preparations from mouse striatum as described previously (German et al., 2012). The enrichment of nSMase2 and DAT in striatum is more pronounced. In Immunoblotting with imunoprecipitation of DAT showed nSMase2, but imunoprecipitation of nSMase2 did not showed DAT. anti-nSMase2 antibody does not have good performance to pull down nSMase2 protein from supernatant. In nSMase activity assay with immunoprecipitated pellet, the IP pellet of anti-DAT antibody show nSMase activity to an extent equivalent to that achieved with the IP pellet of anti-nSMase2 antibody. These results indicate a physiological relevance of the DAT–nSMase2 interaction. Further analysis revealed that GW4869, nSMase2 inhibitor, decreased the surface level of DAT in PC12 cells stably expression DAT. In sum, our findings provide that physiological interaction between DAT and nSMase2 and, nSMase2 is suggested to facillitate relocalization of DAT on the presynaptic surface.

Keywords: Ceramide, Dopamine transporter, neutral sphingomyelinase 2.

SUN-422
Single particle tracking to study the binding of protein misfolded oligomer to membrane ganglioside GM1

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Recent results suggest an important role of ordered lipid domains of plasma membranes in amyloid aggregation process and its pathogenic effect. We investigated the contribution to toxicity of the membrane ganglioside GM1 in SH-SY5Y neuroblastoma cells exposed to oligomeric conformers of $A\beta_{42}$ and HypF-N endowed with different ultrastructural properties. By means of real-time single particle tracking, we show that misfolded oligomers bind GM1, decreasing its lateral diffusion on the plasma membrane of living cells. In turn, the biochemical response to the oligomeric species results from the membrane content of GM1 and its clustering. Overall, our results indicate an altered membrane raft mobility and clustering in neurons experiencing aberrant protein oligomers.

Keywords: Membrane GM1, Protein oligomers, Single particle tracking.

SUN-423
State-dependent H-bonds of exceptionally conserved asparagines in S6 helices of sodium and calcium channels: roles in channel gating and ligand action

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Background: Voltage-gated sodium and calcium channels play key roles in the physiology of excitable cells. The alpha-1 subunit of these channels folds from a polypeptide chain of four homologous repeats. In each repeat, the cytoplasmic halves of the pore-lining helices contain exceptionally conserved asparagines. Such conservation implies important roles, which are unknown. Inherited mutations of the asparagines are associated with some channelopathies. Engineered substitutions of the asparagines affect activation and inactivation gating and the action of pore-targeting drugs and toxins. In the absence of X-ray structures of eukaryotic sodium and calcium channels, underlying mechanisms are unclear.

Methods: X-ray structures of potassium and sodium chandelles in the open and closed states were used as templates to build homology models of the open and closed Cav1.2 and Nav1.4 channels and some of their mutants. The models were optimized with the method of Monte Carlo-energy minimization and state-dependent contacts of the conserved asparagines were analyzed.

Results: In the homology models of the open and closed Cav1.2 and Nav1.4 channels the asparagines do not face the pore. In the open, but not in the closed channels, the asparagine residue in a given repeat forms an inter-repeat H-bond with a polar residue, which is typically nine positions downstream from the conserved asparagine in the preceding repeat. The H-bonds, which are strengthened by surrounding hydrophobic residues, would stabilize the open channel and shape the open-pore geometry. According to calculations, the latter is much more sensitive to mutations of the asparagines, than the closed-pore geometry. Substitutions of the asparagines, their H-bonding partners, or surrounding residues change state-dependent inter-repeat contacts. The changes may affect channel activation, inactivation, and ligand action. Our models suggest the atomistic mechanisms behind a calcium channelopathy (the night blindness) and sodium channelopathies (Braguda syndrome and infantile arrhythmias). We further propose that engineered substitutions of the conserved asparagines influence the state-dependent action of local anesthetics, antiarrhythmic agents, and steroidal agonists like batrachotoxin and veratridine by changing availability of the open channels and affecting the open pore geometry.

Conclusions: The exceptional conservation of asparagines in the inner helices of sodium and calcium channels is due to their involvement in inter-repeat H-bonds that stabilize the open state and shape the open pore geometry.

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Keywords: Channel gating, Molecular modeling, State-dependent drug action.

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SUN-424
Structural characterization of the retromer cargo recognition sub-complex

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Retromer is a peripheral membrane protein complex that plays a key role in cargo export from the endosomal network. It is involved in many physiological, developmental and pathological processes including Wnt signaling, toxin transport and amyloid production in Alzheimer's disease.

The classical retromer complex consists of five proteins that can be classifide into two sub-complexes. One sub-complex contains a heterodimer of proteins from the sorting nexin family (SNX1/SNX2 and SNX5/SNX6), containing Bin-Amphiphysin-Rvs domains (SNX-BAR proteins) that can drive and/or sense membrane deformation and tubulation. By recruiting the cargo-selective sub-complex to the forming tubules, the SNX–BAR coat complex mediates the retrograde transport of proteins from endosomes to the *trans*-Golgi network. The cargo-selective sub-complex is heterotrimeric and consists of VPS26, VPS29 and VPS35. While there is noticeable diversity of sorting nexins between species, the cargo-selective sub-complex is highly conserved across all eukaryotes. Therefore the core functional component of the retromer complex is considered to be the cargo selective trimer.

Using the SAXS/WAXS and MX beamlines at the Australian Synchrotron, we have acquired crystallographic and small angle scattering data to determine how the core cargo recognition sub-complex assembles. Intriguingly, this trimeric complex is able to form a symmetric dimer, which may have implications for functional interactions *in vivo*. We are also currently exploring the structure of these proteins in the thermophilic fungus *Chaetomium thermophilum*. Recently, we crystallized and solved the structure of VPS29. The crystallization of VPS26 and VPS35, as well as co-crystallization experiments are currently in progress. We are using this structural information in combination with biochemical and biological studies in a synergistic approach to understand retromer-mediated endosomal protein sorting and how this fascinating protein complex contributes to a diverse set of cellular processes.

Recent studies have highlighted the molecular and functional diversity of retromer and the identification of new interacting partners, including the WASH complex, has revealed that the role of retromer extends to aspects of endosome-to-plasma membrane sorting and regulation of signaling events. The structural characterization of the cargo-selective complex of retromer and its interacting partners will provide additional insight into retromer specificity and function.

Keywords: membrane trafficking, protein complex, retromer.

SUN-425
Structural dissection of architecture of caveolar protein coat

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Coordinated action of membrane-embedded caveolin and peripheral cavin proteins results in the formation of membrane invaginations called caveolae. Mammalian caveolae play key roles in variety of cell processes, and structural information is critically needed to understand the molecular mechanisms underlying their formation and organization. Using a combination of X-ray crystallography, electron microscopy and biochemical analysis we structurally dissect the architecture of the cavin coat from the atomic to the supermolecular scale. The assembly starts from selective trimerisation of cavins via their conservative helical domain. Resultant formation of the supercoil trimer induces helical folding in disordered regions and cavin association into rod-shaped subcomplexes. Then cavin rods assemble through side-to-side and end-to-end interactions into striated lattices characteristic for native caveolar coat. Subcomplexes' anchoring to lipid membranes involves basic regions located in helical portions of cavins and this association induces membrane curvature characteristic for caveolae.

Keywords: caveolae, cavins, membrane domain.

SUN-426
Structure of the SNX27:VPS26 complex reveals mechanistic details of endocytic recycling

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Retromer is a highly conserved protein complex composed of three core proteins VPS35, VPS26 and VPS29. It plays a central and essential role in endosomal membrane trafficking, acting as a hub for endosomal tubulovesicular membrane trafficking in coordination with members of the sorting nexin (SNX) protein family and regulatory proteins. Mutation of retromer has been found to cause Parkinson's disease, likely due to disruption of endosomal morphology and trafficking, and its down-regulation is linked to neurodegenerative phenotypes in Alzheimer's disease. It has primarily been associated with endosome-to-trans Golgi network (TGN) trafficking of diverse transmembrane cargos such as the cation independent mannose 6-phosphate receptor (CIMPR), but to-date the mechanism by which it engages cargo and regulatory molecules has remained obscure. Recently, retromer has been shown to be important for endosome-to-plasma membrane recycling, retrieving cargo from lysosomal degradation through its association with the adaptor protein SNX27. This interaction is thought to couple retromer to a wide array of cargo, bound to the SNX27 PSD95-disc large-zonula occludens (PDZ) domain via type-I PDZ binding motifs (PDZbms). The binding of retromer to SNX27 also lies at the heart of a network of regulatory interactions that include bin-amphiphysin-rvs (BAR) domain containing SNX proteins that promote membrane tubulation.

Here we show how retromer engages the PDZ domain of SNX27 to facilitate endosomal recycling of a large variety of transmembrane proteins and prevent their lysosomal degradation. The crystal structure of the PDZ domain of SNX27 bound to the retromer protein VPS26A reveals a unique mechanism of PDZ-domain-mediated interaction. It explains how SNX27PDZ can bind VPS26A as it simultaneously recruits transmembrane cargo molecules via their C-terminal PDZ binding motifs, while also allowing VPS26A to be incorporated into the retromer complex by association with VPS35. Structure-based mutagenesis to perturb the SNX27-VPS26 association shows that this is essential for the recycling of the glucose transporter 1 (GLUT1) model cargo receptor from endosomes to the plasma membrane. Mechanistically, the binding of retromer to the PDZ domain of SNX27 increases the binding affinity for cargo PDZ binding motifs by over an order of magnitude, suggesting an important role for

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retromer-SNX27 cooperativity in endosomal cargo selectivity. These results demonstrate a novel mechanism of protein binding by a PDZ supramodule and provide molecular insight into the biologically critical endocytic recycling of cargo proteins via the SNX27-retromer-mediated pathway.

Keywords: endosomal recycling, retromer, sorting nexin.

SUN-427
Substrate rigidity modulates membrane reservoir to alter ion flux in macrophages

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Macrophages sense the environment in the surrounding tissue for signs of possible danger. In our previous work, we showed that the stiffness of a central nervous system (CNS) implant contributed to the development of the foreign body reaction (FBR). Materials significantly stiffer than brain tissue propagated a more robust FBR and this reaction was initiated by microglia, the macrophages in the CNS. It is possible that these macrophages sensed a foreign stiffness as a sign of possible danger. In our current work, we investigate a possible mechanism for this immunomechanosensitivity. Using a star-polyethylene glycol (star-PEG) cross-linked with heparin gel system, seeded bone marrow derived macrophages (BMM) consistently spread on stiff (~19 kPa) substrates in a pattern resembling frustrated phagocytosis. BMM displayed markedly reduced membrane ruffling patterns upon inspection by scanning electron microscopy (SEM), which suggested a decrease in available membrane reservoir. By pulling membrane tethers using atomic force microscopy (AFM), we confirmed that the membrane reservoir was significantly reduced on cells seeded on stiff substrates. We then investigated potassium (K^+) dynamics because K^+ efflux is a common precursor to inflammation. Colorimetric analysis of potassium channel activity suggests increased channel opening in cells on progressively stiff substrates. This activity is at least partially mediated by caveolae, cholesterol rich invaginations that serve as membrane reservoirs. Overall, these results suggests a link between cell spreading and depletion of the membrane reservoir, which may alter ion flux. These results also suggest that by modulating the membrane, one can directly influence the inflammatory potential of immune cells in a biophysical manner.

Keywords: ion channels, Macrophage activation, mechanotransduction.

SUN-428
Super resolution microscopy-based analysis of RhoD GTPase intracellular localization and transcriptional screening in human cancer tissues

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RhoD GTPase is a member of Rho family of small GTPases, which participate in the regulation of various processes such as the formation of cellular protrusions, vesicular trafficking, transcriptional regulation, cell motility and cell and centrosome cycle. These processes are often deregulated in cancer, and indeed some Rho proteins have been implicated in transformation. As most classical rho GTPases, RhoD cycles between GTP/GDP bound states and exert its function via several downstream effectors such as members of the Diaphanous Related Formins, Plexins, Rabankyrin and others. RhoD is in evolutionary terms the youngest member of the family, since it is only found expressed in therians (marsupials and placentalia). Functional analysis has shown that RhoD is more related to RhoA, B, C than Rac or Cdc42. Similar to RhoB, RhoD is the only other member of the family localizing to vesicular structures.

Many studies have implicated RhoD in the early endocytic pathway and RhoB in the late degradative (lysosomal) pathway. In this study, we performed a super-resolution analysis of the localization of the wild type form of RhoD in human umbilical cord endothelial cells (HUVECs), using Stimulated Emission and Depletion confocal microscopy (STED). Moreover, we performed quantitation of the endosomal colocalization status of RhoD positive vesicles versus various markers of the endocytic cell compartments, using specialized quantitation software (MotionTracking). In cancer, deficient termination of the oncogenic signaling of mutated receptors, such as the EGFRs, through altered kinetics of transport from early to the late endosomal compartment and/or suboptimal lysosomal degradation could contribute to the process of malignant transformation. Furthermore, we have previously shown that RhoD is implicated in cell proliferation and regulation of the centrosome cycle, key cellular processes often altered during the transformation process. Therefore, we also sought to identify the expression profile of this protein in various cancer tissues. For this, we performed an extensive screening of the expression levels of RhoD in 384 cancer tissue samples and we present the data herein

Keywords: RhoD, STED, tissue.

SUN-429
Systematic characterisation of PRAF and YIPF proteins involved in the membrane trafficking

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Despite being discovered nearly two decades ago, the membrane protein families PRAF and YIPF, are still poorly characterised in terms of their function in membrane trafficking. The best characterised protein PRAF1 has been associated with the activation of Rab GTPases, switch proteins that regulate membrane trafficking processes, while PRAF3 has been described to interact with Arf proteins, which are also major players in membrane trafficking. In turn, PRAF2 has been shown to have elevated expression levels in different cancer types such as breast, colon, lung, ovary and nervous system. On the other hand there are only a few publications about YIPF proteins and even these report contradicting conclusions.

In this study we systematically characterised proteins from the PRAF and YIPF families. Their subcellular localisation was determined by fluorescence microscopy imaging and unsupervised quantitative image analysis of both GFP-tagged proteins and immunostained samples. Three PRAF proteins localise mostly to endoplasmic reticulum and Golgi complex, while all 7 YIPF proteins localise to Golgi. Interestingly YIPF proteins seem to localise to different microdomains of the Golgi which is being further investigated by electron microscopy and co-localisation techniques. PRAF and YIPF families are membrane proteins with 4 or 5 transmembrane domains respectively, both having their N-terminal region facing the cytosol. The predicted topology has been experimentally confirmed with the fluorescence protease protection assay. These cytosolic regions interact with other cytosolic proteins, with Rabs being of particular importance. Immunoprecipitation experiments followed by mass spectrometry analysis confirmed some previously known interactions between PRAF or

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YIPF proteins and Rabs. Other interesting interactors were also identified: for example, proteins related to heat shock and lipid metabolism were detected in PRAF1 immunoprecipitates.

The work presented here provides a systematic characterisation of PRAF and YIPF membrane proteins in terms of their specific subcellular localisation, topology and function. Moreover, the assembled datasets and tools will allow us and other researchers to further investigate these two families of proteins, especially their role in membrane trafficking and their link with cancer.

Keywords: mass spectrometry, membrane trafficking, microscopy.

SUN-430
The capture and characterization of the Fc receptor complex from cultured macrophages

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Cell surface receptors are of critical importance to biomedicine and the treatment of disease but are notoriously difficult to isolate and identify by classical approaches. Thus there is an urgent need to activate and capture receptor associated supramolecular complexes from the surface of live cells using liquid chromatography and tandem mass spectrometry (LC-ESI-MS/MS). The Fc gamma receptor (FCGR) is responsible for the engulfment of foreign particles and pathogens coated with Immunoglobulin G (IgG). The FCGR complex was captured from live human U937 and murine RAW 264.7 macrophages by presenting the cognate ligand (IgG) on micro chromatography beads. Many co-receptors, including innate immune receptors such as Fc-like receptors, scavenger receptors, toll-like receptors, Fc-like killer cell receptor, lectins, epidermal growth factor, interleukin and colony stimulating factor, chemokine, cytokines, histamine and other receptors that are known to modulate immunological response were observed to copurify alongside the Fc receptors on micro beads. As a second method, direct biotinylation of IgG provides for specific capture of the Biotin-IgG-FCGR complex on strepavidin beads, but the biotinylated receptor complex cannot be specifically separated from the contamination and background binding to the strepavidin resin. However, new affinity chromatography reagents such as NHS-SS-biotin may be cleaved with DTT to specifically release the FCGR complex. The monovalent versus aggregated FCGR complex was bound and activated by its IgG-S-S-biotin probe to activate and capture the receptor before collection over streptavidin followed by specific elution with the reducing agent DTT. After binding and activation of the cell surface receptor by its ligand, the cells were disrupted with a French press and the homogenate applied to a streptavidin agarose affinity column. After washing, the activated and assembled FCGR cell surface complex was eluted from the column with a reducing agent DTT, and digested with trypsin. The peptides were analyzed by LC-ESI-MS/MS using a linear ion trap (Thermo) where the peptides identification using the SEQUEST, MASCOT, OMSSA and X!TANDEM algorithms and quantified using R statistical analysis. We propose to define the role of a subset of the IgG-Fc co-receptors by phagocytosis assays and the measurement of phagocytosis and free radical production in the presence of specific siRNA or controls, mutant constructs and pharmacological agents. We also propose to quantify the assembly of the Fc-IgG receptor complex over time and compared to other innate immune receptors. The analysis of a cell surface receptor complex developed and tested on the model FCGR complex may be applied to a wide variety of receptors crucial in human infection, disease and pain.

Keywords: mass spectrometry, receptor complex.

SUN-431
The dynamics of dynamin in vivo

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Clathrin mediated endocytosis is the process in which cells are able to internalize patches of membrane together with external or transmembrane material. The membrane patch is forced into becoming a vesicle due to the formation of a Clathrin coat, recruited to the membrane by cargo-specific adaptor proteins. The newly formed bud is then severed from the membrane in a fission event mediated by the large GTPase Dynamin.

Dynamin polymerizes around the neck of the bud in form of a helix and, upon GTP hydrolysis, changes conformation and twists and constricts the neck promoting fission.

With the purpose of clarifying the dynamics of memebrane fission in living cells, we selected and characterized conformational antibodies able to recognize the nucleotide-bound state of Dynamin. We are currently using these antibodies by expressing them directly in living cells, coupled with a fluorescent protein. By using TIRF microscopy we can follow over time the colocalization of the antibodies with fluorescent Dynamin or other members of the Clathrin mediated endocytosis machinery.

Keywords: None.

SUN-432
The effects of 53 GHz irradiation on enhancements of antibiotics antibacterial influence on *Escherichia coli*

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Coherent electromagnetic irradiation (EMI) of extremely high frequency - 30 GHz to 300 GHz is widely spread in environment. EMI can cause different biological effects [1, 2]. Because of its disinfecting properties EMI has applications in the environment controlling - water sources, agricultural wastewater, in treatment of food at moderately low temperatures and in therapeutic practice [2].

Bacterial growth has a great significance to understand changed bacterial sensitivity to EMI or antibiotics. Over the years antibiotics have been widely used in food industry, in agriculture and aquaculture, increasing the percentages of antibiotic resistant bacteria [2]. EMI can affect the cellular defense system and makes it vulnerable to antibiotic attacks [2].

The effects of different antibiotics – chloramphenicol (Chl), ceftriaxone (Cef), kanamycin (Kan) and tetracycline (Tet), on bacteria were examined via determination of bacterial growth in a comparative manner – non-irradiated bacteria (control), irradiated bacteria, control and antibiotic, irradiated bacteria and antibiotic, control and irradiated antibiotic (Figure). About the bactericidal effects were inferred from decreased *E. coli* growth [2]. It was shown that 53 GHz irradiation causes bactericidal effects. Also, it changes antibiotic sensitivity or resistance, which is more visible in the cases of Cef and Tet [2].

It was demonstrated that 53 GHz irradiation (1 hour) of antibiotics increased antibiotics antibacterial properties approximately for the same time in the cases of used antibiotics, but was a little more with Kan and Tet. Enhanced bacterial sensitivity to antibiotics by EMI is similar to the effects of higher concentrations of antibiotics.

Interestingly, the enhanced effects of non-irradiated antibiotics on irradiated bacteria are more significant compared with the effects of irradiated antibiotics on non-irradiated bacteria (Fig-

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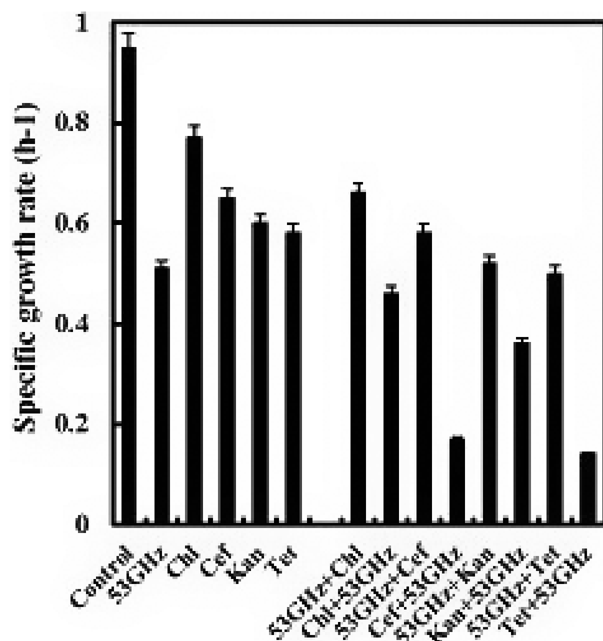

Fig. 1. *E. coli* K12(λ) specific growth rate in different conditions – control (without irradiation and antibiotics); after 53 GHz irradiation; non-irradiated bacteria with antibiotics (Chl, Cef , Kan, Tet); non-irradiated bacteria with irradiated antibiotics (in figure mentioned as 53 GHz + antibiotic); irradiated bacteria with non-irradiated antibiotics (in figure mentioned as antibiotic +53 GHz). Antibiotics Chl, Cef, Kan and Tet non-irradiated and after radiation with 53 GHz frequency added into the grown medium at their minimal inhibitory concentrations (4 μM, 0.4 μM, 15 μM and 4 μM, respectively). Bacteria were grown in peptone medium.

ure). Probably such effects connected with EMI interaction with antibiotics surrounding water molecules.

This study is very interesting new approach, which can be useful in medical, agricultural and food industry applications.

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Keywords: *Escherichia coli*, electromagnetic irradiation, antibiotics.

SUN-433
The interplay between the actin cytoskeleton and plasma membrane organization in B cell activation

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The actin cytoskeleton is involved in numerous cellular events. Thus not surprisingly, increasing evidence also demonstrates the role for the cytoskeleton in various stages of lymphocyte activation. We recently observed that a mere transient alteration of the actin network leads to B cell activation. Here we show that the disruption of the cytoskeleton triggers signaling that not only requires the B cell receptor (BCR), but also the co-receptor CD19. In addition to the cytoskeleton, the co-operation of these two receptors is further coordinated by the CD81-tetraspanin web, which organizes CD19 in the plasma membrane. New insights into the initiation of the BCR signaling are supported by dSTORM super-resolution microscopy, which demonstrates that endogenous IgM, IgD and CD19 exhibit distinct nano-scale organization within the plasma membrane of naïve B cells. Interestingly, these membrane receptor nanoclusters do not change in character during BCR signaling. Thus, we postulate that cytoskeleton reorganization releases pre-existing BCR nanoclusters, which can interact with CD19 held in place by the tetraspanin network. Our results not only suggest that receptor compartmentalization regulates B cell activation by surface-bound antigen, but also imply a potential role for CD19 in mediating ligand-independent BCR signaling necessary for B cell survival.

Keywords: Actin, Lymphocytes, Super-resolution microscopy.

SUN-434
The role of lipid metabolism during the *Drosophila* spermatogenesis

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The existence of similar male sterile phenotype in flies, mice and human strongly suggests that many of the genes required during spermatogenesis have been evolutionary conserved. Male sterile mutations of *Drosophila* exhibit a broad range of phenotypes and affect all stages of spermatogenesis. Spermatid individualization is an especially interesting step of the spermatogenesis because it requires an unusual amount of membrane remodelling using a well-defined actin structure. *Drosophila* spermatids increase 150-fold in length and fivefold greater total surface area following individualization. Increasing number of lipid metabolic enzymes show important role during all stages of spermatogenesis, for example in the biosynthesis of phosphatidylinositol (PI) and their phosphorylated forms. We have started the genetic characterization of mutant lines in which membrane transport related genes are affected. One of them is the *CdsA* gene which encodes for phosphatidate cytidyltransferase, CdsA enzyme, which catalyzes the synthesis of CDP-DAG from phosphatidic acid, which is an important player of PI and cardiolipin (CL) biosynthesis. Phosphoinositides play important roles in lipid signalling and membrane trafficking, while cardiolipin is an important component of the inner mitochondrial membrane. We characterized the $CdsA^{ms}$ mutant with classical and molecular genetic methods and analysed its lipid composition using mass spectrometry.

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Keywords: Drosophila, lipid, spermatogenesis.

SUN-435
TMEM165 is a protein whose mutations in Congenital Disorders of Glycosylation patients alter Golgi Ca^{2+} and pH homeostasis

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TMEM165 is a novel gene involved in Congenital Disorders of Glycosylation (Foulquier *et al.*, Am J Hum Genet 2012, 91). The

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TMEM165 deficient CDG patients present a peculiar clinical phenotype including severe dwarfism; psychomotor retardation, midface hypoplasia, muscular weakness, fat excess, joint laxity, and hepatosplenomegaly. TMEM165 encodes a putative transmembrane 324 amino acid protein whose cellular functions are unknown. TMEM165 belongs to a well-conserved, but uncharacterized, family of membrane proteins named UPF0016.

We first started by examining the effects of naturally occurring mutations on the intracellular localization of TMEM165 and their abilities to complement the TMEM165-deficient yeast, ΔGdt1. We demonstrated that wild-type TMEM165 was localized within Golgi compartment, plasma membrane and late endosomes/lysosomes, whereas mutated TMEM165 were found differentially localized according to the mutations. We also demonstrated that, in the yeast functional assay with TMEM165 ortholog Gdt1, the homozygous point mutation correlating with a mild phenotype restores the yeast functional assay, whereas the truncated mutation, associated with severe disease, failed to restore Gdt1 function. These results highly suggested that these clinically relevant point mutations did not affect the protein function but critically changed the subcellular protein localization. Moreover, the data pointed to a critical role of the YNRL motif in TMEM165 subcellular localization (Rosnoblet *et al.*, Hum Mol Genet 2013, 22). Since Gdt1p, the budding yeast member of this family, contributes to Ca2+ homeostasis via a unique Ca2+ transport pathway localized in the Golgi apparatus, we used yeasts to approach the cellular function of Gdt1p/ TMEM165. One particularity of the gdt1Δ mutant was to be sensitive to high concentrations of Ca2+, and interestingly, this sensitivity was suppressed by expression of TMEM165, the human ortholog of Gdt1p, indicating conservation of function among the members of this family. Patch-clamp analyses on human cells indicated that TMEM165 expression is linked to Ca2+ ion transport. Furthermore, defects in TMEM165 affected both Ca2+ and pH homeostasis (Demaegd, Foulquier *et al.*, PNAS 2013, 110). We thus propose that Gdt1p and TMEM165 could be members of a unique family of Golgi-localized Ca2+/H+ antiporters and that modification of the Golgi Ca2+ and pH balance could explain the glycosylation defects observed in TMEM165-deficient patients.

Keywords: Ca2+ and pH homeostasis, Glycosylation, trafficking mechansims.

SUN-436
Trafficking of the amino acid transporter $B^{(0,+)}$ ($ATB^{0,+}$) to the plasma membrane

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The amino acid transporter $B^{0,+}$ ($ATB^{0,+}$), coded by the *SLC6A14* gene, transports a broad spectrum of neutral and basic amino acids through the plasma membrane, and is up-regulated in malicious cancer cell lines. It was shown to be phosphorylated by protein kinase C (PKC), correlating with an augmented presence of the transporter at the cell surface. Since several members of the SLC6 transporter family are known to interact with SEC24 proteins of the COPII complex along their pathway from the endoplasmic reticulum (ER) to Golgi, the present study focused on a possible involvement of SEC24 proteins in $ATB^{0,+}$ trafficking. Rat $ATB^{0,+}$ was heterologously expressed with C-terminal 3xFLAG tag in HEK293 cells. Immunoprecipitation experiments suggested a possible interaction between the transporter and SEC24C (but not with the A, B and D isoforms of SEC24), confirming the importance of the ER export 'RI' motif in the binding of cargo proteins with SEC24C. This interaction, however, was not altered upon PKC activation. When SEC24A-D were knocked-down using siRNAs, the amount of $ATB^{0,+}$ detected at the plasma membrane by biotinylation studies remained unchanged. Interestingly, another protein found to interact with $ATB^{0,+}$ was caveolin-1, which apart from its structural role in rafts, is also postulated to play a role in cholesterol and protein trafficking. We here propose an alternative mechanism directing the 12TM transporter $ATB^{0,+}$ from the ER compartments to the plasma membrane.

This work was financed by the National Science Centre grant 2012/B/NZ3/000225.
Keywords: amino acid transporter, Sec24 proteins, trafficking.

SUN-437
Translocation of cell-penetrating peptides across the plasma membrane is controlled by cholesterol and microenvironment created by membranous proteins

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Cell-penetrating peptides (CPPs) are considered as one of the most promising tools to mediate the import of membrane-impermeable, biologically active molecules into the cells. Despite the extensive research in the field of CPPs' cell entry, the exact mechanisms underlying their cellular uptake and possible role of the cell surface molecules in the internalization process has remained controversial. The full understanding of their cellular uptake methods is of the utmost importance for the design and development of peptide vectors.

Our research focused on the interactions between CPPs and the membrane constituents of giant plasma membrane vesicles (GPMVs). GPMVs comprise a model system which maintains the compositional complexity and protein content of biological membranes and are considered to be a relevant model to study the translocation of CPPs across the plasma membrane in conditions lacking endocytosis [1]. Cholesterol has been shown to be one of the main determinants in formation of more densely packed and more ordered membrane areas. By lowering and enhancing the cholesterol content in GPMVs' membrane, we showed that higher cholesterol content and tighter packing of membrane components reduced predominantly the accumulation of amphipathic CPPs in vesicles, confirming that the internalization of CPPs takes place preferentially via the more dynamic membrane regions. Furthermore, the significance of the membrane dynamics in CPPs internalization was examined by formation of ceramide in the membrane of GPMVs. Ceramide modulates the structure and properties of cellular membranes, which can result in promoting or inhibiting the translocation of CPPs across the plasma membrane. Accumulation of amphipathic peptides into GPMVs was inhibited by formation of ceramide in GPMVs membrane. Ceramide alters the lateral organization of membrane, resulting in the formation of highly ordered ceramide platforms, and thereby also decreasing the microfluidity of membrane, which in turn reduced the uptake of amphipathic peptides. Additionally, we assessed the role of membrane proteins as possible mediators of CPP uptake. The partial digestion of membrane proteins from GPMVs' surface signifi-

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cantly reduced the uptake of nonaarginine and Tat peptide, demonstrating that proteinaceous receptor is necessary for the uptake of arginine-rich CPPs.

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Keywords: None.

SUN-438

What is the role of the influenza fusion peptide in membrane fusion? A computational study

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Influenza virus (IV) is responsible for worldwide outbreaks of flu, causing hundreds of thousands of deaths every year, which rise to millions in pandemic years. The finding of effective drugs against IV is, therefore, a matter of the utmost importance and urgency. To infect host cells IV fuses its membrane with the host membrane. The fusion process is promoted by the glycoprotein hemagglutinin (HA), which is located on the surface of the virus. HA has a highly conserved N-terminal domain, comprising ~20 residues, which inserts and destabilizes the host membrane during fusion - fusion peptide (FP). To elucidate the molecular determinants that lead to the destabilization of biological membranes by the FP, we used a molecular dynamics approach. These simulations enabled us to analyze the structural properties of the peptide inside the membrane and characterize the interactions between the peptide and the lipids. This knowledge contributes to a better understanding of the role of the FP in the fusion process, which can be useful for the development of anti-viral drugs against influenza.

Keywords: Hemagglutinin fusion peptide, INFLUENZA VIRUS, Molecular dynamics simulation.

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CSI-04 – Optogenetics & Behavior

SUN-440
Engineering new genetically encoded biosensors for quantitative biochemical imaging in the living cell

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Biosensors incorporating fluorescent proteins (FP) are widely used to image and quantify various interactions and biochemical activities within living cells, tissues and whole organisms. These applications require FPs with not only maximum brightness and photostability but also a full control of their photophysical responses to changes in the environment. When this is the case, their calibrated responses may be used to monitor specific parameters, such as the local pH of secretory compartments (Poëa-Guyon et al., 2013a,b), or the generation of ROS species like the OH° radical (Alvarez et al., 2010). In FRET experiments on the contrary, two spectrally matched donor-acceptor FPs are expected to respond exclusively to changes in their relative spatial arrangement. Despite their poor photophysical performances and strong pH sensitivities, cyan and yellow FPs remain in most cases the prefered option to build FRET biosensors, which has led to many controversial data in the litterature.

Major advances have been achieved recently by our lab and others, in the engineering of cyan fluorescent proteins with emission quantum yields close to unity, fluorescence lifetimes greater than 4 ns, an extended photostability and a remarquable environmental insensitivity, notably towards pH over the whole range of physiological pH values (Erard et al., 2013). We have shown that these improvements stem from only a very few critical mutations having well defined structural consequences on the chromophore pocket (Fredj et al., 2012). These newly engineered FPs now reveal unexpected aspects of the functioning of conventional FRET biosensors, and trace promising routes towards the *de novo* rational design of optogenetic reporters.

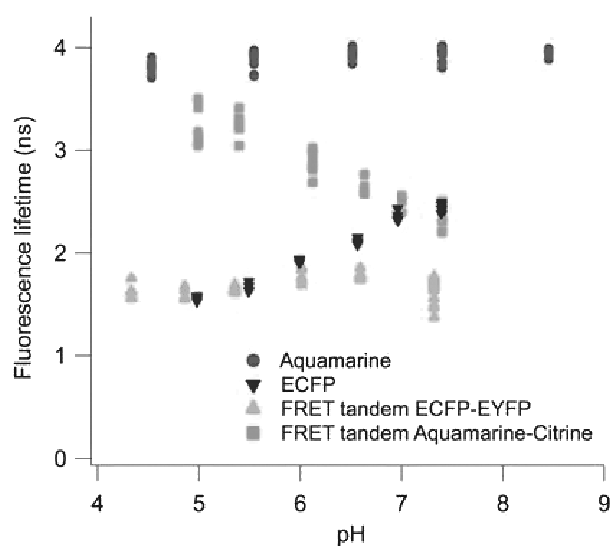

Fig. 1. Fluorescence lifetimes of cyan fluorescent proteins expressed in the cytosol of BHK cells.

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Keywords: Biosensors, fluorescence, FRET.

SUN-441
Excitation transfer along linear chain of small plasmonic particles and exciting collective modes in circular particles chain in biological tissue

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Metallic nanoparticles with plasmonic resonance may unite in biological tissue as finite clusters obeying specific optical eigenmodes or line up to transfer excitation between clusters. It is presented simple analytic and physically transparent results for the problem solution of collective modes exciting inside circular chain and the problem excitation transfer along linear chain of coupled small plasmonic spherical particles. The results are obtained with the aid of a system of equations for self-consistent currents excited inside particles by incident electromagnetic wave field. For circular particles chain the coupling matrix between their currents becomes under definite conditions of such favorite properties that enables one immediately to write out the Bloch-like eigenmodes with corresponding eigenfrequencies and their halfwidthes. Besides we get a simple analytic expressions for coupling factor between inner part of a square structure arranged in a circular shape and its bound part. Considering linear particles chain we use a closest neighbour interaction approach which makes the linear chain like the electric filter. In this approximation an analytic expression is obtained for extinction rate of exciting currents' transfer depending on chain particles number and for resonance filtering frequencies.

Keywords: excitation transfer, plasmonic resonance.

SUN-442
The gap junctions of the thermosensory circuit in the nematode *C. elegans* are responsible for isothermal tracking behavior

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The nematode *Caenorhabditis elegans* moves towards the cultivation temperature when placed in a thermal gradient, tracking isotherms once this temperature is reached (isothermal tracking behavior). AFD is the principal thermosensory neuron described until now and is required to perform all modes of thermotactic

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behavior. But how one neuron can drive the distinct sensorimotor transformations that underlie movement down or up gradients towards the cultivation temperature vs. isothermal tracking near the cultivation temperature is not well understood. To study this question, we explored the downstream synaptic pathways from AFD. AFD has direct synaptic output to only two interneurons, chemical synapses to AIY and electrical synapses to AIB. While AIY role in thermotaxis has been widely studied and its inactivation is known to cause a constitutive cryophilic movement (movement towards colder temperatures), little is know of the AIB role. We have identified a deletion mutant in an innexin gene, *inx-1*, that displays a constitutively isothermal tracking behavior, regardless of cultivation temperature. Innexins are the proteins responsible for gap junctions in invertebrates. *Inx-1* is primarily expressed in the interneuron AIB, the electrical partner of AFD. Surprisingly, cell specific expression of the *inx-1* cDNA in the sensory neuron AFD, but not in the interneuron AIB, suppresses the constitutively isothermal tracking phenotype of *inx-1* mutants. Moreover, inactivation of AIB renders animals with an enhancement of the isothermal tracking behavior. Taken together, these results suggest that electrical and chemical synaptic pathways from the AFD neuron are differentially used to perform distinct modes of thermotaxis.

Keywords: Behavior, *C. elegans*, Neural circuits.

SUN-443

Wdr13 knockout mice shows marginally better memory but impaired ability to withstand social isolation stress

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Wdr13 is a novel WD repeat protein containing gene, knockout of which in mice results in age dependant mild obesity and pancreatic beta cell hyper-proliferation leading to increased insulin secretion (Singh et al, 2012). Our lab also found that this protein interacts in a co-repressor complex with several nuclear receptors and phosphorylation at 70 S of this protein is important for its function. Function of this gene in brain and behavior haven't been studied extensively though literature survey shows association of this gene with memory. RISH localizes wdr13 expression in hippocampal formation, cerebral cortex, cerebellum and striatum. An age window of 2–3.5 months has been chosen for brain and behavior studies in these knockout mice. Using NMR it was found that metabolic changes in brain are not prominent in this interval. Wdr13 -/0 mice in CD1 background show increased anxiety and impaired ability of novel object recognition. In C57BL6/J background also, the knockout mice showed higher anxiety than that in wild types proving that the phenotype was strain-independent. The Wdr13 knockout mice, however, fared marginally better than the wild type mice in memory tests. There was no significant change in the brain morphology of the adult and hippocampal adult neurogenesis (at 2 months) in these mice was not significantly affected under normal conditions. Deep proteomics using iTRAQ revealed increased levels of synapsin1 and down-regulated levels of neurogranin in hippocampus and prefrontal cortex may be causative for this phenotype. Interestingly, upon social isolation for 3 weeks, the anxiety and depression-like symptoms in the knockout mice are aggravated with loss in dendritic branches in hippocampus and significant decrease in synaptic genes like syn1, rab3a, nrxn2 at transcript and protein levels. This was also accompanied with increase in GATA1, a common negative Transcription Factor for the above-mentioned and known to be marker of MDD (Major Depressive Disorder). We are led to believe that Wdr13 regulates GATA1 expression and absence of it leads to de-regulation of the gene and its downstream targets.

Keywords: Mouse Behavioral Biology, NMR Spectroscopy, Proteomics.

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CSI-05 – Stem Cell Differentiation

SUN-444
3D collagen scaffolds hosting neural stem cells: developing neuroimplants for spinal cord injury (SCI) repair

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Many studies have investigated the effects of engraftment of *in vitro*-propagated Neural Stem Cells (NSC) or committed neuronal or glial progenitors on the recovery of injured Spinal Cord (SC). Most of the studies performed in rodents demonstrate that grafted cells display a short-term beneficial effect on locomotor function. One of the major setbacks of these efforts is that transplantation of NSC cell suspensions results in cell spreading and dispersal within the SC, limiting their efficacy for successful local integration and functional networking. Transplantation of a solid scaffold containing NSCs may successfully overcome these limitations. We are developing neuroimplants using 3D collagen I scaffolds hosting embryonic neural stem cells, isolated from the knock in *sox2-egfp* mice strain. A sox2-positive population more than 90% pure is isolated, using fluorescence-activated cell sorting (FACS) analysis. Scaffolds were prepared with homogenization of microfibrilar bovine collagen I in acetic acid, then freeze-drying: freeze-dry parameters control pick porosity and cross-link with various additives control stiffness and pick degradation rate. Collagen I scaffolds are much more stiffer and degradation-resistant than gels scaffolds have an open foam structure, 0.5% mass fraction and 30 to 400 μm pores and *in vivo* degradation rate at 90 days half life. Confocal and electron microscopy analysis of NSC have shown that composition and microstructure of 3D scaffolds play a significant functional role: scaffolds with a combined composition (50% collagen-50% gelatine or 92% collagen-8% chondroitin-6-sulphate) support more effectively NSC survival and proliferation as measured by TUNEL and Ki67 staining respectively, maintaining stemness (sustained sox2 expression) and propagating neurosphere formation. On the other hand, primary sensory neurons from mouse embryonic dorsal root ganglia, grew better in pure collagen scaffolds with less rigid structures. We are now testing the 3D collagen I scaffolds populated with mouse neural stem cells in mice with experimental spinal cord injury.

This work was supported by the ERC01 and Heraclitus II grants from the General Secretariat of Research and Technology (GSRT).
Keywords: Collagen scaffolds, Neural Stem Cells, Spinal Cord Injury.

SUN-445
3D microcarrier cell culture affects proteomic signature of exosomes derived from human dental pulp stem cells

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Microcarrier technology is an efficient method for scaling-up cell production in small volumes, while promoting improved cellular phenotypes in a three-dimensional environment. The main advantage of this system is the possibility to cultivate up to 100 times more cells in the same amount of medium. In addition, these systems could be potentialy used as a factories for the small scale production of different therapeutic factors. Here we present results on the use of BioLevitator™ (Hamilton), a commercially available three-dimensional culturing platform and alginate microcarrier cell culture system (Global Cell Solutions) for the propagation of stem cells derived from the dental pulp of human exfoliated deciduous teeth (SHEDs). Exosomes were purified by ultracentrifugation from SHEDs cultivated under two conditions: standart two-dimensional culture flasks, or from SHEDs grown on the laminin-coated microcarriers in bioreactor. In both cases cells were grown in serum- and xeno- free medium (MSC NutriStem XF, Biological Industries). For proteomic studies liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) analysis was carried out on an EASY-nLC (Thermo Fisher Scientific) connected to a Velos Pro-Orbitrap Elite hybrid mass spectrometer with nano electrospray ion source (Thermo Fisher Scientific). In total we identified 80 proteins in exosomes from standart SHED cultures and 60 proteins in exosomes from microcarrier cultures. The majority of the identified proteins are included in the vesiclepedia database. Importantly, only 28 proteins were common between exosomes from different preparations. These findings indicate, that 3D microcarrier cell culture have a profound impact on the proteomic composition and possibly physiological properties of exosomes.

Keywords: Bioreactor, exosomes, stem cells.

SUN-446
A multidisciplinary approach to studying crypt-villus homeostasis and regeneration in the intestinal epithelium

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The luminal surface of the gastrointestinal tract is folded to form invaginations or crypts, and evaginations, or villi. The epithelial cell layer lining these structures performs a sophisticated dual role in maximising nutrient exchange while maintaining a barrier to intestinal pathogens. The epithelial layer is continuously regenerated; stem cells located at the base of crypts proliferate and differentiate into a variety of cell types which migrate upwards along crypts and villi before being shed into the gut lumen. Maintenance of the functional integrity of the intestinal barrier requires tight coordination of cell proliferation, migration and shedding along the crypt-villus axis, with dysregulation of these processes leading to tumourigenesis and inflammatory disease. How these processes are regulated in homeostatic, disease and recovery states is incompletely understood.

Using a multidisciplinary approach combining experimental measurement and mathematical models we aim to define the mechanisms involved in homeostasis of the intestinal epithelium and its recovery after perturbation. Specifically, the mechanisms controlling cell migration along the crypt-villus axis, those regulating cell shedding, and those involved in translating events on the villus to responses in the crypt. We have developed individual based models (IBMs) to study the spatio-temporal dynamics of

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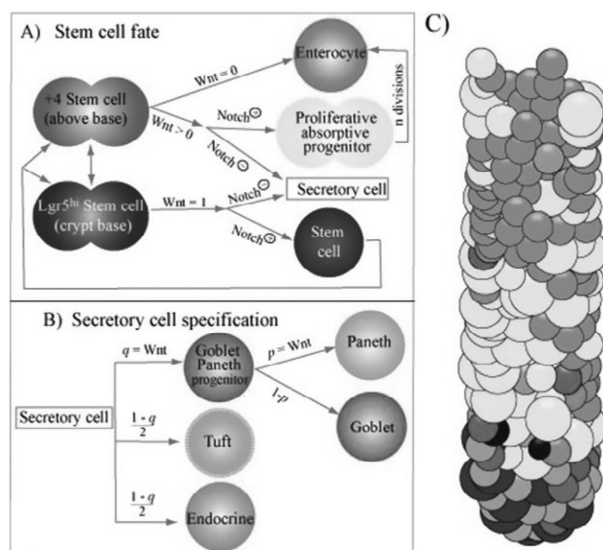

Fig. 1. Model for cellular differentiation and proliferation in the crypt of mouse small intestine.

epithelial cell generation in crypts, providing insight into the dynamics of cell differentiation and localisation, and the propagation of mutations. Using Chaste as a computational framework, and through continual development and refinement of models at different levels of special granularity, we aim to simulate crypt-villus epithelial dynamics in both healthy and diseased mucosa.

In combination with mathematical approaches, we have tracked and manipulated epithelial cell behaviour using a variety of in vivo murine models of healthy and altered epithelia, including inflammatory and pro/anti-proliferative settings. For example, using an LPS–induced model of epithelial damage, we observe intense apoptosis at villus tips and a significant reduction in villus height in the small intestine, with recovery of typical morphology within a few hours. Using in vivo and in vitro microscopic and histological techniques, we have traced epithelial cell proliferation, migration and shedding to better define the processes involved in villus regeneration.

Our ongoing work continues to combine experimental and mathematical approaches to define mechanisms which are essential to maintain lifelong health of the gastrointestinal tract and ultimately aims to identify strategies for intervention in intestinal disease.

Keywords: Intestine, modeling, Stem cells.

SUN-447
A neurofilament-derived peptide targets neural stem cells and alters their self-renewal capacity

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The neural stem cells (NSC) are characterized by their capacity to self-renew, to form neurospheres in culture, to proliferate, and to generate neurons, astrocytes and oligodendrocytes (multipotency). The characteristics of these cells provide new therapeutic approaches for the treatment of neurodegenerative disorders and

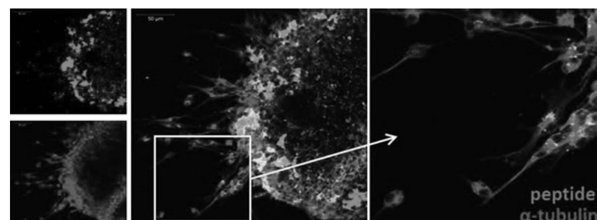

Fig. 1. Confocal microscopy of the peptide (in green) uptake in neural stem cells of new-born rats (stained with anti-tubulin, in red).

for the treatment of malignant glioma. The development of neural stem cell-based therapies may be beneficial to target these cells, increase their mobilization and stimulate neurogenesis for regenerative medicine and for the treatment of brain tumours.

We previously showed that a peptide corresponding to the tubulin-binding sequence located on neurofilament, alone or linked to nanoparticles, is able to target glioblastoma cells *in vitro* and *in vivo*. The selective and massive uptake of this peptide by glioblastoma cells occurs through endocytic pathways and is related to their high proliferative state, whereas a low level of internalization occurs for slow proliferative healthy cells (astrocytes, oligodendrocytes or neurons). Moreover, while the peptide is able to disrupt the microtubule network of glioblastoma cells and inhibit their proliferation, it has no major effect on the microtubule network from healthy cells. Finally, injected in rats bearing glioblastoma, the peptide reduces tumour development (Bocquet et al., 2009; Bergès et al., 2012; Balzeau et al., 2013; L-Chambaud et Eyer, 2013).

In this study we show that this peptide is able to translocate passively in neural stem cells *in vitro*. Moreover, the *in vitro* formation of neurospheres was not altered by the peptide whereas the self-renewal capacity of these cells was slightly reduced and associated with an increase of adherent cells and a decreased of NSC proliferation. Finally, when injected in the cerebrospinal fluid of rats the peptide targets adult neural stem cells *in vivo* without major detectable cytotoxicity. These results indicate that this peptide represents a new molecular tool to target neural stem cells in order to develop new strategies for regenerative medicine and treatment of brain tumours.

Keywords: neural stem cells, self-renewal, targeting peptide.

SUN-448
A novel method to identify and isolate pluripotent human stem cells and mouse epiblast stem cells using lipid body-associated retinyl ester fluorescence

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We describe a characteristic blue fluorescence exhibited by Human embryonic stem cells which helps in identification and isolation of human pluripotent stem cells. The blue fluorescence is easy to observe with basic epifluorescence microscopy. The intensity of blue fluorescence correlates with the expression of pluripotency markers such as OCT4, SOX2 and NANOG. We show that with the help of FACS, undifferentiated pluripotent stem cells can be easily isolated based on their blue fluorescent intensities. This blue fluorescence phenomenon appears early during reprogramming and arises from cytoplasmic lipid bodies by the sequestration of reinyl esters. These blue fluorescent lipid

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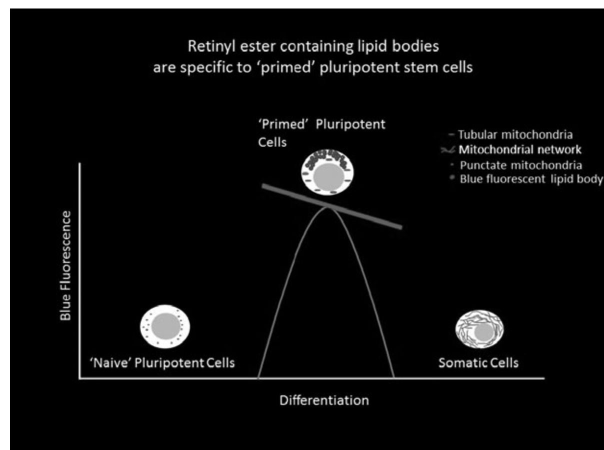

Fig. 1.

bodies are specific to 'primed' or 'epiblbast-like' stem cells and absent in 'naive' stem cells such as mouse ES cells. Retinol, a common component in human ES media can be sequestered in lipid bodies and can also induce the formation of lipid bodies.

Keywords: None.

SUN-449
A novel peptide for triggering differentiation of hepatocyte-like cells from human induced pluripotent stem cells

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Hepatocytes produced by differentiation from human induced pluripotent stem cells (iPSCs) could potentially contribute to the development of toxicological evaluation for pharmaceutical screening and regenerative therapies such as hepatocellular transplantation. Introduction of Activin A and bFGF; BMP4 and FGF4; HGF, OsM and DEX etc. at each stage induces the differentiation of human iPSCs into mesendoderm and later endoderm (stage I), hepatic progenitors (stage II), and mature, hepatocyte-like cells (stage III), respectively. However, hepatocyte-like cells generated using humoral factors have varying differentiation-inducing efficiencies and drug metabolic activities, making them, at present, unsuitable for use in medical applications. To resolve this issue, we propose using a functional peptide to induce the efficient generation of hepatocyte-like cells from human iPSCs instead of the conventional method of stepwise treatment with the humoral factors. We examined whether it was possible to differentiate hepatocytes from human iPSCs through treatment with the peptide, based on the idea that turning on the fundamental module that controls the differentiation from human iPSCs to hepatocytes would consequently produce more reliable hepatocyte-like cells and remove the variation in the differentiation-inducing efficiencies and the drug metabolic activities. We focused on the signal peptide of amyloid precursor protein and synthesized a peptide sequence combination of the signal peptide (15-27aa: LLLLLLVGLTAPA) of amyloid precursor-like protein 2 (APLP2), a protein ordinarily found in the neural cells differentiated from ectoderm, with a nucleolar localization signal (NoLS) region of LIM kinase 2 (LIMK2). It was demonstrated that the combination peptide strongly induced alpha-fetoprotein (AFP), a common marker of the early stage of hepatocytes, and Albumin (ALB), a common marker of the early-to-mature stage. Consequently, these results suggest that a peptide designed based on the APLP2 signal peptide and LIMK2 NoLS has the potential to trigger the essential factors which directly induce differentiation from human iPSCs to hepatocytes.

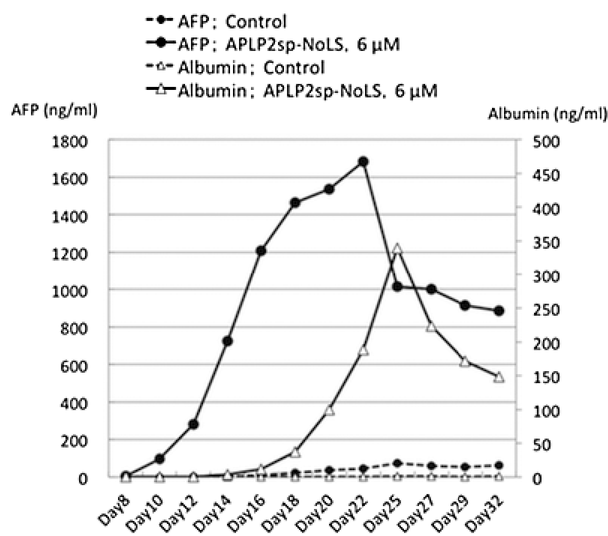

Fig. 1.

Keywords: hepatocytes, induced pluripotent stem cells (iPSCs), signal peptide.

SUN-450
Analysis of a factor that induces neuronal differentiation on small cell lung cancer

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The differentiation of the Small Cell Lung Cancer (SCLC) from neuroendocrine cells based on studying the features of SCLC through biological, pathological and clinical aspects was reported by Shimosato et al. [1]. In a research carried out by Tanaka and Terasaki [2], the morphological change of many SCLC cell lines was found when cultured with the extracellular substrate of human lung adenocarcinoma cell line, PC-9 cells. This phenomenon is thought to be depending on the cell adhesion to the substrate triggering the neurite development on these SCLC cells.

In this study, an experiment of the differentiation from SCLC cells to neuronal cells was conducted using conditioned medium of PC-9 cells. The SCLC cells were clearly changed to neuron-like shape after three days of cell culture. In order to analyze the substrate that causes the differentiation of SCLC cells, the concentrated proteins in the conditioned medium of PC-9 cells were separated by the Native-PAGE and then transferred to a PVDF membrane. Lu-134A cells, one of the SCLC cell lines, were then cultured on this membrane. After three days of culture, the membrane with cells was fixed and stained. As a result, the neurite development of Lu-134A cells was found on several protein bands.

The Two-Dimensional Cell Blot Method [3] was applied to further analyze the protein characteristics on those bands. Concentrated proteins in the conditioned medium of PC-9 cells were separated two-dimensionally by isoelectric focusing and SDS-free

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polyacrylamide gel electrophoresis. Separated proteins were transferred to a PVDF membrane, and Lu-134A cells were then cultured on the membrane. The protein distribution and the Lu-134A cell morphologies on the membrane were observed by microscope. Through the analysis of the protein spots where Lu-134A cells changed their morphologies, the main factor was identified based on the molecular weight and pI value, and further amino-acid sequences of the protein would be examined using the MALDI-TOFMS technique.

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Keywords: Neuronal differentiation, Small Cell Lung Cancer, Two Dimensional Cell Blot Method.

SUN-451

Basal lamina mimetic nanofibrous peptide networks for skeletal myogenesis

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Skeletal muscle is a collection of muscle fibers, which function together as a unit to generate contractile longitudinal forces for physical movement. Traumatic injury, tumor excision, congenital defects or myopathies disrupt muscle function and mobility and lead to muscle tissue reconstruction. One of the promising treatment methods for muscle damages is muscle stem cell transplantation; however, isolated stem cells drastically lose their ability to form myotubes and function appropriately after *in vitro* expansion. Also, there is a need for high numbers of stem cells for transplantation which requires harvesting around 3–4 kg of muscle tissue. The muscle stem cell transplantation method is still challenging due to the lack of donor tissue availability and limited autologous graft surgeries. Thus, regenerative medicine is a promising alternative solution for the treatment of various muscle related problems. It is still challenging to engineer skeletal muscle, yet numerous techniques are constantly being developed such as soft lithography, hot embossing, electrospinning, photolithography etc. Basal lamina of muscle tissue is vital for repair, maintanance and fiber force transmission. Fibrous architecture and biochemical components of this extracellular matrix support development and function of skeletal muscle. Thus, scaffolds mimicking ECM are promising candidates of efficient tissue engineered systems. In this study, we developed a novel ECM mimetic peptide nanofiber scaffold for myogenic differentiation of myoblasts (C2C12 cells) by mimicking the compositional and structural properties of native skeletal muscle basal lamina. The peptide nanofiber scaffolds were designed to mimic active site of laminin (IKVAV) and fibronectin (RGD) which take roles in muscle regeneration and development. Our results showed that self-assembled peptide nanofibers decorated with laminin derived epitopes support adhesion, growth and proliferation of the cells and significantly promoted the expression of skeletal muscle-specific marker genes (MyoD, myogenin, and MHC). Overall, functional peptide nanofibers used in this study present a biocompatible and biodegradable microenvironment, which is capable of supporting the growth and differentiation of C2C12 cells into myotubes. The laminin derived (IKVAV-incorporated) peptide nanofiber material offers a promising platform for future clinical applications due to its biocompatibility, biodegradability, and convenience in delivery by injection to damaged tissue site.

Keywords: Differentiation, Peptide Nanofibers, Skelatal myogenesis.

SUN-452

Cell fusion following cardiomyocyte and mesenchymal stem cell co-culture and functional evaluation of cellular changes

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Stem cell research focusing on cardiovascular regeneration aims to achieve stem cell mediated cardiomyogenesis leading to sistolic and diastolic cardiac functional improvement and/or development of new, functional cardiac tissue. Fusion of mesenchymal stem cells (MSCs) and host cardiomyocytes has been proposed as one of the mechanisms for cardiac regeneration. However, the mechanisms and the functional consequences of cell fusion remain undefined. The aim of this study is to elucidate the role of fusion in cardiac reprogramming characteristics of MSCs with microarray methodology.

We established an in vitro model that stimulates the fusion of h-MSCs and hCM/AC16, which allowed functional evaluation of hybrid cells. Firstly, fusion between hMSCs/hCMs was induced with PEG. After, hybrid cells were examined for their karyotype analysis, immunohistochemical and electrophysiological characteristics. Gene expression changes between hMKH, hCM and hybride cells were analysed with microarray methodology.

Hybrid cells were positive for both MKH and CM markers. As a part of electrophysiological studies, calcium channels were also examined by patch-clamp. No significant difference was found between hybrides and human cardiomyocytes regarding response to ATP and caffeine. According to microarray results, 1494 and 139 genes were found to be expressed differently between MKH/hybride cells and AC16/hybride cells respectively. To investigate the upregulation or downregulation of specific cellular pathways, genes present on the array were assigned to 23 groups, based on their biological function.

Hybrid cells maintain the cardiomyocyte characteristics. Gene analaysis data showed that MSCs support cardiomyocytes in a way to promote their differentiation to cardiomyocytes. Progress in cellular therapy for cardiovascular diseases can be achieved by further analysis of cells with cardiac differentiation and fusion capacity. Cell fusion can be an important therapeutic mechanism in cardiovascular regeneration and also can be applied to translational science.

Keywords: Cardiomyocyte, Cell fusion, Mesenchymal Stem Cells, Microarray.

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SUN-454
Cellular memory in nuclear reprogramming

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As development progresses, cells lose their pluripotent status and enter a differentiation process to give rise to the different cell types of the adult organism. Once a cell has established a differentiated cell identity, it only rarely if ever changes to another type. This stable commitment is usually maintained through many sequential cell divisions, and is likely to depend on the memory of an epigenetic state of chromatin. Xenopus eggs can be used to induce the reversal of differentiation processes. Yet, the egg is not fully efficient in reprogramming a somatic nucleus, as certain genes retain an epigenetic memory of their somatic cell of origin. To identify such genes on a genome wide level, endoderm or mesoderm donor cell nuclei were transplanted to eggs and gene expression was assessed by RNA-sequencing in the individual cloned embryos. Substantial expression of donor cell type-specific genes could be observed in the wrong cell type. For example, embryos prepared from transplanted endoderm cells overexpressed endoderm marker genes like Sox17, GATA6 and A2 m in their neuroectoderm cells. Because, in Xenopus, there is no transcription for the first 12 cell cycles, some somatic cell nuclei must remember a developmentally activated gene state and transmit this to their mitotic progeny independent of ongoing transcription and in the absence of the conditions that induced that state. We will address which epigenetic mechanisms are important for the propagation of the active state of gene transcription using the newly identified memory genes as candidates. Results from this study are expected to help to understand how epigenetic memory is important in stabilizing cell differentiation in normal development, and in resisting nuclear reprogramming.

Keywords: Differentiation, Reprogramming.

SUN-455
Characterization of ABC transporter pattern in human pluripotent stem cells and their differentiated derivatives

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Human pluripotent stem cells are special type of stem cells with a broad differentiation capacity and unlimited cell growth potential. ATP-binding Cassette (ABC) transporters provide chemical defense and stress tolerance in human tissues. The presence of these transporters in human pluripotent stem cells may significantly contribute the stem cell defense mechanisms. Our group previously showed that ABCG2 is functionally expressed in human pluripotent stem cells [1] and this expression is required to tolerate malicious effects resulting from different stress conditions [2]. Recently we have investigated the expression of all ABC genes in human embryonic stem cells (hESCs) and in their differentiated offsprings, such as cardiac, neural and mesenchymal cells. Cellular features regarding pluripotency and tissue identity, as well as ABC transporter expression were studied by flow cytometry, immuno-fluorescence microscopy and qPCR-based low-density arrays. Statistical analysis showed upregulation of pluripotency markers in hESCs, whereas the differentiated offspring showed upregulation of the proper lineage-specific markers. Cluster analysis indicated the upregulation of several ABC transporters in a cell-type specific manner. The cellular localization of the relevant ABC transporters has been established in the pluripotent and differentiated hESC-derived samples by using well-characterized specific antibodies in flow cytometry and in confocal microscopy. The protein and mRNA expression results showed proper correlation in the samples examined. These studies provide valuable information regarding ABC protein expression in human stem cells and in their differentiated progenies. The results may also help to obtain further information concerning the specialized cellular functions of selected ABC transporters.

This work has been supported by the Hungarian Scientific Research Fund [NK83533], HungarianBrain Research Program [KTIA_13_NAP-A-I/6] and by the National Development Agency [KTIA_AIK_12-1-2012-0025 and KMR_12-1-2012-0112].

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Keywords: ABC transporter expression, Differentiation, Human pluripotent stem cell.

SUN-456
Characterization of the slowly cycling label-retaining stem cell population in mouse liver

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Tissue homeostasis in adult organs is typically maintained by undifferentiated stem/progenitor cells with the ability to self-renew and provide more differentiated progeny. In general these tissue specific somatic stem cells (SSCs) are believed to be slowly cycling cells residing in the specific niches. In the liver, however, the normal tissue turnover is achieved by replication of parenchymal cells and the contribution of stem cells in liver maintenance is still under debate. It is currently believed that only when the proliferation ability of hepatocytes or biliary epithelial cells is compromised, e.g. in chronic injury, liver progenitor cells are activated.

Hepatic SSCs are believed to reside in the canals of Hering although the true localization and characteristics of liver SSCs remain unclear. In order to identify slowly cycling label-retaining cell (LRC) population we used transgenic mice expressing 1) reverse tetracycline-dependent transactivator under the control of a ubiquitous promoter, and 2) Histone2B-EGFP fusion (H2B-GFP) controlled by tetracycline response element. By feeding these mice with a tetracycline analogue doxycycline the H2B-GFP expression is induced in all cells. After removing doxycycline from drinking water, dividing cells gradually lose their label whereas quiescent cells retain their GFP label (LRCs).

We performed a time-series experiment to determine the optimal time for the admission of doxycycline, traced the label up to 20 weeks and identified the localization and the repertoire of liver stem cell markers of label-retaining GFP-positive cells under normal conditions. We discovered that slowly cycling liver cells concentrated mainly in the portal areas, particularly in bile ducts, and expressed biliary epithelial cell markers. We also found LRCs among hepatocytes. These parenchymal LRCs, however, were individually scattered throughout the liver and had lower GFP expression. In addition, we investigated the behaviour of

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LRCs in chronic injury and looked at the changes in LRC compartment after partial hepatectomy (PHx). We found that proliferation of liver LRCs, indicated by the loss of GFP signal in bile ducts, occurred after induced chronic injury (DDC diet and bile duct ligation). PHx, on the other hand, did not affect the quiescent state of biliary LRC, although we could detect less parenchymal LRCs after liver regeneration. These results suggest that LRCs take part in liver regeneration after cholestatic chronic injury and that only part of parenchymal LRCs contribute to the recovery from liver resection.
Keywords: labeling, liver stem cells.

SUN-458
Comparative study between the effects of human CD34 + and rat bone marrow mesenchymal stem cells on amelioration of CCL4 induced liver fibrosis

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Human umbilical cord blood (UCB) cells and rat bone marrow mesenchymal stem cells (BM-MSCs) have many advantages as grafts for cell transplantation. Here, we transplanted UCB cells and BM-MSCs into injured liver fibrosis and investigated the reversal of hepatic fibrosis after in vivo transplantation. A CCl4 rat model with liver fibrosis was induced. Human (UCB) CD34+ stem cell was isolated with MACS (magnetic cell sorting). Rat BM-MSCs was isolated, cultured and propagated. Cells were labeled in vitro with green fluorescent protein (GFP). Rats were divided into 4 groups; group (1): control healthy, group (2): CCl4 injected rats, group 3: CCl4/CD34+injected rats with human undifferentiated cells and group 4: CCl4/BM-MSCs injected rats with rat bone marrow undifferentiated cells through intravenous (IV) route. A significant elevation was estimated in serum albumin in CCl4/CD34+ compared to the CCl4 group ($p < 0.001$). Serum ALT had a significant decrease of its level after administration of stem cells (CD34 + or BM-MSCs) compared to the CCl4 group ($p < 0.001$). However, it was still significantly higher than control ($p < 0.001$) with no significant difference between the groups that received different sources of stem cells. Histopathological and immunohistochemical examination of liver tissue showed that stem cells have a significant antifibrotic effect. GFP labeled cells were detected in groups injected with stem cells. Concerning gene expression, the collagen and TGF-β genes were highly expressed in the CCl4 group whereas its expression was significantly decreased after administration of both different sources of stem cells. Albumin, matrix metalloproteinase (MMP2) genes and TNF-α were expressed in liver tissues in the groups that received stem cells. Higher expression was observed in the group that received undifferentiated CD34 + I.V compared to BM-MSCs. Human UCB CD34+ stem cells have antifibotic effect in rats with induced liver fibrosis.
Keywords: None.

SUN-459
Comparison of autolog versus artificial serum albumin usage in the efficiency of dendritic cell isolation and differentiation

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Dendritic cells (DC) are antigen-presenting cells (APCs) which play a critical role in the regulation of adaptive immune response and potentially represent valuable tools for the therapy of tumors. However there is requirement for a method that provides high yield monocytes which are differentiated into clinical grade dendritic cells. Most of the methods which have been tried for enrichment of the yield have not provided enough quantity and quality of monocytes. We believe that the type of serum used is important to increase the yield cell adherence to the flask in the isolation procedure. For this purpose different types of sera were compared for the efficiency of monocyte adherence. Fetal bovine serum (FBS), human serum albumin (HSA) and autolog serum were compared. Peripheral blood mononuclear cells (PBMCs) were isolated from the blood of healthy donors by standard density gradient centrifugation with Ficoll-Paque and differentiated to immature dendritic cells in the medium which contained different type of serum. PBMCs were seeded in different media which had different amount and type of serum. Serum ratio of the media were: group A 10% FBS+ complemented medium, group B 1% HSA + complemented medium, group C 1% autolog serum+ complemented medium, group D complemented medium without any serum supplement. After the maturation period (7 days), DCs maturation were assessed by determining the characteristic markers of PBMC and DC by a flow cytometric assay. We have also analyzed the cytokine production during maturation time by ELISA.

Our findings demonstrate the important of culture medium role in DC generation from peripheral blood monocytes and will make easy to work out a reproducible method that will be the most appropriate for experimental and clinical applications.

This study was supported by Marmara University Scientific Research Commission (SAG-A-110412-0083).
Keywords: Dentritic cells, serum supplement, primer cell culture.

SUN-460
Comparison of three types of cerebellar grafts in cerebellar mutant mouse Lurcher

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Aims: Therapy of central nervous system diseases accompanied with neuronal loss is limited. Neurotransplantation has a great potential for future treatments of these diseases. Lurcher (Lc) mutant mouse is a model of olivocerebellar degeneration suffering from complete loss of Purkinje cells and cerebellar ataxia. The aim of the study was to assess morphology and functional effects of three types of cerebellar grafts in Lc mice.
Methods: Adult Lc mutant or healthy wild type (WT) B6CBA mice received embryonic carcinoma stem cell-derived neuropro-

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genitors (NPG), suspension of embryonic cerebellar (ECB) cells or solid ECB grafts. All types of grafts were injected into the cerebellum (CB) of host mice. Motor skills were tested using rotarod 2 months after the surgery in graft-treated and control mice. The mice were then sacrificed and their brains were examined for presence and morphology of the grafts. Presence of donor–derived Purkinje cells and density of astrocytes in the grafts and their surrounding was assessed using immunohistochemistry.

Results: In Lc mice, the NPG graft did not survive. On the other hand, the NPG graft survived well in WT mice and cerebellar cell suspension and solid grafts survived in most WT as well as Lc mice with similar survival rate. While in WT mice the grafts were always localized inside the CB, in Lc cerebellar location of the graft was rare. The grafts were rather in the mesencephalon. If the grafted tissue was in contact with the Lc CB, no signs of integration were observed. Donor-derived Purkinje cells were found only after transplantation of ECB tissue either in the form of cell suspension or a solid graft. All types of grafts contained numerous astrocytes of both host and donor origin. In none of the graft types improvement of motor performance was seen.

Conclusions: Lc mutant CB seems to provide less permissive milieu for grafts of all types than the healthy tissue. ECB cell suspension grafts showed the most promising appearance compared with the other two types of grafts in WT mice. Nevertheless, in Lc insufficient integration into the host CB developed and improvement of signs of the ataxia was not observed.

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Keywords: Cerebellum, mouse Embryonic stem cells, neurotransplantation.

SUN-461
Control of neural differentiation of progenitor cells with new versions of GDNF

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A complete human GDNF has both pre-pro regions and mature GDNF. Several versions of modified GDNF have been created to study a role of 'pro' region during factor secretion from the cell as well as an ability of factor to stimulate neural cell differentiation. All versions of GDNF were cloned into HEK293 cells, and stable transgenic cell lines were maintained. After culturing the cells harboring modified GDNF, the condition media was added into culture of rat embrional spinal ganglion explant and growth of neural sprouts were analyzed. Deletion of 'pro' region essentially enhances modified GDNF effects as neural inductor.

GDNF therapy is effective against disorders associated with degeneration of dopaminergic neurons such as Parkinson's disease. This treatment not only increases the dopaminergic synaptic neurotransmission in the corpus striatum but also decelerates the degenerative processes in the nigrostriatal projections. We obtained a modified GDNF (mGDNF) to stimulate neural differentiation of progenitor cells. The effect of the transgenic products on growth of sprouts was studied in the spinal ganglia of 14 day rat embryos. Media conditioned by the transgenic cells were used to culture spinal ganglia attached to the bottom of the plate. Spinal ganglia cultured in a medium, supplemented with conditioned media containing mGDNF, demonstrated active growth of sprouts which are immunopositive for neuronal marker, beta-3-tubulin. Therefore modified GDNF is a potent stimulator of neural differentiation. Using mouse model of ischemic stroke induced by endothelin-1, it was shown that the transgenic cells harboring mGDNF actively migrate from the injection zone towards the thrombosed vessel. Using the neurotoxin MPTP we got the model of Parkinson's disease. It was found that in a mouse model of Parkinson's, motor effects significantly restored by transplantation of transgenic cells producing mGDNF. Immunohistochemical staining of brain sections showed that transplantation of transgenic cells harboring mGDNF greatly increases number of tyrosine hydroxylase immunopositive cells in the substantia nigra in animal models of Parkinson's disease.

GDNF can be modified to be applied for the therapy of neurodegenerative diseases and ischemia. Res. is supported by Grants RFBR, MCB

Keywords: None.

SUN-463
Differentiation of human induced pluripotent and mesenchymal stem cells on a 3D scaffold for Anterior Cruciate Ligament regeneration

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In vitro manipulation of pluripotent stem cells, either derived from the early blastocyst or generated upon reprogramming of differentiated cells towards induced pluripotency (iPS), opened the possibility for the development of new reparative and therapeutic approaches. Damaged tissues can be also be regenerated using adult stem cells, which demonstrate a more restricted differentiation and proliferation potency, however, human mesenchymal stem cells (MSC) are considered good candidates for the study and treatment of injuries related to musculoskeletal tissues. Herein, we report an easy and efficient protocol for differentiation of human iPS cells towards the MSC lineage (hiPS-MSC) and further differentiation of hiPS-MSC and MSC for the formation of a 3D structure mimicking the Anterior Cruciate Ligament (ACL). Simultaneous differentiation towards two lineages (osseous and fibrous) of mesenchymal cell types, on a Leeds-Keio 3D scaffold, leads to the formation of a functional ACL structure in vivo. 3D platforms are the milestone for regenerative technology of complex organs and common behavior of hiPS derived cells and MSC in this system confirms the reliability and further development of such approaches to the medical industry.

Keywords: hiPS, MSC, ligament.

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SUN-464
Dysfunctional mitochondrial fission impairs cell reprogramming

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We have investigated the role of mitochondrial dynamics in cell reprogramming. Mitochondrial fission is observed at day 4 after retroviral expression of reprogramming factors in Mouse Embryonic Fibroblasts (MEFs). By day 8, most of the epithelial-like cells undergoing reprogramming displayed fragmented mitochondria. In MEFs lacking expression of the fission factor Gdap1 reprograming-induced mitochondrial fragmentation and mesenchymal to epithelial transition were reduced, and the efficiency of reprogramming was strongly decreased. In the Gdap1-/- MEFs, mitophagy was not activated and mitochondrial mass increased during reprogramming. Finally, Gdap1-/- MEFs showed a 3-fold increase in the percentage of cells in the G2/M cell cycle phase when compared to wild-type controls. We propose that dysfunctional mitochondrial fission impairs reprogramming by interfering with cell cycle progression.

Keywords: cell reprogramming, mitochondrial dynamics.

SUN-465
Effects of OCT4, SOX2 and NANOG expression on imatinib resistance in CML

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Overview: Oct4 and Nanog are homebox transcription factors essential to the self-renewal of stem cells and are expressed in several cancers, the role of OCT4/NANOG signaling in tumorigenesis is still elusive.

Infinitely self-renewing cells could conceivably account for the immortal nature of tumor cells at the population level, and accumulating evidence supports the notion that ESC self-renewal and pluripotency genes, including the transcription factor triad OCT4, SOX2 and NANOG, serve as neoplastic engines driving oncogenesis. Chronic myeloid leukemia (CML) is a clonal disorder of the pluripotent hemopoietic stem cell, in which a reciprocal translocation t(9;22)(q34;q11) forms a Philadelphia(Ph) chromosome and creates a novel fusion gene, bcr–abl. Imatinib mesylate is one of the most widely used drugs for CML. Imatinib resistance, occurring in nearly 33% of patients, has been associated with a variety of mechanisms, subclassified as bcr-abl gene expression or mutations in the bcr-abl gene. In the present study we examined mRNA expression levels of genes which have important roles in self-renewal and pluripotency in K562r and K562s cells.

Methods: K562s(sensitive)and K562r(resistance) were grown in RPMI-1640. K562r cells were maintained in RPMI-1640 medium supplemented with 5 μM imatinib. RNA isolation, cDNA synthesis, RT- PCR was performed respectively.

Results: The results showed that NANOG(3.7),OCT4(2.7) and SOX2(4.7) mRNA expression levels in K562r cells was higher than levels in K562s cells.

According to our findings, enhancement of imatinib doses in K562r teratment increase expression level of NANOG,OCT4 and SOX2, so it can be suggested that up-regulation of these genes and CSC are responsible of imatinib resistance

Keywords: Drug resistance, Chronic myeloid leukemia, Imatinib.

SUN-466
Effects of embryonic neural stem cell therapy on DNA damage products in urine and tissue after spinal cord injury in rats

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We tested the hypothesis that embryonic neural stem cell (ENSC) therapy reduces neuronal DNA damage in the lesional spinal cord region and evaluated the significance of some DNA breakdown products in urine and tissue samples in order to reflect efficiency of the ENSC therapy. DNA damage were measured by using single cell gel electrophoresis (SCGE/Comet Assay) in spinal cord (SC) tissue of rats 7 (acute group) and 28 (chronic group) days after surgical resections of a 10 mm segment of the cord below T8-T9 and sham operations. As biomarkers of DNA damage, urinary 5-(hydroxymethyl) uracil (5HMU) and 2'-deoxyuridine (2dU) were analyzed by high-performance liquid chromatography (HPLC) with electrochemical detection (ED). Comet scores of the lesioned tissues were significantly higher in the acute and chronic spinal cord injury (SCI) compared to the sham operated groups ($p = 0.009$ and $p = 0.009$, respectively). After the ENSC transplantation, tail %, tail length and tail moment (T%, TL and TM) decreased in acute ($p = 0.009$, $p = 0.009$ and $p = 0.009$, respectively) and chronically ($p = 0.009$, $p = 0.009$, $p = 0.009$ respectively) injured rats. 5HMU and 2dU were significantly higher in the acute and the chronic groups than sham operated groups ($p = 0.009$ and $p = 0.009$, respectively). 5HMU concentration in the acute SCI group was found a correlate more strongly with tail moment ($r = 0.9039$, $p = 0.02$). In the chronic injury group, 5HMU and 2dU concentrations were found a correlate strongly with tail length ($r = 0.9990$, $p = 0.001$ and $r = 0.9272$, $p = 0.016$, respectively). While 5HMU seems to be a better biomarker for acute injury, both HMU and 2dU excretions increase in chronic damage.

Keywords: Spinal Cord Injuries; Comet Assay; Chromatography, High Pressure Liquid; 2'-deoxyuridylic acid; 5-hydroxymethyluracil.

SUN-467
Effects of low intensity/very low frequency electromagnetic fields on stem cells proliferation and differentiation

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Background: EMF, at different frequencies and intensities, is used in therapeutical purposes, like teh treatment of chronic inflamamtory diseases, oncology, etc. This study aimed to evaluate the effects of low intensity/low frequency electromagnetic fileds on the proliferation of stem cells and on the differeniation into adipocytic and osteocytic phenotypes, investigating the potential uses of such EMFs in regenerative medicine.

Material and Methods: ADMSC (Adipose Derived Mesenchymal Stem Cells) – ATCC PCS 500-011), were cultivated in Mesenchymal Stem Cells Basal Medium with MSC Growth Kit, according to the suppliers instructions. Cells on pasage 3 were used for monitoring the effect of variants of EMF, by cultivating

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in 96 well tissue culture plates overlapped on a devices generating EMF in the external 1–3 and 10–12 rows, while the internal rows (4–9) were not exposed. Using a low seeding rate (2000/well), the cultures were exposed to EMF for 2 hrs daily, for 4 days. On day 5, cell growth ratio was determined by MTT assay.

Cells on pasage 4 were cultivated for differentiation into adipocytes and osteocytes, according to the instructions and using ATCC kits.

The process of growth and of differentiation was investigated by Luminex-xMAP, with a set of Milliplex MAP-mates for total signaling molecules.

Results and Discussion: Exposure to EMF resulted in a statistical significant improvement in mesenchymal stem cell proliferation at one of the four experimental frequencies tested; average 23% stimulation, $p < 0.05$. Other frequencies resulted in lower rates of stimulation or no stimulation.

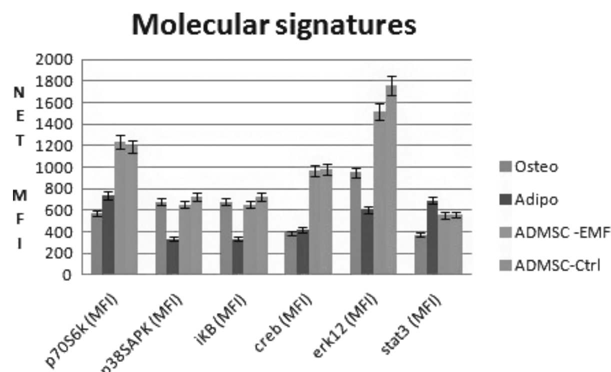

Fig. 1.

The differentiation process, monitored similarly by the MTS assay, showed some stimulatory effects for the same EMF settings, however, lower in intensity and statistical significance. Aparently, the effect is more intense in those cells that undergo more active proliferation than on low growing, differentiating cells.

The xMAP assays revealed a cvasi-constant profile of signaling molecules for the MSCs cultivated in standard conditions and exposed to EMF. The same preservation of profile appeared also for the process of differentiation, however, with particular profiles for each differentiation phenotype (Figure 1)

Certain EMF settings in the very low frequency range can stimulate the proliferation of stem cells, while not affecting the overall signature of signal transduction molecules.

Acknowledgment: The study was supported by Grants POS-CCE Nr. 502/2013 and P-N 09.33-03.10/2009.
Keywords: ADMSC, signal transduction, EMF.

SUN-468
Effects of MK2 gene silencing on vitality, proliferation and differentiation of mesenchymal stem cells under hypoxic culture conditions

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Bone marrow derived mesenchymal stems (BM-MSCs) are expanded in long-term culture with maintained proliferation capacity, differentiation potential, immune-modulating property and have limited tendency to form tumors. BM-MSC are main candidate cellular source in stem cell therapy applications. Cultivation of cells is performed under atmospheric oxygen tension, in recent years, many researchers reported hypoxic conditions have positive effects on stem cell proliferation and differentiation. For this reason, in our study, MAPKAPK2 (MK2; mitogen-activated protein kinase-activated protein kinase 2) gene, which belongs to MAP kinase family member related with proliferation, cellcycle, apoptosis, differentiation and inflamatory response, was silenced permanently with shRNA lentiviral vector. Its effect on proliferation and differentiation were analysed both in 20% (normoxia) and 3% (hypoxia) oxygen levels. The physiological roles of MK2 activation are most clearly revealed by the targeted disruption of theMK2 gene. We investigate the effects of long-term hypoxia (72 h) on cell proliferation and osteogenic differentiation in both oxygen concentrations, and the role of MK2 gene under hypoxic condition was focused. MK2 gene was silenced in rat BM-MSCs by lentiviral shRNA vector permanently. Cells were cultured in two different culture conditions (20% normoxia and 3% hypoxia); western blotting, real-time PCR analysis, proliferation and viability tests, WST-1, Annexin V-7AAD and cell cycle analysis with flow cytometry and immuno-histochemical analyses were performed before and after differentiation. MK2 protein is activated by phosphorylation under hypoxic condition, and shRNA was used for silencing. But this process does not change MK2, it left in inactive form. By silencing MK2 gene in MSCs, the cell proliferation capacity and anti-apoptotic effects were observed to improve cell proliferation capacity. Furthermore, the expression of osteogenic markers decreased in silenced cells before and after osteogenic differentiation compared to normal cells, but the decline is more significant in 3% hypoxic oxygen concentration. By revealing the crucial role of MK2 gene in the proliferation and differentiation of silenced rBM-MSCs cultured under different oxygen concentrations, the importance of this gene and related pathways were demonstrated with their possible mechanisms. This study provides an evidence for the importance of hypoxic conditions in the cell cultures. In future aspects, possible new mechanisms, like MK2 pathway, might be used in pathological processes for cell therapies in ischemic conditions.

Keywords: cell differentiation, Gene silencing, MK2.

SUN-469
Enhancement of angiogenic and cardiomyogenic potential of murine mesenchymal stem cells by MCPIP1 expression

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Monocyte chemoattractant protein-induced protein 1 (MCPIP1) has been identified as a silencer of inflammatory response. However, MCPIP1 was also described as new modulator of angiogenesis and adipogenesis. Mesenchymal Stem Cells (MSCs) are multipotent subset of adherent bone marrow (BM) cells and are capable of osteogenic, chondrogenic and myogenic differentiation. In this study, we examined angiogenic and cardiomyogenic potential of MCPIP1- transduced MSCs *in vitro*.

MCPIP1 overexpression was obtained by double retroviral infection of MSCs (passage 3 to 4). We investigated MCPIP1 influence on metabolic activity, cytotoxity, apoptosis and proliferation of MSCs. We also executed the expression of Oct-3/4,

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Sox-2, Klf-4 as well as angiogenic potential via capillary tube formation assay. Then, MCPIP1-overexpressing cells were differentiated into endothelium or cardiomyocytes. Expression of differentiation-related genes was analyzed by real-time PCR. Moreover, after 5 and 10 days of angiogenic differentiation, capillary tube formation assay was performed. We measured the level of selected angiogenesis-related proteins secreted by MSCs as well as proteomic analysis of MCPIP1- overexpressing MSCs was performed.

We found that MCPIP1-overexpressing MSCs exhibited elevated angiogenic potential, which was accompanied with change in expression of genes guiding their angiogenic differentiation (Gata-2, Tie-2, vWF, VE-cadherin). These cells in cardiomyocytes differentiation displayed increased level of mRNA for Gata-4, Nkx2.5, Myl-2, Myh-6. Moreover, MCPIP1 did not influence morphology, metabolic condition and apoptosis. Antigenic profile of MCPIP1-overexpressing cells was comparable to control cells. However, MCPIP1 overexpression slightly decreases proliferation of MSCs.

We showed for the first time that MCPIP1 positively modulates conversion of MSCs into endothelium cells and cardiomyocytes. Therefore, MSCs with overexpression of MCPIP1 may be potentially applied in regenerative medicine.

Keywords: MCPIP1, mesenchymal stem cells (MSCs), regeneration.

SUN-470

Enhancing adipose-derived stem cell proliferation and differentiation into β-cells by fibroblast growth factors

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Insulin deficiency resulting from pancreatic β-cell destruction leads to type 1 diabetes which accounts for 5–10% of all cases of diabetes. Due to the developmental plasticity of mesenchymal stem cells (MSC), MSC-based therapeutic intervention has become a promising strategy to replace injured tissues such as islets. However, a major obstacle of applying MSC is required large amounts of MSC for transplantation and significant cell loss after transplantation. To tackle this issue, we seek for adipose-derived stem cells (ADSCs) owing to their ease of isolation, abundant sources and multipotency and would like to further enhance their proliferative ability and differentiation characteristics.

We isolated ADSCs from porcine back fat region of subcutaneous adipose tissues (pADSCs) and showed that these ADSCs expressed the MSC surface markers including CD29, CD44, CD90 and MHC I. These pADSCs also sustained the pluripotency to differentiate into different kinds of cells originated from three distinct germ layers such as adipocytes, chondrocytes and osteocytes. We cultured pADSCs in basal medium supplemented with or without growth factors such as fibroblast growth factor 2 (FGF2) or FGF4 to determine the proliferation by MTT analysis and cell counting. Our results demonstrated that both FGF2 and FGF4 increased the proliferation of pADSCs. In our experiments, FGF2 also induced ERK phosphorylation. Therefore we speculate that FGF2 may work on ERK pathway to promote pADSC proliferation. Furthermore, FGF-supplemented pADSCs could be induced to differentiate into insulin producing cells. Determined by immunofluorescent staining and examined by confocal microscopy, quantitative PCR and ELISA, β-cells markers such as islet-1 and insulin were highly expressed in FGF-supplemented pADSCs. These results indicated that FGF2 increases pADSC proliferation without compromising the β-cell differentiation characteristics of pADSCs.

To conclude, in this study, we determined the optimal culture condition for pADSCs with either FGF2 or FGF4. These preconditioned pADSCs provide a potential strategy to increase pADSC proliferation for transplantation in treating type 1 diabetes or other autoimmune diseases.

Keywords: fibroblast growth factor, proliferation, β-cells.

SUN-471

Epigenetic and genetic profile of human mesenchymal stem cells from adipose and synovial tissue

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Adult stem cells have more restricted differentiation potential than embryonic stem cells (ESCs), but upon appropriate stimulation can differentiate into cells from different germ layers. Genetic and epigenetic control of ESCs differentiation is well characterized, while adult stem cells remain poorly explored. For better understanding of molecular pathways involved in mesenchymal stem cell (MSC) differentiation, RNA and miRNA expression was profiled in MSCs from adipose tissue and synovial membrane. DNA methylation and hydroxymethylation marks in stemness genes were analysed in MSCs in comparison to cancer cell lines (T24, MX-1, CX-1).

Genome-wide RNA and miRNA expression profiling was performed on Agilent microarrays. Expression of more than 800 genes and 23 miRNAs were significantly changed during MSCs differentiation into osteogenic lineages in both cell types. Among the known targets of identified miRNAs, 143 genes were also deregulated in our data-set. The main biological processes targeted during cell differentiation were cell signalling, innate immunity and regulation of cell proliferation. Of interest, expression levels of the main pluripotency factors *OCT4*, *SOX2,* and *NANOG* remained unchanged during MSCs differentiation. This result was validated by real-time PCR. Bisulphite-based DNA methylation analysis of the pluripotency-associated loci showed partial methylation of *OCT4* and *NANOG* promoters in MSCs, while the promoter of *SOX2* was rarely methylated. More specific analysis of DNA modifications in MSCs, revealed an accumulation of 5-hydroxymethylcytozine (5hmC) in parallel with 5-methylcytosine (5mC) in the pluripotency-associated loci, with the most abundant levels detected in regulatory regions of *OCT4* and *NANOG*. However, no significant changes in 5hmC levels were observed during MSCs differentiation. DNA modification profile of the same loci was quite different in cancer cell lines. Promoters of the pluripotency genes contained more 5mC marks and reduced levels of 5hmC. Expression of the TET1 enzyme was also decreased in cancer cell lines in comparison to MSCs. In summary, the data of our study shows that the plasticity of MSCs is regulated by an interplay between genetic and epigenetic factors also involved in regulation of pluripotency of ESCs.

Keywords: 5-hydroxymethylcytosine, DNA methylation, mesenchymal stem cells (MSCs).

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SUN-472
Exosomes from dental pulp stem cells suppress 6-hydroxy-dopamine-induced apoptosis in human dopaminergic neurons

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Recent findings demonstrated the importance of paracrine mechanisms in the therapeutic action of human mesenchymal stromal cells (MSCs). It must be noted, however, that MSCs isolated from different tissues are not equivalent in their therapeutic potential and display tissue-specific differentiation capacity. Several studies demonstrated that in contrast to the MSCs derived from other tissues, MSC-like cells isolated from dental pulp (also known, as stem cells derived from the dental pulp of human exfoliated deciduous teeth, SHEDs) have unique neurogenic properties which could be potentially exploited for therapeutic use.

In the present study we investigated neuroprotective effects of exosomes derived from SHEDs on human dopaminergic neurons during oxidative stress induced by 6-hydroxy-dopamine (6-OHDA). For this purpose, ReNcell VM human neural stem cells (Millipore) were differentiated into dopaminergic neurons and treated with 100 μM 6-OHDA alone, or in combination with exosomes purified by ultracentrifugation from SHEDs cultivated under two conditions: standart two-dimensional culture flasks, or from SHEDs grown on the laminin-coated microcarriers in bioreactor (BioLevitator, Hamilton). Real-time monitoring of apoptosis was performed using Leica SP8 (Leica Microsystems) confocal microscope and CellEvent™ Caspase-3/7 green detection reagent (Life technologies). We found that exosomes derived from SHEDs grown on the laminin-coated three-dimensional alginate microcarriers significantly postponed 6-OHDA-induced apoptosis in dopaminergic neurons. Strikingly, no such effects were observed for the exosomes derived from SHEDs grown under standart culture conditions.

Our results demonstrate that exosomes derived from SHEDs are potential new therapeutic tools against Parkinson's Disease.

Keywords: dental pulp, exosomes, Stem cells, neuroprotective strategies.

SUN-473
Expression of cancer-testis antigens of Mage family in mouse pluripotent stem and teratocarcinoma cells

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Embryonic stem (ESCs) and embryonic germ cells (EGCs) are pluripotent while embryonic teratocarcinoma cells (ECCs) are their malignant counterparts. Despite similar growth rate of ESCs, EGCs and ECCs, their differentiation and developmental potentials are significantly different. Moreover, different ECC lines show variable differentiation and tumorigenic potentials. Previously it was demonstrated that cancer-testis antigens (CTA) may be involved in lineage determination in course of differentiation of mouse and human embryonic stem cells. CTAs have specific expression pattern in cancer and normal adult somatic and germ cells, as well as in different embryonic cells. Therefore, changes in CTA profiles may be considered as marker for transformed and abnormally differentiated cells.

In order to understand a role of CTAs in the differentiation of normal and cancer cells the gene expression profiles of Mage-a and Mage-b families were studied in undifferentiated and differentiating upon retinoic acid stimulation mouse ESCs, EGCs and ECCs. Quantitative real time PCR analysis of Mage-a2, Mage-a4, Mage-a6, Mage-a8 and Mage-b1, Mage-b3, Mage-b4, Mage-b5 expression identified similar expression patterns in ESCs, EGCs and ECCs. However, the expression levels of Mage-a2 and Mage-a6 were significantly higher in the undifferentiated nullipotent ECC F9 than in pluripotent stem cells and ECC P19, which are capable to differentiate. On the other hand, expression patterns of all genes studied were almost identical in the ESCs, EGCs and both ECC lines differentiating after retinoic acid exposure. Positive correlations of gene expression levels of Mage-a4 and C-myc were also found for all cells studied. In general, undifferentiated and differentiating ESCs, EGCs and ECCs express higher level of Mage-a family genes than the Mage-b. Based on these data we suggest that Mage-a2 and Mage-a6 expressed at higher levels in nullipotent teratocarcinoma cells may be considered as markers for malignant pluripotent cells with abnormal differentiation potentaial.

Keywords: pluripotent stem cells, cancer, oncogenes, differentiation.

SUN-474
Generation and characterization of neuralized mesenchymal stem cells from multiple sclerosis patients

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Background: Lot of preclinical studies showed the theraputic potential of MSC in MS. Moreover, few clinical stuies showed positive results with the use of MSC in MS. The mechanisim of action of MSC in the disease model are mainly via immunomodulation and neuroprotection. The possibility of tissue repair with the use of MSC is very debatatble and seems to be not a major mechanisim in the action of disease amelioration. Therefore, the employment of neuroprecursors derived from MSC (MSC-NPs) instead of naïve MSC can be useful also in terms of neuro-regeneration and remyelination (while keeping the immunomodulatory properties) similar to potential of neural stem cells without ethical hurdles that neural stem cells have in clinical applications.
Methods: MSC were isolated from the BM of five MS patients. The MSC were cultured in a medium contatining EGF, bFGF and B27 supplement to generate MSC-NPs. The MSC-NPs were characterized using FACS. The neural differentaion of MSC-NPs by culturing with neural differentiation commercial media (NeuroCult™ NS-A). The immunomdualtory effects of MSC-NPs were tested with lymphocytes suppression assay in vitro.
Results: We were able to generate MSC-NPs successfully from 5 donors. The spheres stained positively for the neurosphere markers Nestin and PS-Ncam (>90%) while loosing the mesenchymal markers CD90 and CD105 (<5%). As well the MSC-NPs lost their ability to differentiate in mesodermal tissues (adipocytes and osteocytes). MSC-NPs differentiated successfully to neurons (MAP2 marker), astrocytes (GFAP marker) and oligodendrocytes (CNPase marker). Morevover, MSC-NPs induced dose-dependent suppression of lymphocytes.
Conclusions: The generation of MSC-NPs (which hold the neural differentiation potential of neural stem cell) from naïve MSC isolated while keeping there immunomodulatory properties open for us new possibilities for neuroregeneration and remyelination in future autologous cell-therapy treatments for multiple sclerosis.

Keywords: None.

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SUN-475
hASCs exhibit chondrogenic potential of differentiation inside collagen-sericin hydrogels improved with hyaluronic acid and chondroitin sulfate designed for cartilage reconstruction

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Aim: The aim of this study was to investigate human adipose derived stem cells (hASCs) chondrogenic potential in contact with novel 3D collagen hydrogels improved with sericin, hyaluronic acid and chondroitin sulfate (CollSSHACS), which were designed for cartilage tissue engineering (CTE). hASCs biological activity in contact with CollSSHACS was permanently compared to hASCs behavior in contact with a control hydrogel of pure collagen (Coll).
Materials and Methods: 3D cell-scaffold culture was achieved, exposed to chondrogenic induction cocktail for 28 days and the *in vitro* differentiation process was monitored at 7, 14 and 28 days post-induction. Cell distribution and scaffold structure before and during differentiation were analyzed by scanning electron microscopy (SEM). Actin filaments visualized by confocal microscopy revealed cell cytoskeleton architecture in contact with biomaterials. Safranin-O and Alcian Blue histological stainings were performed to monitor the chondrogenic differentiation process. Chondrogenic markers SRY (sex determining region Y)-box 9 (Sox9) and cartilage oligomeric matrix protein (COMP) gene expression was assessed via qPCR, while the protein levels of the same markers were qualitatively evaluated by confocal microscopy.
Results: SEM confirmed that hASCs populated the pores of the hydrogels and adhered to the CollSSHACS better than to control Coll, probably due to the sticky properties of sericin protein. Safranin-O and Alcian Blue stainings revealed matrix accumulation around cells during differentiation starting from 14 days. Sox9 was found to be upregulated starting with 7 days post induction in CollSSHACS as compared to control, where these markers were expressed later. Extracellular matrix marker COMP displayed an increasing profile of gene and protein expression from 14 to 28 days post osteogenic induction, with higher levels registered in cells cultivated in CollSSHACS than in cells embedded in Coll.
Conclusion: hASCs/CollSSHACS could be an appropriate 3D bioconstruct for CTE, considering its good biocompatibility and ability to support hASCs chondrogenic differentiation.
Acknowledgments: These studies were supported by research project funds PCCE248/2010 and ESF for Human Resources Development POSDRU/159/1.5/S/133391.
Keywords: cartilage tissue engineering, chondrogenic differentiation, human adipose derived stem cells.

SUN-476
Hippocalcin promotes neuronal differentiation and inhibits gliogenesis through the activation of PLD1 in rat neural stem cells

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The purpose of this study is to investigate how hippocalcin induces neuronal differentiation in rat neural stem cells (rNSCs). When hippocalcin was overexpressed in rNSCs, Tuj1 was increased, and GFAP was decreased. This result implies that hippocalcin facilitates neuronal differentiation while it suppresses astrocyte differentiation in rNSCs. We found that hippocalcin binds to PKCα, and down regulation of hippocalcin inhibited phosphorylation of PKCα (Thr 638/642). Interestingly, overexpression of hippocalcin promotes dephosphorylation of STAT3 (Y705). Furthermore, we elucidated that SHP-1, a tyrosine phosphatase, is located at upstream of STAT3. To find out molecular mechanism of PKCα on activation of SHP-1, we focused on phospholipase D1 (PLD1) which is activated by PKC. Down regulation of PLD1 decreased SHP-1 activation and consequently blocked dephosphorylation of STAT3 (Y705) resulting in neuronal differentiation. These results suggest that hippocalcin promotes neuronal differentiation of rNSCs through the activation of PLD1 by PKCα during differentiation, and followed by SHP-1 which dephosphorylates STAT3 (Y705). >
Keywords: Hippocalcin, Neuronal differentiation, Phospholipase D1.

SUN-477
Identification and characterization of murine dermal precursor cells with myogenic potential

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We have recently shown that bona fide, pulsating skeletal muscle myofibres may be generated from dermis-derived cells through recreation of 3D myogenic niche (Garcia-Parra et al., 2014). Interestingly, and after one month culture engineered muscle constructs showed progressive degradation of the myofibres concomitant with fatty infiltration, paralleling the natural course of muscular degeneration. However a critical point to translate these results to humans is to determine the origin and identity of myogenic precursor cells enriched within murine dermal cultures. Knowing that dermal and muscle cells share a common embryonic origin at the dermomyotomal stage, and taking into account that there might be different types of cells within adult skin presenting myogenic potential, our main objective was to identify and characterize the origin and identity of myogenic cells present in dermal cultures. To this end, we tested as working hypotheses the enrichment of (i) satellite cells from the dermal *Panniculus carnosus* (PC) muscle, (ii) dermomyotome-derived adult stem/precursor cells, (iii) perivascular cells, and (iv) neural crest-derived precursor cells. In order to trace the origin and identity of dermal myogenic cells, we took advantage of the following transgenic mice to perform lineage tracing experiments: (i) Pax3-GFP and $Pax7^{CE}$ (tracers of PC-derived satellite cells); (ii) Myf5-Cre (dermomyotome), (iii) Cspg4-Cre (perivascular and glial marker), and (iv) Sox10-Cre (neural crest). Cell tracing combined with FACS-based isolation and myogenic differentiation assays showed a major contribution of

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Myf5 + cells to the dermis-derived myogenic precursor cell subset, which was at least in part derived from PC satellite cells.
Keywords: 3D cell culture, Adult dermal precursor cells, Myogenic differentiation.

SUN-478
In vitro generation of endothelial progenitor cells differentiated from mouse induced pluripotent stem cells

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Autologous iPS cells after reprogrammed into endothelial progenitor cell (EPC) may offer several advantages in the treatment of cardiovascular disorders because of their cardiogenic and vasculogenic differentiation potential. To reach that purpose, we reprogrammed and then characterized mouse iPS cells into Flk-1^{+} cells. Further maturation of EPC was characterized by the expression of CD31 and CD133. We analyzed Flk-1 gene and protein expression levels with qRT-PCR and immunocytochemical methods on each days between 2.5 and 7.5. Flk-1 expressing cells were selected by magnetic activated cell sorting (MACS) at day 5. Purified Flk-1^{+} cells were cultured in differentiation medium with 2.6 nM human $VEGF_{165}$ to induce EPC formation. On day 2 following induction, CD31 and CD133 gene expression were analyzed with qRT-PCR and immunocytochemical methods.. In the second step, CD31 and CD133 positive cells were generated and enriched during day 2–3 of induction. We concluded that optimal time for harvesting Flk-1^{+} cells on by MACS was is day 5 of initial differentiation. Following isolation of Flk-1^{+} progenitor cells they were further matured into CD31^{+}/CD133^{+} cells within 2–3 days of induction. In conclusion, we showed that early EPC cells could be successfully derived from mouse fibroblast-driven iPS cells. We suggest that those iPS cell-derived EPC cells may be used in the treatment of heart failure, ischemic heart disease, and critical limb ischemia by remodeling the blood vessels and could be considered for an in vivo model for the translational research.

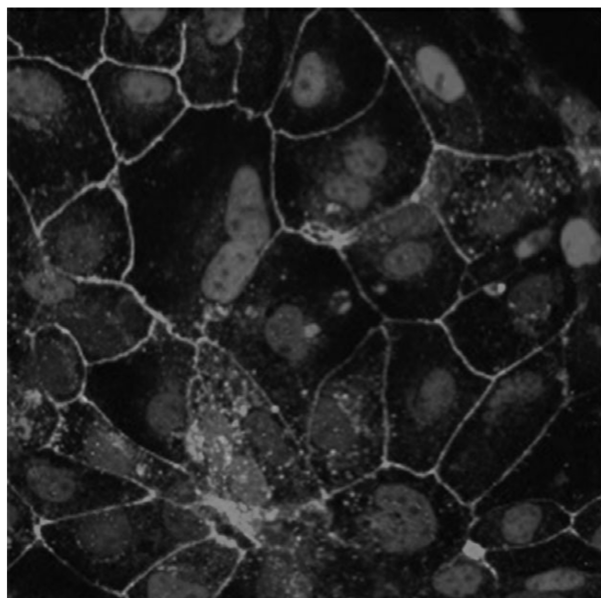

Fig. 1.

Keywords: Angiogenesis, Endothelial progenitor cells (EPCc), induced pluripotent stem cells(iPSc).

SUN-479
In vivo modelling of TSC1 SEGAs for intraventricular mTOR inhibitors administration

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Tuberous sclerosis (TS) is an autosomal dominant genetic disorder caused by mutations of either TSC1 gene, encoding for the protein hamartin, or TSC2 gene with the consequent constitutive activation of the mammalian target of rapamycin complex 1 (mTORC1) [1]. This results in the loss of control of cell proliferation and differentiation and, thus, the onset of several benign tumors, called hamartomas, on various organs [1]. An important pathological characteristic of TS is the presence of subependymal giant cell astrocytomas (SEGAs) in the brain that is the main cause of patients' death [2]. The standard therapy for SEGAs, represented by the surgical resection, may cause several complications during and post surgical intervention [1,2]. At present, the best approach to overcome surgical intervention is to orally administer mTOR inhibitors (mTOR-Is) [1–3]. However, this strategy is hindered by the immunosuppressive properties of mTOR-Is. This side effect can be overcome by establishing intraventricular administration of mTOR-Is [4]. The limiting factor for developing this TS treatment is the lack of suitable cellular or animal models.

We first tried to produce a TS cell line to be used as *in vitro* SEGAs model. We isolated neural stem cells (mNSCs) from brain of *Tsc1^{tm1Djk}*/J mouse that carries a "flox box" within TSC1 gene. Successively, we deleted a portion of this gene by transient transfection of mNSC cells with a GFP. Cre plasmid to express Cre recombinase. Neurospheres were screened by genomic PCR to identify clones displaying loss of the flox box. Immunoblotting analysis confirmed that hamartin protein was not expressed in selected clones. The resulting TSC1^{-}/$^{-}$ mNSCs showed mTOR pathway activation, as attested by immunoblotting analysis of phospho p70S6K (T389) and 4E-BP1 (T 36/47). They are stably self-renewing in typical neurospheres and are nestin-positive, as assessed by IF staining. We are currently performing experiments to assess

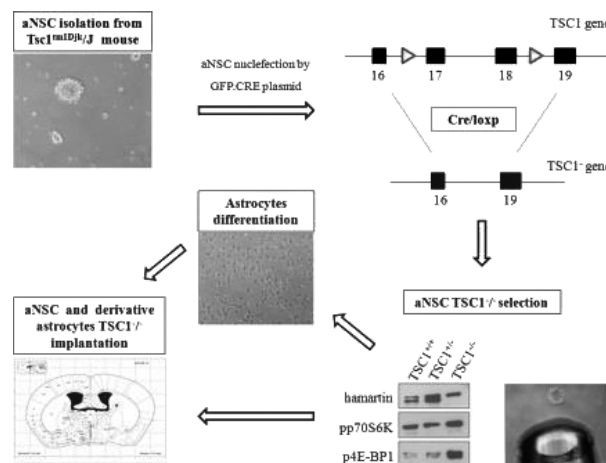

Fig. 1.

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if mTOR-Is treatment gives rise to a restored mTOR pathway, and its implication on proliferation rate.

TSC1^{-}/$^{-}$ mNSCs or derivative astrocytes have been implanted in the brain parenchyma of syngenic *Tsc1*^{tm1Djk}/J mouse by using stereotaxic surgery and *in vivo* SEGAs generation will be assessed by MRI.

Acknowledgements: Work supported by: AIRC 5 per mille 2011 Pr. 12214; 2007–2013 ESF Umbrian ROP; liberal donation of Dr C. Pigiona for the research project "Intrathecal administration of mTOR-Is for the therapy of neurological diseases".

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Keywords: Intraventricular administration of mTOR Inhibitors, Subependymal giant cell astrocytomas, Tuberous sclerosis.

SUN-480
Irisin modifies the differentiation program of subcutaneous human white adipocytes and induces "browning"

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Although brown adipose tissue (BAT) can be found only in small amounts in the human body after infancy, recent studies revealed that it has a major importance in regulating the energy balance of the entire body. Its oxidative metabolism contributes to energy expenditure during cold exposure and diet or physical exercise induced thermogenesis by triggering the differentiation of "brown adipocyte-like" or "beige" cells interspersed in subcutaneous white adipose tissue depots in a process called "browning". A very strong negative correlation has been found between obesity and BAT amount in humans. Targeting the currently known and unknown regulatory systems of "browning" might open up better strategies to specifically stimulate BAT in obese individuals to aid weight reduction.

Irisin is a recently identified peptide hormone which is cleaved from the Fndc5 transmembrane protein and induces a "browning" program in subcutaneous white adipose tissue in mouse models. In humans, however, an escalating debate revolves around the secretion and metabolic effects of irisin. Our aim was to clarify whether human recombinant irsin was able to induce a browning program on differentiating human adipocytes.

Human primary preadipocytes obtained from herniotomy were differentiated into white or brown adipocytes with or without long or short-term irisin treatment. Expression of white, brown and general adipocyte markers were determined by RT Q-PCR, immunoblotting or immunocytochemistry and changes in morphology (size and number of lipid droplets, expression of Ucp1 and Cidea *in situ*) were visualized and quantified by Laser Scanning Cytometry. Functional analysis was carried out using a Seahorse Bioscience XF-96 Analyzer.

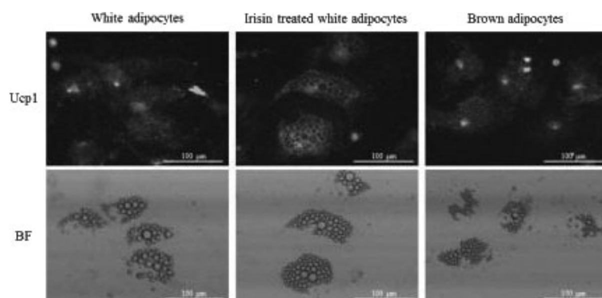

Fig. 1.

Irisin administration during white adipogenic differentiation resulted in a significant overexpression of several brown adipocyte marker genes (UCP1, ELOVL3, CIDEA, CYC1, PGC1A). Irisin treated cells had more and smaller lipid droplets, more mitochondrial DNA, higher functional mitochondrial respiration and expressed more Ucp1 and Cidea *in situ* than the *in vitro* differentiated white adipocytes. We conclude that irisin treatment is able to induce a browning program in differentiating human subcutaneous white adipocytes in culture conditions.

This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/ 2-11-1-2012-0001 'National Excellence Program' which provided personal support to Kristóf E.K., TÁMOP-4.2.2.A-11/1/KONV-2012-0023 grant and the Hungarian Academy of Sciences.
Keywords: Browning, Irisin, Laser Scanning Cytometry.

SUN-482
microRNA-9 maintains adult neural stem cells in a quiescent state

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Adult neurogenesis is the generation of new neurons from neural stem cells (NSCs) present in the adult brain. Fundamentally, this process is a balance between maintaining a quiescent NSC pool and recruiting them into the neurogenesis cascade. Using the adult zebrafish telencephalon as a model, we aim at deciphering the molecular mechanisms governing this balance. We place special focus on microRNA-9 (miR-9), which we previously showed to control the transition between commitment states in embryonic neural progenitors. Our recent work demonstrates that miR-9 is expressed and fluctuates over time in quiescent NSCs of the adult zebrafish telencephalon. Moreover, abrogation of miR-9 function *in vivo* leads to a significant increase in actively dividing NSCs, indicating that miR-9 is necessary for maintaining NSC quiescence. Previous work from our laboratory demonstrated that active Notch signaling maintains NSC in a quiescent state. We further show that miR-9 and Notch signals belong to the same regulatory cascade. Lastly, we have observed that miR-9 expression in the quiescent NSCs is highly concentrated in the nucleus. We also detected nuclear localization of Argonaute2 (Ago2), the effector protein of microRNAs. These data raise the intriguing possibility of a non-canonical mechanism of miR-9 action in quiescent NSCs, which we are currently investigating by modulating the subcellular localization of Ago2.

Keywords: microRNA, Neural Stem Cells, Quiescence.

SUN-483
MicroRNA-mediated mechanism for direct conversion of dental pulp cells to endocrine lineage cells

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To further evaluate the multipotency of dental pulp cells, and to investigate the possible direct reprogramming of these cells, we

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examined their *in vitro* induction of direct conversion to an endocrine cell lineage. *In vitro* induction was carried out using similar conditions to those reported for regulating the differentiation of undifferentiated intestinal cells into endocrine progenitor cells. Specifically, the transcription factors *Pdx1* and *Neurog3* were transfected into rat dental pulp cells to induce their direct conversion to endocrine lineage cells. The degree of induction was evaluated by detecting insulin-producing cells. Using microRNA arrays, the microRNA expression profiles were comprehensively analyzed. At 10 days after induction, insulin-producing cells were detected. Specifically, at 10 days after induction, the expression levels of *Ins1* and *Ins2* were each increased by about 7-fold compared with their levels before induction. Based on the expression profiles, eight microRNA probes showing significant differences at 10 days after induction compared with their pre-induction baseline values were extracted after filtering. Notably, miR-183 was downregulated by less than 40% after induction. Following a target scan of miR-183, we identified 242 conserved targets, including molecules crucial for the development of pancreatic beta-cells such as Forkhead box O1 (*Foxo1*). *Foxo1* was upregulated by more than two-fold after induction. These findings indicate that dental pulp cells have potential for direct reprogramming to insulin-producing cells. This potential ability for the direct reprogramming of dental pulp cells shows promise for clinical applications.

Keywords: dental pulp, direct conversion, multipotency.

SUN-484
miR-140-5p suppresses BMP2-mediated osteogenesis in undifferentiated human mesenchymal stem cells

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Human mesenchymal stem cells (hMSCs) have self-renewal and differentiation capabilities but the regulatory mechanisms of MSC fate determination remain poorly understood. Here, we aimed to identify microRNAs enriched in hMSCs that modulate differentiation commitments. Microarray analysis revealed that miR-140-5p is commonly enriched in undifferentiated hMSCs from various tissue sources. Moreover, bioinformatic analysis and luciferase reporter assay validated that miR-140-5p directly represses bone morphogenic protein 2 (BMP2). Furthermore, blocking miR-140-5p in hMSCs increased the expression of BMP signaling components and critical regulators of osteogenic differentiation. We propose that miR-140-5p functionally inhibits osteogenic lineage commitment in undifferentiated hMSCs.

Keywords: bone morphogenic protein 2, human mesenchymal stem cells, miR-140-5p.

SUN-485
Molecular and histological analysis of osteoblast induced by novel collagen helix fibrils

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Background: Osteoblasts are distinguished by the characteristic morphology and by the deposition of the mineralized matrix. Another characteristic is the synthesis of type I collagen and other specialized bone proteins. In particular, collagen that is the principal component of matrix provides the deposition of mineral with a template. Since collagen has biocompatibility with bone, collagen is a poor bone graft material when used alone. Probably, collagen is an inactive biomaterial against osteoblast. Therefore, much attention has been directed to find the biomaterial that induces osteogenic progenitor cells. However, best material still remains to be developed. It is essential to clarify the molecular mechanism of osteogenesis and understand how it induces. We have developed a novel collagen type I (N-Col) with enzyme treatment. Interestingly, N-Col has ability in spheroid formation. In this study, we report that N-Col markedly facilitated osteogenic differentiation of mouse osteogenic cells (MC3T3-E1) and Sprague-Dawley (SD) rat mesenchymal stem cells (rMSC). Furthermore, we investigated bone wound healing effects of N-Col graft by in vivo experiments.

Methods: Collagen was treated with an enzyme X, and we obtained a cleaved collagen preparation (N-Col) (patent pending). Culture dish was coated with N-Col or pepsin-treated collagen (Pep-Col). Subsequently, MC3T3-E1 cells or rMSC cells were cultured on each coated-dish with osteogenic basal medium. We observed cell morphology by using a phase-contrast microscope for a period of 20 days. To evaluate the osteogenic differentiation, we investigated the mineralization by Alizarin red S reagent. Furthermore, we transplanted each collagen material into 2.5 mm critical-sized defects (CSDs) of SD rat shinbone. After 15 days, the bone repair efficiency of CSDs in rat was evaluated by the histological observation of shinbone horizontal section with HE staining.

Results: MC3T3-E1 cells formed the spheroid-like morphology by only culturing on the dish coated with N-Col. Adhesion to N-Col significantly promoted the activity of mineralization and alkaline phosphatase of MC3T3-E1. The results of rMSC were the same as those of MC3T3-E1. In our experiments, we showed that N-Col graft induces bone regeneration of rat shinbone and is more bioabsorbable material than Pep-Col. The precise mechanism of osteogenic differentiation with N-Col culture system is not yet fully understood. However, Our results demonstrated that N-Col has remarkable effects in bone wound healing.

Funding: This work was supported by the Adaptable and Seamless Technology Transfer Program through target-driven R&D, Japan Science and Technology Agency [AS2414037P to K.M.].
Keywords: mineralization, osteoblast, spheroid.

SUN-486
Nanoscaffolds with immobilized bio-factors enhance the induction of stem cell differentiation into chondrocytes

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Chitosan nanoscaffolds (8% chitozan and 30% PolyCaproLactone) for cartilage regeneration endowed with *in vivo* biotinylated factors immobilized via streptavidin/biotin interaction induce dental mesenchymal stem cells into chondrocytes. In particular, the differentiation of human dental MSCs (DPSCs) into chondrocytes with recombinant biotinylated bone morphogenetic protein-2 (BMP2), Transforming Growth Factors TGFβ1 and TGFβ2 as well as the LAP-TGFβ (TGFβ -Latency Associated Peptide) has been shown to be proceeded effectively by all of the above mentioned factors all together or separately. The aforementionmed factors after being cloned into pAN5 plasmids, were purified and refolded for adopting their functional conformation,

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e.g the correct disulfidge bridges. The factors were purified and refolded by following the combination of different methodologies in a two step procedure. Mesenchymal stem cells were growth in their presence and their differentiation into chondrocytes has been visualized by RT-PCR or by identification of the typicall cartilage extracellular matrix components (ECM). The presence of aggrecan and collagen type II in high-density cultures (after 21 days) upon stimulation with BMP2 (50 ng/ml) and TGFb1 and TGFb2 (10 ng/ml each of them) confirmed undoubtely the induction of chondrocytic phenotype. In addition, staining of proteoglycans with Alcian blue after 21 days, revealed the deposition of typical cartilage extracellular matrix components indicating the effective functionality of the immobilized biotinylated factors and the low expression levels of osteocalcin revealed the absence of osteocytes. Remarkable is the contribution of the LAP-TGF, which exhibited in vitro an opposite behavior as that has been shown in vivo. Namely, LAP-TGFβ was fully active and did not prevent the binding of TGFβ to the appropriate receptors as it was firstly expected based on the international literature. To summarize, we propose the use of all three factors in order to endow effectively the nano-scaffods with the required properties for reconstruction of injured chondro tissue.

Acknowledgment: The authors would like to thank the NATIONAL ACTION: «COOPERATION 2009» Program – NanoArthroChondros (09ΣΥΝ-41-1150).
Keywords: None.

SUN-487
Neuronal differentiation of mouse ES cells by Rho kinase inhibitor

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Mouse embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of 3.5-day-old blastocysts of preimplantation mouse embryos and have a pluripotent ability to differentiate into various cell lineages *in vitro* including neurons. Recently, it was reported that Y-27632, a specific inhibitor for Rho-dependent protein kinase (ROCK), permits the survival of human ES cells by inhibiting the dissociation-induced cell death. Because ROCK regulates the function of several target proteins through its kinase activity, the inhibition of ROCK activity may provide new possibilities of controlling the *in vitro* differentiation of the ES cells into neurons. Therefore, we investigated the effect of ROCK inhibitor on the differentiation of mouse ES cells into neurons.

We investigate the effect of ROCK inhibitor addition on differentiation into neurons and muscle cells from ES cell colonies (approximately 200 μm in diameter). When ES cell colonies at 12 days of cultivation were labeled with an antibody against βIII-tubulin (a marker of postmitotic neurons), they efficiently differentiated into neurons by the addition of ROCK inhibitor. In addition, ES cell colonies differentiated not only into neurons but also into muscle cells by the addition of ROCK inhibitor. Because the percentages of neurons differentiated from ES cells as a result of incubation with ROCK inhibitor were 60–70%, we concluded that the ES cells primarily differentiated into neurons. We characterized the types of neurons that differentiated from the ES cells by immunofluorescence analysis. ROCK inhibitor promoted the differentiation of the ES cells into motor and sensory neurons.

Mitogen-activated protein kinase (MAPK) /extracellular signal-regulated kinase (ERK) signaling pathway was known to require for neuronal differentiation of ES cells. We investigated whether the addition of ROCK inhibitor promoted phosphorylation of ERK in ES cells by western blotting. When ROCK inhibitor was added to the culture medium, the amount of p-ERK was higher than that without ROCK inhibitor. Furthermore, when ES cells were treated with MAPK/ERK kinase (MEK) inhibitor, ROCK inhibitor did not promote the neuronal differentiation of the ES cells.

ROCK inhibitor promoted the differentiation of ES cells into neurons and muscle cells. We found that the ROCK inhibitor may promote the neuronal differentiation of the ES cells by activating the ERK signaling pathway. These results show that the addition of ROCK inhibitor to the culture medium is useful for the differentiation of ES cells.

Keywords: Differentiation, ES cell, ROCK inhibitor.

SUN-488
Osteogenic differentiation of mesenchymal stem cells by bioactive self-assembled peptide hydrogels

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Replacement and repair of bone lost due to trauma, degenerative diseases and infections still remain major clinical challenges. Autografting, allografting and xenografting are indicated as current strategies for the treatment of bone defects. However, these strategies cause problems in clinical applications such as immunological response and disease transmission, together with the donor site morbidity. To overcome these limitations, regeneration of new bone can be induced by the usage of new synthetic bioactive materials. One of the most promising strategies is to develop synthetic scaffolds mimicking functional components of extracellular matrix (ECM). ECM plays important roles in bone regeneration and mineralization. Collagens and hydroxyapatite provide the bone mechanical strength and flexibility. Glycosaminoglycans, one of the non-collagenous components of ECM, promote bone remodelling by affecting cellular proliferation and differentiation through stabilizing growth factors and enhancing growth factor-receptor interactions. In this study, we aimed to direct the differentiation of mesenchymal stem cells into osteogenic lineage by peptide amphiphile nanofiber scaffolds decorated with various functional groups mimicking chemical and morphological characteristics of glycosaminoglycans and collagen. Peptide amphiphile molecules self-assembled into a supramolecular nanofibrous network similar to natural ECM. We observed that the peptide nanofiber system induced osteogenic activity of mesenchymal stem cells and calcium deposition, which offers novel platforms for selective induction of mesenchymal stem cells into osteogenic lineage.

Keywords: Mesenchymal Stem Cells, Osteogenic Differentiation, Peptide Nanofibers.

SUN-489
Phospholipase D1-induced Bcl-2 expression is required for neuronal differentiation in rat neural stem cells

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We studied the possible role of phospholipase D1 (PLD1) in the neuronal differentiation, including neurite formation of neural stem cells. PLD1 protein and PLD activity increased during neuro-

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nal differentiation. Bcl-2 also increased. Down regulation of PLD1 by transfection with PLD1 siRNA or a dominant-negative form of PLD1 (*DN-PLD1)* inhibited both neurite outgrowth and Bcl-2 expression. Moreover, treatment with arachidonic acid (AA) which is generated by the action of PLA2 on phosphatidic acid (a PLD1 product), increased the phosphorylation of p38 MAPK and CREB, as well as Bcl-2 expression, indicating that PLA2 is involved in the differentiation process resulting from PLD1 activation. PGE2, a cyclooxygenase product of AA, also increased during neuronal differentiation. Moreover, treatment with PGE2 increased the phosphorylation of p38 MAPK and CREB, as well as Bcl-2 expression, and this effect was inhibited by a PKA inhibitor (Rp-cAMP). As expected, inhibition of p38 MAPK resulted in loss of CREB activity, and when CREB activity was blocked with CREB siRNA, Bcl-2 production also decreased. We also showed that the EP4 receptor was required for the PKA/p38MAPK/CREB/Bcl-2 pathway. Taken together, these observations indicate that PLD1 is activated by PLCγ/PKCα signaling and stimulates Bcl-2 expression through PLA2/Cox2/EP4/PKA/p38MAPK/CREB during neuronal differentiation of rat neural stem cells.

Keywords: Bcl-2, Neuronal differentiation, Phospholipase D1.

SUN-490
Rapid, unbiased and reproducible large scale gene expression profiling from limited samples: analysis of individual embryoid bodies and pluripotent stem cell colonies

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Transcriptional profiling during stem cell differentiation and cellular reprogramming has become a powerful tool for studying biological mechanisms of lineage determination and reprogramming progression. Current workflows require tedious column-based RNA purification from high amount of starting materials; projecting in either limitation of analyzed targets or high running costs for conducting a thorough study. Here, we present a novel workflow using validated reagents that allow researchers the ability to assess up to 100 specific gene targets from as little as a single embryoid body (EB) or a small biopsy of human pluripotent stem cell (hPSC) colonies.

The 4-step workflow is initiated by a single step sample preparation enabling high throughput isolation of genomic DNA-free RNA lysates directly from the cultured cells, without the need for column purification. The lysate is then directly reverse transcribed to cDNA. To allow analysis of a large number of targets from limited samples, such as a single EB, the cDNA is pre-amplified for a panel of 100 pre-validated targets previously shown to be essential for human pluripotency and lineage determination. Lastly the pre-amplified targets are analyzed by SYBR Green qPCR.

Using a single EB (~1000 cells) as the starting sample, we demonstrated that the above workflow enables the successful analyses of differentiation progression and lineage determination. We proved excellent correlation and reproducibility in gene profiling between the pre-amplified cDNA samples compared to the non-amplified starting cDNA samples. Out of the 58 expressed targets 97% show a Cq difference below 0.75 cycles from predicted pre-amplified cDNA values, thus confirming that the workflow introduces no or minimal bias. Similarly, we successfully applied this workflow to gene expression profiling of small hPSC biopsies (<1000 cells) to monitor culture quality or reprogramming progression. This would not have been possible using the conventional column based RNA purification approach. In summary, we have developed a complete workflow enabling rapid, unbiased and reproducible gene expression profiling of a large panel of targets from very limited samples. The flexibility and adaptability of this workflow facilitates integration in a high throughput screening environment. Furthermore, the minimal amount of material required by this workflow highlights a more cost effective way for culturing and analyzing hPSC.

Keywords: limited sample quantification, gene expression profiling, pluripotent stem cells, embryoid body differentiation, target-specific preamplification.

SUN-491
Regulation of embryonic genome activation

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Embryonic genome activation (EGA) describes the onset of gene expression in the early embryo. Before this event maternal factors expressed during oogenesis control the development. The onset of EGA is species-specific and controlled in time and space. Across species several factors have been implicated in EGA regulation including chromatin regulators, transcription factors and RNA binding proteins. However, a regulatory mechanism and the evolutionary conservation still remain largely unknown. We use the model organism *Caenorhabditis elegans* to identify new factors involved in EGA regulation. As a tool to visualize EGA a reporter strain was created expressing GFP from the promoter of *vet-4,* one of the early embryonic genes. In a genetic screen for EGA repressors performed in our lab, several mutants with precociously misexpressed EGA in the germline have been identified. A first class of mutants forms relatively normal oocytes expressing embryonic genes, whereas in a second class of mutants the EGA expressing germline cells form a teratoma. These findings propose EGA as a first reprogramming event towards pluripotency. However, at the same time, the phenotype of the class I mutants indicates that an additional reprogramming step is needed for the teratomatous differentiation as these mutants show embryonic gene expression but no differentiation. I aim to dissect the reprogramming event leading to EGA via identification of the mutated genes with Whole Genome Sequencing (WGS), further characterization of the mutants and identification of the pathways they are involved in. Furthermore, after several EGA repressors have been identified in this first screen I perform a second genetic screen to identify EGA activators. I will use the already mentioned reporter strain and screen for embryos that lost GFP expression and therefore EGA. The finding of EGA repressors and activators will shed light on the regulation in *C.elegans* but may also reveal evolutionary conserved mechanisms of regulation.

Keywords: embryonic genome activation, germline, pluripotency.

SUN-492
Role of melatonin on differentiation of mesenchymal stem cell dreived from 3rd molar germ tissue

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Stem cell based applications have become a popular and a promising approach for therapy of a number of disorders including

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neurodegenerative diseases, degenerative muscle diseases and osteoporosis as well as trauma, inflammations, burns and injuries. Human tooth germ stem cells are an adult stem cell source; they have mesenchymal stem cell properties and show high proliferative and differentiation capacity. Melatoninhas been demonstrated to regulate differentiation of human and mouse mesenchymal stem cells into various cell lineages in addition to its other functions in the body. In the current study, the effects of melatonin on osteogenic, neurogenic, adipogenic, chondrogenic, myogenic and odontogenic differentiation of human tooth germ stem cells were investigated. The results showed that melatonin increases the viability of cells. It significantly augments osteogenic, neurogenic, chondrogenic, myogenic and odontogenic of the cells whereas it reduces adipogenic differentiation capability. These results suggest that melatonin has a great potential to increase differentiation capacity of human tooth germ stem cells and might be useful in regenerative therapy applications involving stem cell differentiations in addition todefining potential treatments for obesity because of its suppressor effects on adipogenesis.

Keywords: Melatonin, Mesenchymal Stem Cells, Stem Cell Differentiation.

SUN-493

Role of STRO-1 positive porcine dental germ stem cells in dental stem cell-mediated bone tissue engineering

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Collection of bone marrow stroma from patients is an invasive precedure. Thus, scientists focused on minimally invasive procedures for collection of mesenchymal stem cells. In humans, tooth germ tissues derived from third molars undergo organogenesis to give rise to dental structures at around age 6. As a result of this, some of the progenitor cells reside in dental tissues meaning that these cells remain developmentally and replicatively young. In addition, third molar tooth germs are routinely extracted as an orthodontic treatment and discarded as surgical waste. Therefore, stem cell harvesting from this source causes no ethical controversy.

In this study, pig was used as an experimental model for the isolation of tooth germ stem cells (TGSCs) due to its anatomical, physiologic, and metabolic similarities and its similar diphyodont and heterodont dentition to that of humans.

Mandibular third molar tooth germs were surgically removed from the jaws of 6-month-old domestic pigs. Tooth germ tissue, including the dental mesenchyme and its surrounding follicle, was minced into small pieces and used for the explant culture. Passage 1 (P1) porcine TGSCs (PTGSC) were analyzed by flow cytometry and found positive for CD105, CD90, and CD44 and negative for CD45 and CD34 surface markers showing their MSC characteristics. P1 PTGSCs were also tagged with STRO-1 antibody and 21.6% of cells were detected and sorted as positive for STRO-1. STRO-1 positive (STRO-1+) cells identifies a subpopulation of MSCs which are highly clonogenic and can differentiate into adipocytes, osteoblasts, chondrocytes and smooth muscle cells. Unsorted and Unsorted (US) and STRO-1+ cells were induced for osteogenesis and analyzed for their proliferation behaviors and differentiation capacities towards osteogenic lineage by assessing ALP activity, calcium content and mineralized nodules in cultures.

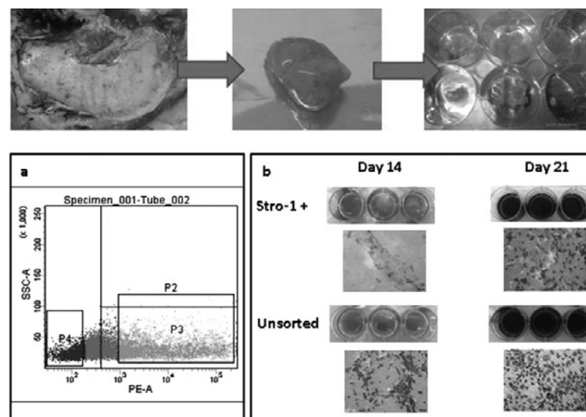

Fig. 1. Extraction of tooth germ from porcine third molars and isolation of cells by explant culture. (a) Stro-1 selection of PTGSCs by FACS (b) von Kossa staining of PTGSCs osteogenically induced for 21 days.

There was no significant difference between proliferation rates and ALP activities of STRO-1+ and US cells whereas calcium deposition by US cells were significantly higher. von Kossa staining of mineralized nodules of both STRO-1+ cells and US cells showed high levels of dark-black staining of mineralized nodules especially after 21 days of incubation being slightly higher in US cells. Results showed that after 21 days of culture, both groups differentiated towards osteogenic lineage without any significant difference created by STRO-1+ cells. Besides, US cells even deposited more calcium as shown by calcium assay and von Kossa staining. This might indicate that STRO-1+ cells might require a heterogeneous population of cells in their niche to perform their proposed role in osteogenesis.

Keywords: osteogenic differentiation, Stro-1, tooth germ stem cells.

SUN-494

Rspondins are required for adrenal gland homeostasis

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Wnt/β-catenin signaling is a key pathway in organ development and tissue homeostasis and is implicated in proliferation and differentiation of stem cells. Constitutive activation of β-catenin is the most frequent event that induces adrenocortical tumors. We are interested in Rspondins, a family of proteins that have been reported to act as activators of canonical Wnt/b-catenin signaling. Our analyses show that expression of *Rspo1 and Rspo3*, two members of this family, are maintained within the adrenal capsule throughout life, a site that has been proposed to contain a stem/progenitor cell compartment. To address the role of *Rspondins* in development and homeostasis we performed loss-of-function approaches using genetically modified mice. Analysis of *Rspo1* knockout mice did not reveal an overt adrenal phenotype.

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In contrast, deletion of *Rspo3* at embryonic day E11.5, lead to significantly smaller adrenals exhibiting a disrupted cortex structure at E16.5. Moreover, removing *Rspo3* in adult mice caused a reduction of cortex size within 6 weeks after deletion suggesting a requirement for this gene in adrenal homeostasis. To complement these loss-of-function studies, we ectopically expressed *Rspo1* within the developing adrenal cortex. Strikingly transgenic mice displayed dramatic overgrowth of the adrenal cortex as early as E15.5. Interestingly, the size of the adrenal cortex increased in a time- and dosage-dependent manner without an overall disturbance of the cortical architecture. At later stages of life (>6 months), however, a proportion of mice developed adreno-cortical tumors. Taken together, these data demonstrate an essential role for *Rspo3* in adrenal gland development and homeostasis and reveal a growth promoting and oncogenic capacity of *Rspondins*. Our results might also suggest that overexpression of these genes may be at the basis of adrenocortical tumors in human patients.

Keywords: adrenal glands, Rspondin, stem cells.

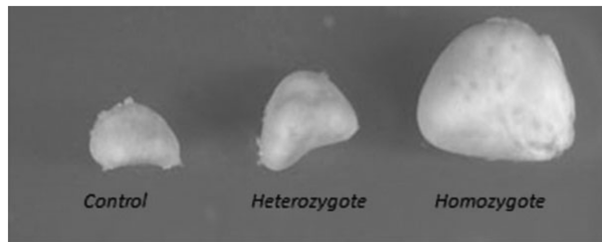

Fig. 1. Ectopic expression *of Rspo1* induces dosage-dependant hyperplasia of the adrenal gland.

SUN-495
Spatial regionalization and heterochrony in the formation of adult pallial neural stem cells

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Little is known on the origin and related heterogeneity of adult neural stem cells (aNSCs) in the vertebrate brain. We used the zebrafish to investigate the contribution of embryonic neural progenitors to the population of aNSCs of the dorsal telencephalon (pallium). We first assessed the adult fate of a large population of embryonic radial glial progenitors expressing the E(spl) transcription factor Her4 (orthologous to mouse Hes5), which highlights actively neurogenic zones in the early embryo. Using a conditional Cre/lox genetic strategy driven by the *her4/Hes5* promoter, we could specifically and permanently fate map this population at chosen developmental stages. We report that this population, when traced from embryonic stages onwards, generates the entire aNSC compartment of the dorso-medial pallium, but fails to contribute to aNSCs of the lateral pallium. In contrast, we demonstrate that the lateral aNSC population is established progressively, from larval stages onwards, following an anterior to posterior gradient. We identify its origin in a restricted pool of neuroepithelial progenitors present at 30 hours-post-fertilization at the dorsal telencephalic roof, and amplifying post-embryonically. These progenitors express signalling pathways components such as Wnt3a/Wnt8b. The dorso-medial and lateral aNSC populations remain strictly segregated in space, and we further demonstrate that these differential origins progenitors differ in their sensitivity to Notch signaling. Together, these results demonstrate a dual origin and formation mode of pallial aNSCs, which derive from spatially and molecularly distinct as well as strongly heterochronous progenitors. This allows the temporally organised building of pallial territories as a patchwork of juxtaposed compartments.

Keywords: Embryonic origin, Lineage tracing, Neural Stem Cells.

SUN-497
Studying epithelial-mesenchymal plasticity of human somatic cells in 3D culture

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It is known, that epithelial-mesenchymal transitions (EMTs) which were first described in embryogenesis, are also taking place during physiological and reparative regeneration, fibrosis, oncology and cell reprogramming. Studying epithelial-mesenchymal plasticity (EMP) of human somatic cells (HSC) has an enormous scientific and practical significance. The aim of this research was to study and compare EMP of epithelial and mesenchymal HSC.

In this work we used epithelial (retinal pigment epithelium, placental cytotrophoblasts) and mesenchymal cells (limbal stromal cells, umbilical cord multipotent mesenchymal stromal cells). It was shown that initially epithelial cultures had classical epithelial "cobblestone" morphology. But after the third passage they acquired mesenchymal phenotype, which was accompanied by redistribution of E-cadherin expression from cell membrane to cytoplasm and acquiring N-cadherin and vimentin expression. Addition of dexamethasone to culture medium resulted in changing cell phenotype to epithelial. Mesenchymal cells in monolayer culture didn't change their phenotype even in presence of dexamethasone, expressed N-cadherin and vimentin.

Transfer of cultures from 2D to 3D non-adhesive conditions let us use resulting single spheroids as a model for studying EMP of HSC. Live time-lapse microscopy (Cell-IQ, Finland) revealed regularities in the process of spheroid formation from epithelial and mesenchymal cells. Regardless of cell phenotype and initial cell concentration aggregation and compaction of spheroids took place in the first 7 days; diameter of resulting spheroids didn't grow.

Scanning electron microscopy showed that the surface of spheroids from epithelial and mesenchymal cells consisted of epithelial-like polarized cells, whereas central zone was represented by small non-polarized cells. According to transmission electron microscopy spheroid compaction was accompanied with formation of tight junctions in surface cell layer.

Immunocytochemical analysis showed that surface cells of mature spheroid co-expressed E-cadherin (localized primary on cell membranes) and N-cadherin (mainly in cytoplasm). Central cells didn't express E-cadherin, but highly expressed N-cadherin. According to real-time PCR analysis spheroid formation resulted in gradual increase in expression of three pluripotency markers Oct4, Sox2 and Nanog.

Thus, this study showed that HSC *in vitro* can be either in epithelial or mesenchymal status. 2D culture promotes EMT, whereas 3D conditions stimulate reversibility of EMT which results in formation of mesenchymal-epithelial spheroids. Such spheroids can serve as a unique model for studying EMT phenomena.

Keywords: 3D cell culture, Epithelial-Mesenchymal Transition, spheroid.

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SUN-498

Suppression of the SOX2 neural effector gene by PRDM1 promotes human germ cell fate in embryonic stem cells

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The mechanisms of transcriptional regulation underlying human primordial germ cell (PGC) differentiation are largely unknown. Transcriptional repressor Prdm1/Blimp-1 is known to play a critical role in controlling germ cell specification in mice. Using human embryonic stem cells (hESCs) as an *in vitro* differentiation platform, we previously established that the BMP and/or WNT signaling pathways promote the formation of meiotic germ cells from hESCs and that PRDM1 is induced by BMP/WNT signaling. Here, we show that PRDM1 contributes to the determination of germline vs. neural fate in early development. We show that knockdown of PRDM1 in hESCs impairs germline potential and up-regulates neural genes. Conversely, ectopic expression of PRDM1 in hESCs promotes the generation of cells exhibiting phenotypic and transcriptomic features of early PGCs. Furthermore, PRDM1 directly suppresses transcription of *SOX2*; overexpression of *SOX2* in hESCs under the condition favoring germline differentiation skews cell fate from the germline to the neural lineage. Collectively, our results demonstrated that PRDM1 serves as a molecular switch to modulate the divergence of neural or germline fates through repression of *SOX2* during human development.
Keywords: Transcription factor, Germ cell, Human embryonic stem cell.

SUN-499

Tenascin-C mimetic peptide nanofibers direct in vitro differentiation of stem cells to osteogenic lineage

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Regeneration of damaged bone tissue is essential for the treatment of large bone defects associated with reconstruction surgery of trauma, cancer, and total hip arthoplasties. Nanofiber scaffolds made of self-assembling peptides offer a promising approach for regenerative medicine due to their biocompatible and biodegradable properties. Extracellular matrix (ECM) contains several signals for cell surface receptors contributing to cell fate through regulation of cellular activities such as proliferation and differentiation. Cues from ECM components can be used for the development of new materials to direct the stem cell fate. Short peptides derived from ECM proteins are commonly used to incorporate bioactivity to synthetic scaffolds. Bioactive scaffolds can induce adhesion, migration, proliferation or even differentiation depending on the epitope selection. In this study, we exploited the functional role of extracellular matrix on osteogenic commitment and differentiation by mimicking bone extracellular matrix by incorporating functional sequence of Fibronectin Type III domain from native Tenacin-C, an important glycoprotein found in the matrix on bone surfaces, on peptide nanofiber network. Overall, our results showed that the attachment, proliferation, and osteogenic differentiation of rat Mesenchymal Stem Cells (rMSCs) were enhanced by peptide nanofibers including Tenascin-C mimetic peptide even in the absence of any external bioactive factors and regardless of their mechanical properties, which provides a promising new platform for bone regeneration.
Keywords: Mesenchymal stem cells, Peptide amphiphile, Tenascin-C.

SUN-500

The correlations between growth factors release and stem cells maintenance in hippocampus of amyloid exposure rats

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Purpose of the Study: The pathogenesis of dementia-linked neurodegenerative disorders like has it's specify and various from disease to disease, but the general concerns are probably the same. In the most of them there is the disturbance in metabolism of growth factors, neurotransmitters, up-regulated oxidative stress, and the weakness of the potency of the adult stem cells for regeneration. A wide range of biological active substrates are aprobated as neuroprotectors, but just few of them have less or more efficiency in clinic. In this study we tried to find out correlations between the release of growth factors like IGF-1 and NGF, and adult stem cells maintenance in hippocampus after experimental modeling of Alzheimer's disease (AD).

Mathods Used: Experiments were performed on 40 mature white Sprague-Dawley male rats. The control group consisted of vehicle-treated animals. The experimental model of AD was made in rats by intracerebroventricular (*i.c.v.*) injection of aggregated amyloid-betta (Ab) 1-42. IGF-1 and NGF were determined by ELISA in hippocampus of rats' brain. Markers of neurogenesis (Vimentin, Nestin), dividing cels (BrdU, Wnt) were determined in hippocampus of rats' brain on the 90th day from *i.c.v.* injection of Aβ by flow cytometric detecti

Summary of Results and Conclusion: In hippocampus after *i.c.v.* injection of Aβ1-42 there were no significant changes in fluorescence intensity of Nestin, Wnt and BrdU/Wnt double-labeled cells, but the quantity of Vimentin, Nestin/Vimentin double-labeled cells decreased compared with the control cells, which suggested the inhibitory effect of neurodegeneration on neural stem cells maintenance and survival. On contrary, the expression BrdU positive cells increased, which suggested that neurodegeneration could play a trigger role in stem-cells proliferation. These findings are in line with changes of growth factors: it was observed a significant up-regulation of IGF-1 and slight increase of NGF in hippocampus. Increase in growth factors level and in particularly IFG-1, evidently, is an adaptive response of brain neurons to damage, something like a "cry to survive", which is accompanied by high proliferation of hippocampal adult stem cells.

Summarizing the study, we conclude that the new therapeutic strategies in neurodegeneration have to be focused on activation the adult stem cells proliferation and differentiation via growth factors.
Keywords: amyloid, growth factors, neurogenesis.

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SUN-501
The differentiation of Wharton's jelly mesencymal stem cells into hepatocyte cells

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Liver diseases are amongst the crucial health problems the world and they cause a loss in hepatocyte functions. Despite the number of methods used to treat liver diseases, an effective treatment hasn't yet been found. Therefore, alternative methods based on stem cells are currently being investigated. Mesenchymal stem cells give promising results to cure liver diseases with their high reproduction potential, low immunogenecity and capacity to differentiate. In order to treat patients with liver diseases; different protocols containing cytokines, growth hormones and chemical agents were used to differentiate mesenchymal stem cells into hepatocyte cells.

The purpose of this study is to investigate a relatively effective new protocol to differentiate mesencyhmal stem cells into hepatocyte cells using human Wharton's Jelly.

The methods used in the experiments were; isolation, microscopic, Periodic Acid Shift Dye, Real Time Polymerase Chain Reaction and Flow cytometric. The data was evaluated using SPSS version 16.0 for Windows.

As a result of the microscopic and flow sytometric analysis, surplus number of healthy mesencyhmal stem cells was isolated from Wharton's Jelly using explant method. According to the histological and molecular results, with this new protocol; using bFGF, HGF, FGF-4, EGF, Dexamethasone, Nicotinamide, ITS, Napyruvate, OSM, DMSO containing as the differentiating and maturing culture medium the most effective hepatogenic differentiation was conducted. Comparing with the other protocols, the hepatogenic cells obtained by using this new protocol in comparison to the control (mesenchymal stem cell) and hepatocyte (Hep 3B) cell groups; Real Time Polymerase Chain Reaction made as a result of albumin (ALB) and alpha fetoprotein (AFP) gene expression levels in a statistically significant way to express that most were determined.

As a result of studies; dissimilar to the previous protocols, this new one enabled the differentiation of a more hepatocyte characterized hepatogenic cells from Wharton's Jelly mesencyhmal stem cells.

Keywords: Hepatocyte, Mesencyhmal stem cell, Wharton's Jelly.

SUN-502
The human foetal globin genes exhibit a two-wave pattern of transcription in primary erythroid cultures

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Foetal haemoglobin, the major haemoglobin type produced in the human foetus, is almost entirely replaced by adult haemoglobin shortly after birth. This transition is the result of switching from high-level expression of the foetal globin genes *HBG1* and *HBG2* to predominant expression of the adult *HBB* gene in erythroid cells. Several reports, however, suggest that the foetal globin genes are not completely silenced in adult erythroid cells but rather experience a period of expression during adult erythroid differentiation. We have used primary transcript RNA fluorescent *in situ* hybridisation to study the transcription of the foetal globin genes in cultures of primary erythroid cells. We show that foetal globin gene transcription changes in the course of culture progression following a two-peak pattern. We present evidence suggesting that this is the result of parallel differentiation of two haematopoietic progenitors that take different periods of time to reach the differentiation stage when the foetal globin genes are expressed. Our results support the notion of the existence of a short window of *HBG* activity during adult erythroid differentiation.

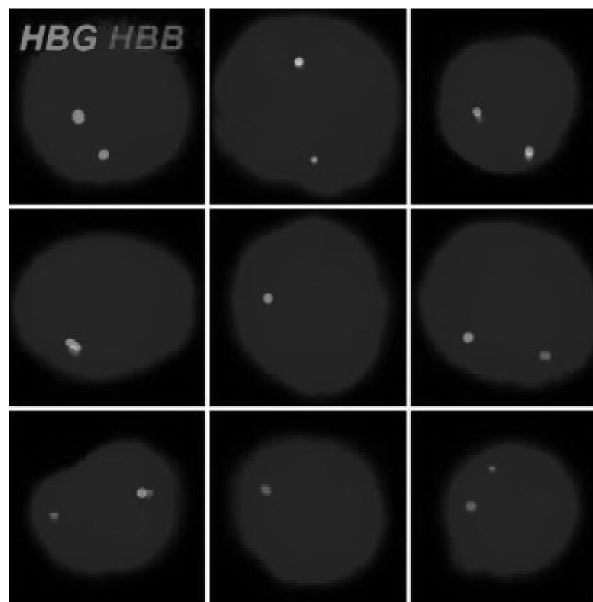

Fig. 1. Detcetion of β-globin primary transcripts by RNA FISH

Keywords: erythroid differentiation, gene transcription, primary transcript RNA FISH.

SUN-503
The role of glycans in osteogenesis

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Multipotent stromal cells/mesenchymal stem cells (MSCs) are capable of differentiating into various cell types; osteoblasts, adipocytes and chondrocytes. This ability has resulted in the use of MSCs in clinical trials to treat a wide variety of diseases, from osteoporosis to heart disease. Much research into the role of cell signalling pathways in directing stem cell fate is underway, but relatively little is known about the role of glycans. *N*-glycosylation is the addition of sugar residues to proteins in the endoplasmic reticulum and Golgi, which can alter their properties including their ability to bind to target proteins. Consequently, glycosylation has a major role in many cellular mechanisms including cell adhesion, signal transduction, and non-self-recognition. Previous studies have described distinct glycan profiles of cells at different stages of development. However, whether glycans play a functional role in directing stem cell differentiation is unknown. To identify the importance of glycosylation during differentiation, we determined the glycan profiles of multipotent stromal cells/mesenchymal stem cells (MSCs) and then disrupted the *N*-glycan synthesis pathway and tested if the ability of MSCs to differentiate had been altered. Telomerase (hTERT)-immortalised clonal MSC lines were generated, to account for the broad glycan-heterogeneity often observed in cell cultures and to generate sufficient material for in-depth analysis of glycans. *N*-glycans were isolated from hTERT-MSCs using filter aided *N*-glycan separation (FANGS) method. This low-throughput method, followed by permethylation to stabilise glycans, allowed

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us to repeatedly profile samples using mass spectrometry (MALDI-TOF/TOF) and quantitatively compare relative glycan abundance in different cellular states.

The *N*-glycan profile of osteoblasts derived from hTERT-MSCs had increased complex type glycans, as well as a higher abundance of fucosylated glycans compared to undifferentiated hTERT-MSCs. To assess if this change is functionally important the Golgi synthesis pathway was genetically disrupted, by transducing hTERT-MSCs with shRNA for Cog4, a subunit of the COG complex. COG is an 8 subunit protein involved in the sorting of Golgi resident enzymes, therefore required for correct glycan synthesis. Interestingly, Cog4 knock-down cells (Cog4KDs) had reduced osteogenic potential, with significantly less calcium staining and less alkaline phosphatase activity compared to controls. Crucially, these results show, for the first time, that disruption of glycan synthesis can effect MSC differentiation. Since COG defects disrupt all glycosylation pathways, not just *N*-glycosylation, further work using specific inhibitors of *N*-glycan processing to elucidate the role of *N*-glycans in the loss of osteogenic capacity of Cog4KDs,will also be discussed.

Keywords: Glycosylation, Mesenchymal stem cells, Osteogenic Differentiation.

SUN-504

Time-dependent modulation of mesenchymal stem cells osteogenic differentiation by FGF2

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Bone regeneration during fracture healing or following implant surgery is essential for the efficient recovery in orthopaedic pathology. To this end, the efficient direction of proliferating mesenchymal stem cells (MSCs) towards the osteogenic fate is mandatory. FGF-activated signalling pathways are known to play essential roles in endochondral and intramembranous bone development[1,2]. Moreover, FGFs are considered potent regulators of cell growth and wound healing that are produced in bone by osteoblasts, macrophages, and endothelial cells, and are stored in their active form in the extracellular bone matrix[3]. FGF2 was already described as an efficient proliferation enhancer when added in the expansion media of MSCs[4]. FGF2 was shown to maintain osteogenicity[5] and increase osteogenic and chondrogenic differentiation *in vitro*[6]. There are also contradictory reports stating the inhibitory effect of FGF2 on osteogenesis[7,8]. However, little is known about the potential molecular mechanisms involved. We have used a combinatorial approach to study the role of FGF2 addition to the osteogenic induction medium (OIM) on MSCs that were pre-treated or not with 1 ng/mL FGF2 for two weeks. The effect of FGF2 on MSCs proliferation and osteogenic differentiation was characterized by MTS assay and Alizarin Red mineralization staining. We have screened for optimal time and dose of growth factor treatment that would be beneficial for the efficient generation of bone tissue. Using image cytometry, we quantified cell differentiation during treatment with FGF2. Our results revealed a dose- and time-dependent effect of FGF2 on MSC-derived osteoblast proliferation and mineralization. We correlated this effect with an increase in beta-catenin expression and with an upregulation of pERK1/2 and pp38 following FGF2 priming. The impact of signalling pathways modulation on Runx2 transcription factor-driven osteogenic commitment was investigated by flow cytometry and Real-time PCR. Our studies will guide the design of laser-transferred polymeric scaffolds embedding FGF2 and other growth factors to improve osseointegration and angiogenesis during bone healing by controlled release of these molecules.

Acknowledgements: The study was supported by the National Grant PN-II-PT-PCCA-2011-3.2-0898, 153/2012.

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Keywords: FGF2, mesenchymal stem cells (MSCs), Osteogenic Differentiation.

SUN-505

Tumor stem cells markers for intracranial tumour evaluation

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Tumor stem cells (TSCs) involvement in brain tumorigenesis has gained field during the last years. The present study is focused on the markers involved in identifying TSCs; their molecular markers have shown differences between malignant/benign intracranial tumors, as well as future clinical application. Several markers that characterize the stem cells of intracranial tumors were identified in this study: CD133, NESTIN, C-kit, Sox-2, CD44, NOTCH. 80% of the malignant tumors and 35% of the benign ones have revealed positive expressions for at least one stem cell marker. The array of studied biomarkers revealed statistical differences for CD133, nestin and notch signaling between malignant and benign intracranial tumors. These markers have been correlated with angiogenic factors, leading to aggressiveness and invasiveness of these tumours. When associated with malignancy potential, they can be considered prognostic markers for intracranial tumor patients. The similarities and differences in the TSC involvement in benign/malignant intracranial tumors should be determined in the following few years. These cells may be responsible for intracranial tumor initiation and aggressiveness, either malignant or benign. Considering their complex pathophysiology and diversity, their decisive role in intracranial tumor evaluation is to be established in the following years.

This work is partially supported by Grants 09.33-03.10 and POSDRU 141531/2014.

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Keywords: intracranial tumors, markers, Stem cells.

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CSI-06 – Ubiquitination and Protein Turnover

SUN-507
20S proteasome gating, cellular chronological life span and resistance to oxidative stress

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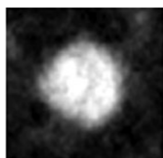
It has recently demonstrated that two Cys residues in the α5-subunit of the yeast 20S proteasome are post-translationally modified by S-glutathionylation when yeast cells grow to stationary phase into glucose-containing medium. Moreover, such modification implies on the opening of the 20S gate and on the increased degradation of oxidized model proteins, as studied in vitro. The set of data accumulated allowed hypothesizing S-glutathionylation as a redox regulation of proteasomal gating. In the present work it is shown that the mutation of those two Cys residues (α5-C76 and α5-C221) to Ser renders opposite 20S proteasome structures regarding their gating status. The closed conformation (Figure, left panel) prevails in the C76S mutant 20S particle when purified from cells grown to stationary phase into glucose-containing medium opposing the C221S mutant counterpart where the open conformation (Figure, right panel) represents 90% of the 20S pool. The cells carrying these mutated 20S forms present opposite phenotypes regarding oxidative stress response and chronological life span. Cells carrying the C76S mutant 20S core particle are either less resistant to oxidative stress or present reduced life span when compared to the wild type cells. Opposite results were obtained with cells carrying the C221S mutant proteasome. The rate of in vitro degradation of model proteins by the C221S-mutated 20S proteasome was twice that observed with the wild type counterpart. Our conclusion to date is that S-glutathionylation of the C221 residue, placed on the 20S surface, is actually a negative regulation of proteasomal gating while the C76 residue, highly conserved through yeast to human, is the true redox regulator of proteasomal gate opening. Most importantly, the difference of phenotype and gate conformation between cells carrying the mutated 20S proteasome together to the ability of the C221S-mutated form to degrade non-ubiquitinylated substrates, clearly demonstrate the importance of the 20S gate conformation on cellular response to redox imbalance. This work has been supported by the São Paulo Research Foundation (FAPESP) and the National Institutes of Science and Technology of Redox Processes in Biomedicine (FAPESP, CNPq, CAPES).



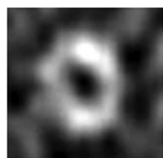


Fig. 1. Top-view of the 20S proteasome through Transmission Electron Microscopy.

Keywords: Oxidative stress, proteasome, redox signaling.

SUN-508
A new pathway regulating DNA replication and common fragile site stability: the RBBP6/ZBTB38/MCM10 axis

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The faithful replication of DNA is essential to maintain the integrity of the genome. Incomplete genome replication leads to DNA breaks and chromosomal rearrangements, which are causal factors in cancer and other human diseases. In spite of their importance, the molecular mechanisms controlling human genome stability are incompletely understood. Here we report a new pathway required for the replication and the stability of the human genome. This pathway has 3 components: an E3 ubiquitin ligase, a transcriptional repressor, and a replication protein. The E3 ubiquitin ligase, RBBP6, ubiquitinates and destabilizes the transcriptional repressor, ZBTB38. This repressor negatively regulates the transcription and the amount of the MCM10 replication factor on chromatin. Cells lacking RBBP6 experience reduced replication fork progression and increased damage at common fragile sites because of ZBTB38 accumulation and MCM10 down-regulation. Our results delineate a new pathway ensuring genome-wide DNA replication and chromosomal stability (Miotto et al, Cell Reports, in press).

Keywords: Replication, Transcription Repression, ubiquitination.

SUN-509
A novel pathway regulating CD20 level – influence of the inhibition of HDAC6 on the efficacy of anti-CD20 antibodies

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CD20, an integrate membrane protein expressed on the surface of normal and malignant B-cells is used as a molecular target for monoclonal antibodies (mAbs) in the therapy of non-Hodgkin's lymphomas and chronic lymphocytic leukemia (CLL). CD20 antigen has been reported to be regulated epigenetically e.g. by histone deacetylases (HDACs). The results of our preliminary experiments show that blocking the activity of a single HDAC isoform - HDAC6 with selective inhibitors (tubacin, ACY-1215 and tubastatin) leads to up-regulation of CD20 protein in B-cell lymphoma cell lines, EBV-transformed B-cells and primary cells from CLL patients but not in normal B-cells. We observed that HDAC6 silencing with shRNA also increases CD20 surface level. On the contrary, overexpression of HDAC6 does not induce changes in CD20 level. The observed up-regulation of CD20 level correlates with increased efficacy of anti-CD20 mAbs - rituximab and ofatumumab in complement-dependent cytotoxicity (CDC) assays. Neither tubacin nor HDAC6 silencing with shRNA does alter the expression of complement inhibitors. However, the increase in CD20 level does not influence antibody-dependent cellular cytotoxicity of NK cells.

In order to elucidate the mechanism by which HDAC6 inhibition increases CD20 level we performed experiments assessing HDAC6 inhibition on CD20 transcription. The results of qRT-PCR using SyBR Green and hydrolysis probes indicate that

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HDAC6 inhibition with tubacin and its silencing with shRNA does not alter CD20 mRNA. HDAC6 inhibition does not change CD20 promoter activity and leads to increase of CD20-tagged protein level expressed in Raji under CMV promoter. HDAC6 inhibition and silencing lead to increase in total CD20 protein level assessed in Western blotting.

Since HDAC6 is a unique member of HDAC family reported to be engaged mainly in the acetylation of non-histone substrates, protein degradation and transport we sought to determine HDAC6 role in CD20 protein transport. Using nocodazole we observed that the effect of HDAC6 on CD20 relies on stable microtubules. What is more, we observed that blocking protein anterograde protein transport with Golgistop abrogates tubacin effect. These results suggests that HDAC6 may be implicated in CD20 transport/degradation pathways.

The results of our study suggest that combining HDACi with anti-CD20 antibodies can be a successful modality for patients suffering from B-cell malignancies. The use of isoform-selective inhibitors may be an effective strategy in enhancing the efficacy of anti-CD20 mAbs. Potentially these compounds would have less adverse effects than HDAC pan-inhibitors. However, their use in the therapy requires further investigation.

Keywords: histone deacetylase, monoclonal antibodies, protein trafficking.

SUN-510
An original approach to identify E3 ubiquitin ligase substrates to be degraded

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Ubiquitin-mediated protein degradation is the major controlled proteolysis pathway in Eukaryotes that ensures that specific protein functions are turned off at the right time and in the right place. In this pathway, E3 ubiquitin ligases (E3s) are involved in the recruitment of specific substrates to be polyubiquitylated and subsequently degraded. Despite the identification of many E3s, the identities of their substrates remained largely unresolved.

In this work, we provided an original approach to identify substrates of E3s to be degraded. ASB2α is the specificity subunit of an E3 complex of the Cullin 5 RING E3 family that exerts its effects in hematopoietic cells through the targeting of specific substrates for degradation by the proteasome. Specifically, a global quantitative proteomic strategy applied to primary cells of wild-type and ASB2α knockout mice was used to identify new substrates of the ASB2α E3. Label-free quantitative proteomic analyses were performed to measure relative changes in protein abundance between primary hematopoietic cells isolated from ASB2-/- and ASB2 + /+ mice. Indeed, potential substrates of ASB2α correspond to proteins detected only in ASB2-/- cells but not in ASB2 + /+ cells or detected to a higher level in ASB2-/- cells compared to ASB2 + /+ cells. The robustness of this label-free quantitative proteomic approach was highlighted by a decrease of known ASB2 substrates, filamins A and B, in ASB2α-expressing cells.

Altogether, our results contribute to the understanding of the mechanisms of action of ASB2α, unravel new regulators of hematopoiesis in primary cells and will aid future studies aimed to the identification of novel E3 substrates.

Keywords: ubiquitin, E3 ubiquitin ligase, proteasome.

SUN-511
Bci is a new component of the autophagy pathway

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Autophagy, a mechanism for clearance of intracellular components in lysosomes, plays an essential role regulating and maintaining cellular homeostasis. Thus defects of this process contribute to the development of a series of pathologies including cancer and neurodegenerative diseases. Recently, a proteomic analysis of the autophagy interaction network (AIN) in human cells under conditions of basal autophagy, revealed a network of 751 interactions among 409 candidates, for many of those no function was assigned. Thus we decided to characterize the gene KIAA0226L that was initially described as a potential interactor of Beclin-1. This gene, here termed *bci* (Beclin1 interactor) has been previously studied only in the human cervical cancer context, as one of the most methylated genes present in human samples, suggesting a tumor suppressor function of the encoded protein in this tissue. In-silico analysis of the amino acid sequence of Bci showed several phosphorylation sites and a domain of unknown function (DUF4206). Bci expression exhibits a tissue-specific pattern with high expression levels in the brain and spinal cord. Moreover, Bci expression levels changed when autophagy is induced. Using immunoprecipitation experiments, we confirmed the binary interaction of Bci with Beclin1, a key regulator of autophagy. Furthermore, when Bci was overexpressed, we observed a decrease in LC3-II conversion, under nutrient deprivation as well as treatment with lysosome inhibitors, suggesting that BCI negatively regulates autophagy. In localization studies, we showed that Bci is localized to the Golgi apparatus, similar to other autophagy pathway components, such as Beclin1. Together, our data suggest that Bci is a potential new key regulator of autophagy.

Funding: Conicyt Ring Initiative ACT1109, Fondecyt Postdoctoral Fellowship 3140110.
Keywords: autophagy.

SUN-512
Bilirubin oxidase: enzyme of choice to biofuel cells

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Enzymatic miniature membrane-less glucose/O_2 Biofuel Cells (BFCs) are of particular interest because they may be used, in the near future, as *in-vivo* power source for implantable medical devices requiring low-power density. At the anode glucose oxidizing enzymes are used while at the cathode it is oxygen reducing enzymes such as bilirubin oxidases (BODs) or laccase. BODs will be preferred because unlike laccases they show high activity and stability in physiological conditions. Even tough, their performances are continuously improving, the power output and lifetime of BFCs are still not sufficient for direct applications, especially because the cathode is the limiting part of the cell.

BODs belong to the multi-copper oxidase family, catalyze the oxidation of bilirubin to biliverdin and reduces oxygen to water without producing toxic oxygen intermediates. The catalytic center consists of four copper atoms classified according to their

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optical and magnetic properties. They are of three types. The type I (TI) copper allows the transfer of electrons from the substrate to the trinuclear center (TCN), composed of a type II copper and the two type III copper, where the O_2 is reduced to water. We recently discovered two news BODs, from fungus (2) and bacteria (3). The use of these enzymes to the cathode of the glucose/O_2 biofuel cell is promising (2, 3, 4), because there are more tolerant to chloride ion and are more active in neutral pH than laccase, which is an enzyme extensively studied in this field of application.

By combining different analytical techniques (stopped-flow, circular dichroïsm,..) our work consist in characterizing these new enzymes and determine the rate-limiting step of the reactions, to be able to improve those enzymes for our applications.

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Keywords: Bilirubin Oxidase, Biofuel Cells, enzyme kinetics.

SUN-513
Biological consequences of intron 6 retention for LAT (linker for activation of T-cells) adaptor function

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Intron retention (IR) is a frequent phenomenon in the human transcriptome. Mostly it leads to a reduction in gene expression by triggering intron retaining mRNA degradation by the nonsense-mediated decay (NMD) pathway. However, number of mRNAs retaining short introns, especially in the open reading frame with adjacent exons have been shown to undergo productive expression. In the present study we assessed biological consequences of intron 6 retention within the human *LAT* gene. We found that intron 6 retaining *LAT* mRNA, here denoted as LAT_{i6}, undergoes productive expression but its level is approximately 20-fold lower than that of canonical *LAT* isoform. Since LAT is a key transmembrane adaptor protein controlling TCR signaling during development and activation of T cells, it seems crucial to understand if additional amino acids encoded by the retained intron 6 may affect LAT function. Western blotting analysis of human LAT_{i6} transduced lymphoid cells revealed that alternative LAT isoform can be translated into protein. Moreover it was targeted to the outer plasma membrane similarly to the canonical LAT isoform. Additionally, confocal microscopy of human lymphoid cell line transduced with C-terminal fusion proteins composed of LAT isoforms and a fluorescent protein (Zs-Green) showed a proper LAT accumulation in the immunological synapse area. Thus, seemingly intron 6 retention does not impair LAT function, however as revealed by the cycloheximide chase assay the half life of LATi6 isoform is significantly shorter (5 hours) in comparison with the canonical LAT isoform (8 hours). We propose that a possible consequence of an increased turnover of LATi6 protein is a more efficient proof reading control of T cell receptor signaling. We discuss how this evolutionary innovation may influence the immune response in various mammalian species expressing orthologous LATi6 proteins.

Keywords: intron retention, LAT, protein half-life.

SUN-514
Central domain of yeast transcription factor Rpn4 facilitates degradation of green fluorescent protein in human cells

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Background: Little is known about universal protein degradation signals. It was shown that mammalian ornithine decarboxylase (ODC) contains portable universal degradation signal (degron) which when fused with a stable protein induces fast proteasomal hydrolysis of chimera in both mammalian and yeast cells. No universal degrons from yeast, capable to stimulate protein degradation in mammalian cells are known.

The Rpn4 is 531 amino acid (aa) long yeast transcription factor with a short half-life. It contains ubiquitin-dependent and –independent degrons. We investigated degradation of the wild type yRpn4 in human cells and compared activity of its degradation signals with mammalian ODC degron.

Methods: First, eukaryotic vectors encoding green fluorescent protein (GFP), yRpn4 and GFP-ODCsignal chimera were obtained. Next, eight vectors were designed to express chimeras, containing ubiquitin-dependent and –independent degrons, as well as N-terminal and central domains of yRpn4 fused with either N- or C-terminus of the GFP. HEK 293T cells were transfected with the plasmids and gene expression was analyzed by Real-time PCR and Western blot (WB). Nuclear and cytoplasmic fractions of cells transfected with yRpn4 were examined for the protein localization. Proteasome inhibition assays with MG132/lactacystin and cycloheximide/puromycin chases were performed to evaluate stability and degradation kinetics of recombinant proteins, fluorescent microscopy and flow cytometry were used to confirm the obtained results. To affirm fast hydrolysis of yRpn4 in transfected cells accumulation kinetics of the recombinant protein in presence of proteasome inhibitor was examined. The yRpn4 accretion was compared with that of two fast and two slow degrading proteins.

Results: Fast proteasomal degradation of recombinant yRpn4 was shown in human HEK 293T cells and yRpn4 was detected in both nuclear and cytoplasmic fractions. N-terminal ubiquitin-independent and canonical ubiquitin-dependent degrons of yRpn4 did not induce proteasomal degradation of the yRpn4-GFP chimeras in mammalian cells. In contrast the central domain (aa 177–327) of yRpn4 effectively stimulated degradation of yRpn-GFP fusion protein (half-life 1–1.5 h); half-life of the GFP-ODCsignal was estimated to be 2–3 h.

Conclusions: Yeast transcription factor Rpn4 undergoes fast proteasomal degradation in human cells and its central domain contains a universal degron that is capable to destabilize GFP more efficiently than the ODC degron. Obtained results not only extend our knowledge about evolution of the degradation processes in micro and macro organisms, but also reveal a new universal degron, which can be used to direct highly efficient proteasomal degradation of required proteins in human cells.

Keywords: Degradation signal, Rpn4, Ubiquitin-proteasome system.

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SUN-515
Chaperone activity of monomeric human 14-3-3 Zeta protein on different model protein substrates

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14-3-3 proteins are ubiquitous, conservative and relatively small (~30 kDa) proteins found in many eukaryotes and have multiple diverse cellular functions. First of all, members of the family are known for their ability to bind hundreds of different protein partners specifically phosphorylated at certain motifs including phosphoserine or phosphothreonine. By this means 14-3-3s regulate stability, enzymatic activity, subcellular localization and many other properties of target phosphoproteins. 14-3-3 can form stable dimers and it has long been discussed that dimeric 14-3-3 can serve as adaptor or scaffold, bridging together two partners simultaneously. Moreover, recently, 14-3-3s were found as chaperones being able to dissolve pre-formed protein aggregates and to prevent aggregation of a range of partially denatured proteins, thereby participating in protein quality control. However, structural elements responsible for the chaperone action of 14-3-3 have not been identified and the question remained whether the dimeric structure is necessary for the chaperone activity of 14-3-3.

Our recent work was devoted to investigation of properties of an engineered mutant of 14-3-3ζ (14-3-3ζm) unable to form dimers and imitating 14-3-3 monomer, which may accumulate under unfavorable cellular conditions. 14-3-3ζm was properly folded, however demonstrated increased surface hydrophobicity at different temperatures, presumably, due to the exposed interface hydrophobic residues normally involved in 14-3-3 dimerization. Despite decreased thermostability and stability to proteolysis, 14-3-3ζm retained phosphosubstrate binding ability and therefore seemed to be functionally active. Moreover, we surprisingly found that it displays pronounced chaperone-like activity by preventing heat shock-induced aggregation of myosin subfragment 1 (S1), alcohol dehydrogenase (ADH), and phosphorylase kinase (PhK) and DTT-induced aggregation of insulin. Importantly, the effect of 14-3-3ζm was concentration-dependent and at all tested chaperone/substrate ratios 14-3-3ζm was more effective chaperone comparing with the wild type dimeric 14-3-3ζ. Moreover, in some cases chaperone-like activity of 14-3-3ζm was even higher than that of the specialized chaperone proteins HspB6 and HspB5. We believe that chaperone-like activity of 14-3-3 monomers can at least partially account for the general chaperone function of 14-3-3 and is presumably due to the hydrophobic clusters located near the 14-3-3 subunit interface that become exposed upon 14-3-3 dissociation, which could therefore be benefitful for the cell upon stress conditions.

Keywords: 14-3-3 proteins, anti-aggregating activity, small heat shock proteins.

SUN-516
Characterization of an E3 liagse, RING finger protein 182 (Rnf182) in the zebrafish embryos

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Ubiquitin proteasome system (UPS) largely associated with most common neurodegenerative conditions including Alzheimer's (AD) and Parkinson's disease (PD). Recent reports have shown, an E3 ligase RING finger protein 182 (RNF182) is a brain abundant cytoplasmic protein and upregulated in AD brains. Little is known about the RNF182 roles in vertebrate embryogenesis. We are reporting the expression and function of *rnf182* in zebrafish. Annotation of domain architectures found that Rnf182 is a RING-finger E3 ligase with a RING domain at N-terminal region together with two transmembrane domains at C-terminal region. WISH analysis detected that *rnf182* is expressed at the end of the gastrulation. Interestingly, and are abundant in the brain, pharyngeal arches, and lateral line system at 46 hpf through 98 hpf. Considering that Rnf182 has a RING finger domain, we ectopically expressed Rnf182 and performed ubiquitylation assays in HEK293T cells, demonstrating that Rnf182 undergoes self-polyubiquitylation. Furthermore, when Rnf182-R lacking the RING domain was overexpressed, it failed self-ubiquitylation of Rnf182, suggesting that Rnf182 might function as an E3 ubiquitin ligase via its RING domain. Overexpression of Rnf182 in zebrafish embryos caused defects in the developing CNS. We are currently generating a zebrafish germ line mutant using CRISPR-CAS9 system.

Keywords: RING, UPS, Zebrafish, Neurogenesis.

SUN-517
Convergence of ubiquitylation and phosphorylation signaling in rapamycin-treated yeast cells

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The target of rapamycin (TOR) kinase senses the availability of nutrients and coordinates cellular growth and proliferation with nutrient abundance. Inhibition of TOR mimics nutrient starvation and leads to the reorganization of many cellular processes, including autophagy, protein translation, and vesicle trafficking. TOR regulates cellular physiology by modulating phosphorylation and ubiquitylation signaling networks, however, the global scope of such regulation is not fully known. Here, we used mass spectrometry (MS)-based proteomics approach for the parallel quantification of ubiquitylation, phosphorylation, and proteome changes in rapamycin-treated yeast cells. Our data constitutes a detailed proteomic analysis of rapamycin-treated yeast with 3,590 proteins, 8,961 phosphorylation sites, and 2,498 di-Gly modified lysines (putative ubiquitylation sites) quantified. The phosphoproteome was extensively modulated by rapamycin treatment, with more than 900 up-regulated sites one hour after rapamycin treatment. Dynamically regulated phosphoproteins were involved in diverse cellular processes, prominently including transcription, membrane organization, vesicle-mediated transport, and autophagy. Several hundred ubiquitylation sites were increased after rapamycin treatment and about half as many decreased in abundance. We found that proteome, phosphorylation, and ubiquitylation changes converged on the Rsp5-ubiquitin ligase, Rsp5 adaptor proteins, and Rsp5 targets. Putative Rsp5 targets were biased for increased ubiquitylation, suggesting activation of Rsp5 by rapamycin. Rsp5 adaptor proteins, which recruit target proteins for Rsp5-dependent ubiquitylation, were biased for increased phosphorylation. Subcellular localization and stability of transmembrane permeases and transporters is known to be regulated by Rsp5. We found that permeases and transporters were biased for reduced ubiquitylation and reduced protein abundance. The convergence of multiple proteome-level changes on Rsp5 indicates a key role for Rsp5 in the response to rapamycin treatment. Collectively, these data reveal new insights into the global proteome dynamics in response to rapamycin treatment and provide a first detailed view of the co-regulation of phosphorylation and ubiquitylation-dependent signaling networks by this compound.

Keywords: mass spectrometry, PTMs, TOR.

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SUN-519
Endoplasmic reticulum-associated degradation controls the levels of amyloid precursor protein

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Alzheimer's disease (AD) is characterized by the overproduction of pathogenic amyloid-b peptide (Ab), which is generated by proteolytic cleavage of the b-amyloid precursor protein (APP). The action of b-secretase on APP produces a C-terminal fragment (C99) that is subsequently processed by g-secretase to release Ab. It has been proposed that substrate availability contributes to Ab production, and that this may in turn be affected by the rate of APP/C99 turnover. We have demonstrated that the degradation of C99 can be triggered at the ER in an ubiquitin- and proteasome-dependent manner. In this study we investigated the contribution of the ER-associated degradation (ERAD) machinery on APP/C99 endogenous levels. It is known that misfolded glycoproteins that fail to attain their correct conformation at the ER are recruited by the ER Degradation Enhancer Mannosidase alpha like 1 (EDEM1) from the calnexin/calreticulin folding cycle, and are directed to the dislocation channel for cytosolic degradation by ERAD. This last step relies on the hexameric ATPase p97/VCP, and on a small p97/VCP-interacting protein (SVIP) that functions as an inhibitor of the ERAD pathway. The aim of this study was to investigate the outcome of ERAD inhibition on APP and C99 levels by either stable expression of an EDEM1 shRNA, transient overexpression of SVIP, or pharmacological inhibition of p97/VCP. Our results support a model in which the levels of APP and C99 are highly controlled at the ER.
Funded by FONDECYT 1130929, Beca CONICYT 21110499 and DID-UACh.
Keywords: APP, ER, ERAD.

SUN-520
Enhanced proteasome degradation extends *Caenorhabditis elegans* lifespan and ameliorates neurodegeneration

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Aging and various age-related diseases are associated with progressive decline in proteostasis and accumulation of damaged macromolecules. The proteasome is the major cellular protease implicated in the disposal of normal and damaged proteins, having an impaired function during aging. In previous reports, using human primary cells, we demonstrated that proteasome activation through overexpression of proteasome subunits confers extension of replicative senescence and resistance to oxidative stress. Herein, we have investigated the impact of enhanced proteasome function on organismal longevity and aggregation-related pathologies by employing *Caenorhabditis elegans* as a model system. We have found that overexpression of a single core proteasome subunit in wild type worms enhanced proteasome content, assembly and function. The activation of the proteasome extended animal lifespan, healthspan and survival under proteotoxic conditions. The longevity prolonging effect of the ectopic expression of the core proteasome subunit was found to depend on the FOXO transcription factor DAF-16 and was associated with its elevated transcriptional activity. Finally, we have unveiled a major role of enhanced proteasome activity in aggregation-related pathologies underlying neurodegenerative diseases. Genetic activation of the proteasome minimized the detrimental effect of polyglutamine-induced toxicity, whereas knockdown of a key component of the proteasome exaggerated the disease phenotypes. Similar results were obtained by using a temperature inducible model of Amyloid beta (Aβ) –induced toxicity mimicking Alzheimer's disease. Collectively, our findings demonstrate that enhanced proteasome function alleviates proteotoxicity and promotes longevity in synergy with other key nodes of lifespan regulation in *C.elegans*. Understanding the mechanism by which preservation of proteostasis, via enhancement of proteasome function, decelerates the aging process and alleviates age-related pathologies may lead to new therapeutic and anti-aging interventions.
Keywords: aggregation-related pathologies, longevity, proteasome activation.

SUN-521
Expression of SUMO negatively regulates interferon signaling

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Interferons (IFNs) exhibit pleitropic functions including antiviral, growth inhibitory and apoptotic activities. IFNs activate the JAK/STAT pathways to trigger the transcription of IFN-inducible genes (ISGs) whose products mediate their biological functions. It has been established that ubiquitin or ubiquitin-like modifiers such as SUMO or ISG15 modify many ISGs or key regulators of IFN signaling. However, little is known on the role of SUMO in IFN signaling, cell regulation and antiviral response.

Here, we report that stable expression of each of the SUMO paralog in different human cell lines altered type II IFN signaling. SUMO1, SUMO2 or SUMO3 stable expression led to a lower STAT1 phosphorylation and binding to DNA in response to IFNγ, resulting in a selective inhibition of IFNγ-induced transcriptional activity. Indeed, the expression of SUMO did not affect the increase of IRF1 mRNA in response to IFNγ whereas it blocked IP10 mRNA expression. Importantly, enhanced SUMOylation reduced the capacity of IFNγ to inhibit cell growth and to protect cells from viral infection, a finding that implicates SUMO as a negative regulator of IFNγ responses.

Taken together, these results identify SUMOylation as a mechanism that attenuates cell sensitivity to IFNγ by decreasing STAT1 activation and its binding to DNA, preventing hyperresponsiveness to this cytokine, thus adding the SUMO-mediated inhibition as part of other negative IFN signaling pathways known to date.
Keywords: interferon, STAT1, SUMO.

SUN-522
Functional and structural studies of the BiP chaperon protein and his role in protein translocation

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BiP (Binding inmmunoglobulin protein) also known as Kar2p in yeast, is a Chaperon involved on protein translocation from the citoplasm to the endoplasmatic reticulum (ER), the first step on protein trafficking. BiP helps to the correct folding of various

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proteins and promote of destruction of missfolded ones. Moreover, BiP facilitates assembling of antibodies. BiP has two domains, one ATPase domain and a peptide binding domain that binds the polypeptide while is translocating (We know the polypeptide domain has a triptophan at position 662, although the crystal structure isn't complete). Until now, it's unclear if BiP uses the energy of ATP and transforms it in mechanical energy, in order to translocate polypeotide through the ER. We have developed a strategy to purified the BiP protein in only two steps, with very good yields and very pure for structural studies. The method consists by a nickel affinity column and subsequently an ATP-agarose column that binds specifically proteins that binds ATP, and after these two steps BIP is obtained pure. We follow the intrinsic fluorescence of tryptophan (W) at different concentrations of guanidinium chloride (GdHCl) and found that BiP shows an increase in tryptophan fluorescence and a red-shift of the maximum emission wavelength longitude as concentration of GdHCl have higher values. Therefore, these results imply that the tryptophan is buried within the protein structure and is a good probe to follow structural changes in this protein upon unfolding. Finally, we have developed an in vitro translocation assay with detergent soluble translocon and pure BiP in order to determine the translocation rates. This method will allow us to test the different components in order to determine how BiP uses the energy of ATP binding/hydrolysis to move a polypeptide chain through the Sec61 translocon complex at the single molecule level by optical tweezers. FONDECYT 11130263

Keywords: Bip protein, optical tweezers, translocation.

SUN-523
Functional roles of the lysine residues in the AXH domain of the ataxin-1 protein

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Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurodegenerative disease that leads to the loss of Purkinje neurons in the cerebellum. SCA1 is associated with expansion of a polyglutamine tract of ataxin-1 which is found predominantly in the nucleus and cytoplasm of neurons. Previously, we found that the E2-conjugating enzyme UbcH6 interacts with the AXH domain and that the AXH domain is preferentially ubiquitinated. Human AXH domain contains six lysine residues. We speculated that the lysine residues of AXH domain may play important roles in the interaction and ubiquitination. As a first step toward characterizing biological functions of the lysine residues, we employed the site-directed mutagenesis techniques to replace lysine residues with arginines. Co-IP and ubiquitination experiments showed that a lysine residue among six lysine residues is mostly ubiquitinated and is important for the interaction between AXH domain and UbcH6. Our study will contribute to elucidating roles of ataxin-1 in the SCA1 pathogenesis.

Keywords: Ataxin-1, AXH, SCA1.

SUN-524
Galectin-8 as a novel regulator of proteasomal activity

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Galectin-8 (Gal-8) is a lectin that regulates diverse cellular processes relevant to the function of the immune system and to diseases such as autoimmunity and cancer, in which the ubiquitin proteasomal system is also involved. Gal-8, like other galectins, is secreted through an unconventional mechanism. Secreted Gal-8 interacts with beta-galactosides (poly-N-acetyllactosamines) present on glycoproteins and glycolipids at the cell surface. The proteasome (20S) is a large protein complex that degrades cytosolic and nuclear proteins. The 20S proteasome binds to different regulators (activators), which affect its catalytic activity. The 19S regulator binds to the 20S proteasome forming the 26S proteasome, which degrades proteins conjugated with polyubiquitin chains. The 11S regulator attaches to the 20S proteasome forming a complex that promotes the production of antigenic peptides. Here we show that Gal-8 increases the activity of the proteasome, both when overexpressed or hexogenously added. Such an effect was inhibited by lactose, indicating its dependency on interactions with glycans at the cell surface, and was associated with an increment in the 19S proteasomal regulator, while the 11S regulator, as well as the proteasomal mass, both remained unchanged. These results are the first to show that a member of the galectin family regulates proteasomal activity. (Financed by CONICYT PFB12/2007, Fondecyt N° 1131122).

Keywords: galectin-8, proteasome.

SUN-525
GATA3 is a novel target for an E3 ligase SCF-Fbw7 in T-cell development

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We have studied cellular targets and functions of SCF-Fbw7, an SCF-type E3 ubiquitin ligase (thereafter Fbw7) and had reported that Fbw7 promotes ubiquitin-dependent degradation of c-Myb, a hematopoietic transcription factor (Kitagawa et al. ***Oncogene*** **28,** ***Cell Div.*** **5**). Moreover, it has been reported that Fbw7 targets other important cellular proteins such as c-Jun, Notch1, c-Myc, NFkB2 and MCL1. Here, we show that GATA3, one of GATA family transcription factor is a novel target for Fbw7 in T-cell development. Fbw7 bound to, ubiquitylated, and destabilized GATA3 using overexpressed proteins in cultured cells. Two Cdc4 phosphodegron (CPD) candidate sequences, consensus Fbw7 recognition domains, were identified in GATA3 and phosphorylation of Thr-156 in CPD was required for Fbw7-mediated ubiquitylation and degradation. Phosphorylation of Thr-156 GATA3 was detected in mouse thymocytes and CDK2 was identified as a respondent for phosphorylation at Thr-156. Moreover, we found that augmented GATA3 in CD4/CD8 double negative (DN) stage 4, CD4 single positive (SP), and CD8 SP lineages in Fbw7-deficient thymocytes. The conditional KO mice exhibited reduced thymic CD4 SP, and splenic $CD4^+$ and $CD8^+$ subcell proportions. Furthermore, the Fbw7 deficiency skewed CD8 SP lineage differentiation, which exhibited a higher incidence of apoptosis. Similar perturbations during development of CD8 positive cells were reported with transgenic mice, which enforced GATA3 expression throughout T-cell development (Nawijn et al. ***J Immunol.*** **167**). Therefore, our observations suggest Fbw7-mediated GATA3 regulation with CDK2-mediated phosphorylation of CPD contributes to the precise differentiation of T-cell lineages.

Keywords: ubiquitin, Fbw7, GATA3.

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SUN-526
ISG'ylation increases stability of hundreds proteins including STAT1 that prevents premature termination of immune responses in LPS stimulated microglia

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The initiation, progression and termination of brain inflammation requires changes in gene expression, posttranslational protein modifications and regulation of protein degradation. Microglia are brain resident macrophages which become activated in most neurological diseases. Inflammatory activation could be mimicked in primary microglial cultures by treatment with lipopolisacharide (LPS). We demonstrated the increase of Uba7 expression – an E1 enzyme that is crucial for ISG'ylation – posttranslational modification similar to ubiquitination in inflammatory microglia. ISG'ylation is an important part of antiviral response and inflammatory processes, but unlike ubiquitination, ISG'ylation do not lead to protein degradation and its function remains unclear. We found the increased level of ISG'ylation after LPS stimulation. Silencing of UBA7 expression in immortalized BV2 microglial cells led to decrease in a steady state level of hundreds proteins as demonstrated by mass spectrometry. many of these proteins have been described as ISG'ylated proteins in earlier studies. One of such proteins is transcription factor STAT1, which is also a main activator of UBA7 expression. We demonstrated that in microglia stimulated by LPS STAT1 is ISG'ylated and the levels of both total and phosphor- STAT1 decrease after UBA7 silencing. This is accompanied by premature termination of the immune response, as shown by the reduction of *iNos* and *Ccl5* expression. Those observations suggest that ISG'ylation increases stability of many proteins, including STAT1, that prevents premature termination of immune responses.
Keywords: Inflammation, ISG'ylation, STAT1.

SUN-527
Ki-1/57 SUMOylation and its function in the cell

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Ki-1/57 is a nuclear and cytoplasmic human protein that was first identified in malignant cells from Hodgkin's lymphoma. It is involved in gene expression regulation on both transcriptional and mRNA metabolism levels. Ki-1/57 belongs to the family of intrinsically unstructured proteins, undergoes phosphorylation by PKC and methylation by PRMT1. Previous characterization of protein interaction profile by yeast two-hybrid screening showed that Ki-1/57 interacts with proteins of the sumoylation machinery: the SUMO E2 ligase UBC9 and the SUMO E3 ligase PIAS3. We aimed to investigate whether Ki-1/57 is sumoylated and its possible roles in the cell. We have indentified seven potential SUMO target sites (lysines residues) on Ki-1/57 sequence through program SUMOplot Prediction. In fact, we have observed that Ki-1/57 is sumoylated by both SUMO-1 and SUMO-2/3 *in vitro* and *in vivo*. Through immunocytochemistry we have found that EGFP-Ki-1/57 co-localizes with SUMO-1 and SUMO-2/3 in both the nucleus and the cytoplasm of HEK293 cells. To identify which lysine residues are covalently attached to SUMO-1 and SUMO-2/3, site-directed mutagenesis of the sumoylation target lysines was performed. Ki-1/57-Flag wild-type and mutants were superexpressed in HEK293 cells, immunoprecipitated with the antibody anti-Flag and probed with anti-SUMO-1 or anti-SUMO-2/3. We have identified three lysines as the major sumoylation sites by immunoprecipitation. Moreover, we have found that Ki-1/57 sumoylation is important to the regulation of the E1A pre-mRNA processing. These results can help to better understand the mechanisms underlying the sumoylation of Ki-1/57 regulate the protein functions of the cells.
Keywords: Ki-1/57, post-translational modification, SUMOylation.

SUN-528
Low expression level of OB-Rb results from constitutive translocational attenuation attributable to less efficient signal sequence

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OB-Rb is a crucial factor for leptin signaling. This study was initially motivated by the observation that OB-Rb expression is constitutively inhibited at early secretory pathway. Our analyses reveal that OB-Rb contains less hydrophobic, but functionally active N-terminal signal sequence. Constitutive translocational attenuation attributable to less efficient signal sequence proved to be a reason for low protein level of OB-Rb. By contrast, enhanced signal sequence efficiency rescues translocation and cell surface expression of OB-Rb, and eventually potentiates leptin signaling. These observations provide considerable insight into the therapeutic enhancement of OB-Rb translocation as a potential strategy for leptin resistance.
Keywords: Leptin receptor, Signal sequence, Translocation.

SUN-529
Multivesicular body pathway-dependent amino acid recycling is required for metabolic homeostasis and survival during starvation

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The ESCRT machinery drives the multivesicular body (MVB) pathway in eukaryotic cells, which regulates cell signaling by selectively targeting ubiquitinated cell surface receptors for degradation in lysosomes. How ESCRT-dependent membrane proteome remodeling contributes to cellular homeostasis was unknown. Using an unbiased quantitative proteomics and transcriptomics approach in yeast, we show that the ESCRT pathway is essential to maintain free amino acid levels, particularly during nutrient limitation. Early during starvation membrane protein degradation via MVBs supplies amino acids for the efficient synthesis of vacuolar hydrolases, which is required to boost the catabolic activity of vacuoles. This is essential to enhance intracellular amino acid recycling further and thereby – together with autophagy – maintains protein synthesis and promotes the extensive proteome remodeling processes that allow cells to complete cell division and to enter quiescence. These findings reveal an important mechanism by which ESCRT-dependent membrane

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proteostasis maintains cell growth and survival during nutrient limitation.

Keywords: Amino acid recycling, Multivesicular body pathway, Starvation response.

SUN-530
Non-degradative and non-lysine mediated ubiquitination of the Dopamine D4 receptor

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G protein-coupled receptors are integrated in a complicated network of interacting proteins and are often regulated by post-translational modifications. Ubiquitination is a post-translational modification that targets proteins for degradation but can also regulate other cellular processes such as endocytosis, trafficking and DNA repair.

We are investigating the dopamine D_4 receptor (D_4R) signaling. Several polymorphic variants of the D_4R exist, which differ in the number of 16-amino acid repeats in the third intracellular loop. The functional role of the receptor polymorphic region is not known but persons with the seven-repeat allele show a predisposition to develop Attention Deficit Hyperactivity Disorder (ADHD) [1].

We have identified a protein, KLHL12, which specifically interacts with the polymorphic region [2] and enhances ubiquitination of all common D_4R variants but not of the D_4R with seven repeats. This differential ubiquitination can have functional implications. First, KLHL12-mediated D_4R ubiquitination does not lead to receptor degradation [3]. Next the influence of ubiquitination on receptor signaling was explored and a role in modulation of cAMP levels upon D_4R activation was found. To obtain a broader picture of the involvement of ubiquitination in receptor signaling, gene expression profiling using microarray technology will be performed.

In this study we are also testing the hypothesis that KLHL12 promotes ubiquitination on non-lysine residues of the D_4R. First, the ubiquitination pattern of the receptor with all intracellular Lys mutated to Arg was verified. Next, the importance of the Cys and Ser/Thr residues in the ubiquitination process of the receptor was examined. The obtained results suggest that Ser/Thr residues are the preferred ubiquitin-conjugation sites in case of KLHL12-mediated ubiquitination, but further confirmation by mass-spectrometry is necessary.

Acknowledgments: This work was supported by Research Foundation – Flanders (FWO).

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Keywords: dopamine D4 receptor, GPCR, ubiquitination.

SUN-531
Nuclear role of human endocytic adaptor intersectin

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Internalization of different molecules via clathrin-mediated endocytosis, their intracellular transport and cellular signalling events are intimately interwined. The mutual coregulation of endocytosis and mitogenic signalling enables finely tuned regulation of information processing within the cell, cell-dependent and context-dependent cellular responses.

More than a decade ago adaptor protein intersectin (ITSN) was discovered. It was named after «intersection» because of its predicted position on crossroads between signalling and endocytosis. ITSN is a pivotal regulator of clathrin-mediated endocytosis, enhancer of cell proliferation and survival. The project is focused on regulation of mitogenic signalling by ITSN. Recently endocytic proteins were shown to be transported to the nucleus; certain proteins from this cohort regulate gene expression.

Biochemical and cell biology studies demonstrated nucleocytoplasmic shuttling of ITSN. In the nucleus ITSN-positive signal with increased molecular weight was observed. We showed that this is due to monoubiquitination of ITSN. Monoubiquitinated form of ITSN is predominantly localized in nucleus, moreover recombinant fusion protein Ubiquitin-ITSN demonstrated nuclear localization. We have identified ubiquitin ligase AIP4 responsible for modification of ITSN.

We identified heterogeneous nuclear ribonuclear protein K (HNRNPK) as binding partner for ITSN. HNRNPK is involved in regulation of gene expression and transport of transcripts from the nucleus. First, we investigated whether ITSN could be associated with specific transcripts. Antibodies against ITSN precipitated transcripts of proto-oncogene Fyn. These transcripts are specifically bound with HNRNPK to be transported in the cell. Thus, for the first time we showed that endocytic protein could be bound to the transcripts.

To investigate whether ITSN can affect level of expression of HNRNPK targets, nuclear-targeted variant of ITSN was obtained. A set of genes regulated by HNRNPK crucial for cellular proliferation and survival was screened for changes in the level of expression. Overexpression of wild-type ITSN and its nuclear-targeted variant enhanced expression of p53 and c-Myc genes. Notably, both forms of ITSN, wild-type and nuclear-targeted, affected expression levels of mentioned genes. Thus, ITSN may contribute to signalling events in the cytosolic and nuclear parts of signalling cascades. Moreover, nuclear localization of ITSN had significantly higher impact on the level of expression of c-Myc proto-oncogen than wild-type one.

Keywords: adaptor protein, transcription, ubiquitination.

SUN-532
Phosphorylation of Ser8 promotes zinc-induced dimerization of amyloid-β metal-binding domain

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Zinc-induced aggregation of the amyloid-b peptide (Ab) is a hallmark molecular feature of Alzheimer's disease (AD). Recently it was shown that phosphorylation of Aβ at Ser8 promotes the for-

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mation of toxic aggregates. In this work, we have studied the impact of Ser8 phosphorylation on the mode of zinc interaction with the Ab metal-binding domain 1–16 using isothermal titration calorimetry, electrospray ionization mass spectrometry and NMR spectroscopy. We have discovered a novel zinc binding site (^{6}HDpS^{8}) in the phosphorylated peptide, in which the zinc ion is coordinated by imidazole ring of His6, phosphate group attached to Ser8 and a backbone carbonyl group of His6 or Asp7. Interaction of zinc ion with this site involves His6, thereby withdrawing it from the interaction pattern observed in the non-modified peptide. This event was found to stimulate dimerization of peptide chains through the ^{11}EVHH^{14} site, where the zinc ion is coordinated by the two pairs of Glu11 and His14 in the two peptide subunits. The proposed molecular mechanism of zinc-induced dimerization could contribute to the understanding of initiation of pathological Aβ aggregation, and the ^{11}EVHH^{14} tetrapeptide can be considered as a promising drug target for the prevention of amyloidogenesis.

Keywords: Alzheimer's disease, Beta amyloid, Phosphorylation.

SUN-533
Pin1 modulates the ADP-induced migration of human dental pulp cells through P2Y1 stabilization

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The present study was aimed to investigate the importance of Pin1 expression in human dental pulp cells (hDPCs) and to understand the possible involvement of Pin1 in the regulation of P2Y1 and the subsequent activation of ADP-mediated P2Y1 signaling. Here, we show, by Pin1 pharmacological inhibition and by using small interfering RNAs, Pin1 is involved in the P2Y1-mediated hDPC migration. In addition, Pin1-P2Y1 mediated migration is associated with the mitogen-activated protein kinase pathways as evidenced by inhibitors of ERK1/2, JNK, and p38 kinase. Functionally, Pin1 directly binds and stabilizes P2Y1 and high levels of Pin1 sustain P2Y1 signaling, which correlates with cell migration. These results strongly suggest Pin1 mediates cell migration through the P2Y1 stabilization and Pin1-P2Y1 signaling pathways may serve as a novel mechanism for the cell migration progression in human dental pulp cells (This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government MSIP (No.2008-0062283).

Keywords: human dental pulp cells, P2Y1, Pin1.

SUN-534
Pupylation and proteasomal degradation in mycobacteria

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Mycobacteria encode a proteasome assembly restricted to the actinobacterial phylum. Proteins are targeted for degradation by this proteasome complex through a post-translational modification termed pupylation that shows functional analogies to ubiquitination in eukaryotes (for review see 1–3). For the human pathogen *Mycobacterium tuberculosis*, the Pup proteasome system contributes to pathogenicity by supporting the bacterium's persistence within host macrophages, rendering it a feasible drug target.

Pupylation involves the covalent attachment of a small protein termed Pup (prokaryotic ubiquitin-like protein) to lysine residues of the target substrate. A single ligase termed PafA

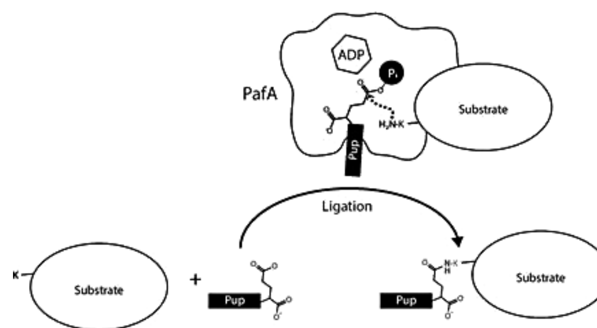

Fig. 1.

(proteasome-accessory factor A) is responsible for the formation of the isopeptide bond. PafA catalyzes the covalent attachment of Pup's C-terminal glutamate residue to the side chain of the target lysine. We are studying the Pup-proteasome system by biochemical and structural techniques with a particular focus on how the intrinsically disordered Pup is recognized by the pupylation enzymes and how target proteins are selected for modification and degradation.

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Keywords: Mycobacteria, Prokaryotic Ubiquitin-like Protein, Proteasome.

SUN-535
Quantitative proteomics and phosphoproteomics of human LRRK2 (R1441C) *Drosophila* model of Parkinson's disease

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Parkinson's disease (PD), occurring in late mid-life, is the second most prevalent neurodegenerative disorder after Alzheimer's disease. *Drosophila melanogaster*, the fruit fly, is already an accepted PD model and has been investigated vastly in the last couple of years. Stable isotope labeling of amino acids in cell culture (SILAC) is a widely used powerful tool for the quantification of proteomics based on mass spectrometry and recently SILAC was also extended for labeling of living animal including mouse, zebra fish and fruit flies. In this study we showed a quantitative proteomics study of human LRRK2-R1441C overexpressing transgenic *Drosophila melanogaster* as a PD model at different disease stages. In total 3570 proteins were identified from fly head and 1753 proteins were quantified. Overall 183 (>10%) proteins were found to be significantly regulated at 30 days old flies. Up-regulated proteins are mostly associated with cytoskeleton and mitochondrion that may presage the development of PD symptoms. In the fraction of down-regulated proteins, we found protiens linking with catecholamine synthesis which determined the selective vulnerability to hLRRK2 (R1441C). Protein phosphorylation

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is a crucial regulatory event for most biological process and since LRRK2 has a kinase activity, we performed phosphoproteomics experiment in order to have a deeper understanding about signal transduction pathways during PD development in *Drosophila*. In total, 5518 phosphorylated sites were identified and among the significant candidates, we quantified Futsch which was already known to be phosphorylated by LRRK2. As a summary, this study provides dynamic and temporal proteomic and phosphoproteomics changes due to over-expressed human LRRK2 (R1441C) in *Drosophila melanogaster* as a PD model that may shed light on the fundamental etiology of PD.

Keywords: Drosophila melanogaster, Mass Spectroscopy, Parkinson Disease.

SUN-536
Relevance of disulfide bridge upon IFNAR1 flexibility

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Members of the Type I interferon acts through a single cell surface receptor composing of IFNAR1 and IFNAR2 subunit inducing measurable antiviral and cell growth inhibition. Since last two decades many research groups have been trying to dissect the mechanism of how the members of the type I interferon which has 90% sequence similarity bind with the same receptor resulting in the inhibition of different activities. Recently we solved the structure of the bound receptor complex and its unbound component, showing significant structural arrangements upon ligand binding.

Here we propose to investigate the relationship between receptor flexibility, domain movement and signalling upon interferon binding. In order to restrict the receptor flexibility and domain movement we introduce single disulfide bridge between different extracellular domains of IFNAR1 ie SD1, SD2, SD3 and SD4. Corresponding proteins with N349C mutation were produced using the baculovirus expression system. The binding affinities of type I interferon mutant (YNS) toward the IFNAR1-EC mutants were measure by Surface Plasma Resonance. All SD12 and SD23 mutants exhibit lower affinity when compared to the SD34, which is mainly due to slower dissociation. At present we are in process to label the IFNAR-EC mutants with ATTO655 in order to study the conformational flexibility of receptor upon interferon binding using FRET or florescence quenching.

In order to analyse change in signalling pathway and gene induction upon interferon stimulus due to different disulphide bonds we examine the interferon stimulating genes using realtime qPCR. Differential up-regulation is clearly observed for the IFI6 and MX1 which are antiviral related, while TRAIL and CXCL11 maintain almost basal expression level. Additions to single additional disulfide bond we have HUH7 stable cell line expressing multiple additional disulfide bonds on same IFNAR1 to further rigidify the receptor.

Keywords: Interferon receptor, protein Protein interaction.

SUN-537
Relevance of disulfide bridge upon IFNAR1 flexibility

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Keywords: Interferon receptor, Protein - protein interactions.

SUN-538
Structure of the DDB1-CRBN E3 ubiquitin ligase in complex with thalidomide: insights into CRL4 inhibition by small molecules

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In the 1950s the drug thalidomide administered as a mild sedative to pregnant women led to the birth of thousands of children with multiple defects. Despite its teratogenicity, thalidomide and its derivatives lenalidomide and pomalidomide (together known as Immunomodulatory Drugs: IMiDs) recently emerged as effective treatments for multiple myeloma and 5q-dysplasia. While the discovery of the $CRL4^{CRBN}$ ubiquitin ligase as the primary cellular target of thalidomide and its lenalidomide mediated ubiquitination of Ikaros/Aiolos transcription factors has provided a conceptual framework for understanding drug action, a detailed molecular understanding remains elusive. We will present (i) the crystal structure of the DDB1-CRBN complex bound to thalidomide, lenalidomide and pomalidomide. Our structures establish CRBN as a $CRL4^{CRBN}$ substrate receptor, which enantioselectively binds IMiDs. (ii) We further provide evidence that the COP9 Signalosome (CSN) mediates the inhibitory effects of thalidomide. (iii) Using an unbiased biochemical screen we identify the homeobox transcription factor MEIS2 as an endogenous substrate of $CRL4^{CRBN}$. We find that IMiDs block endogenous substrates (e.g. MEIS2) from binding to $CRL4^{CRBN}$ when recruiting Ikaros/Aiolos for degradation. This dual activity implies that small molecules can principally modulate a ligase to up- or down-regulate the ubiquitination of proteins. We expect these insights into the molecular workings of thalidomide and its

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derivatives to be exploitable in the development of new classes of targeted CRL ligase inhibitors.

Keywords: CANCER, structural biology, ubiquitin.

SUN-539

Study of the role of the proteasome in senescence of stem cells Marianna Kapetanou, Niki Chondrogianni and Stathis Gonos

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Ageing is an intricate phenomenon which remains a major challenge to modern biology. It can be defined as a multifactorial process leading to a gradual decline of self-defensive mechanisms, reduced regenerative capacity of all tissues and organs and an exponential accumulation of damage at the molecular, cellular and organismal level. Stem cells are responsible for tissue renewal and loss of their stemness may contribute to the physiological decline in tissue homeostasis during ageing. The proteasome, being the main cellular proteolytic system, plays a key role in maintenance of cellular homeostasis and its alterations are associated with various biological phenomena including cellular senescence and ageing. However, little is known regarding the role of proteasome and other antioxidant responses in senescence of stem cells. In order to shed light on the limited data on stem cell ageing, we employed both Wharton-jelly and Adipose derived adult mesenchymal stem cells. We observed a significant decrease in proteasomal peptidase activities accompanied by a reduction of proteasomal subunits and alterations of proteasomal complexes in senescent hMSCs. Simultaneously, a senescence-related increase in levels of oxidatively modified proteins was detected. Additionally we assessed the expression levels of some well-know stemness markers during the ageing process of MSCs. Analysis of antioxidant defense mechanisms will open the road to innovative stem cell-based interventions to improve the quality of human life in old age ('healthspan'), including treatment of late-onset diseases.

Keywords: ageing, proteasome, stem cells.

SUN-540

The delineation of the role of ATP13A2, a P5 type ATPase with unknown function in melanoma

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In the last decade, melanoma has become a global issue due to its aggressive progression, as well as the poorest prognosis of all metastatic cancers. The accumulation of many epigenetic or genetic changes results in intrinsic and/or extrinsic signaling events culminating in the malignant transformation of melanocytes. Additionally, the poor prognosis of melanoma is a consequence of its highly metastatic and aggressive potential and notorious resistance to current therapies. Likewise, the limits and undesired side effects of current cancer therapeutics create a great interest to investigate and develop new targets.

ATP13A2 is a lysosomal/late endosomal P5-type ATPase with undefined function. Recent research however demonstrates an important role for ATP13A2 in efficient lysosome and autophagy functioning, although the molecular mechanisms by which ATP13A2 exerts these effects are still elusive. Loss of function effects of mutations in ATP13A2 underlie the Kufor-Rakeb syndrome, a form of autosomal Parkinsonism, and several studies have pointed out a relevant protective role of ATP13A2 in the suppression of α-synuclein toxicity. Intriguingly, epidemiological studies have reported co-occurrence of melanoma and Parkinson's disease, suggesting shared common genetic components. Of note α-synuclein, a Parkinson's disease-related protein is expressed in both malignant and benign melanocytic lesions. Interestingly according to CCLE database, ATP13A2 is expressed highly in melanoma cell lines as compared to any other cancer cell lines.

The purpose of this study is to delineate the putative role of ATP13A2 in melanoma cell, under homeostatic basal and stress conditions. Preliminary data shows that human melanoma (A375p) cell lines overexpressing either wild type or a catalytically dead ATP13A2 mutant display difference in autophagic flux following loss of proteostasis, induced by the proteasomal inhibitor bortezomib. Moreover, our data show that ATP13A2 over expression reduces the accumulation of ubiquitinated proteins following bortezomib treatment, whereas ATP13A2 silencing increases it. To determine if the reduced amount of ubiquitinated proteins was a consequence of changes in endosome-exosome trafficking, we tested the total protein secretion carried by exosomes in these melanoma cells. Initial results show a reduction in the release of exosomal proteins when ATP13A2 is overexpressed and heightened in the absence of ATP13A2. Moreover, preliminary data points to an increase in the number of lysosomes in ATP13A2 overexpressed cells but a reduction in number of lysosomes in ATP13A2-silenced cells. Taken together these preliminary results suggest an important role of ATP13A2 in the maintenance of cellular proteostasis and endosomal-lysosomal extracellular trafficking.

Keywords: autophagy, exosome, Ubiquitin-proteasome system.

SUN-541

The highly flexible and heterogeneous nature of E1A from human Adenovirus (HAdV) characterized at atomic resolution through NMR

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The small DNA tumor viruses encode some of the most versatile hub proteins like the E1A protein from human Adenovirus (HAdV). The E1A protein is essential for productive viral infection in human cells and a vast amount of data are available on its interactions with host proteins. Up to now no high-resolution information on the full-length E1A protein is available despite its important biological role.

Here we present the NMR characterization of the entire 243 residue long 12S isoform of the E1A protein from HAdV (E1A-12S). The protein results very heterogeneous in terms of structural and dynamic properties with highly flexible modules. This study opens the way to characterize the many interactions in which this protein is involved.

Keywords: IDP, NMR.

SUN-542

Towards the deciphering of LBSL causing mutations impacts on mitochondrial Aspartyl-tRNA synthetase properties, in a cellular environment

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Mammalian mitochondria (mt) have their own translation machinery, composed of RNAs mainly encoded by the mt gen-

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ome and of proteins coded by the nuclear genome. Among the nuclear-encoded and imported proteins of this machinery, aminoacyl-tRNA synthetases (mt-aaRSs) play a crucial role by charging amino acids on their cognate tRNA.

In the last years, increasing number of mutations affecting mt-aaRSs and leading to severe disorders in human has been reported (as reviewed in [1]). The link between mutations and disorders is, however, not so obvious. Several of these mutations affect mt-Aspartyl-tRNA synthetase (mt-AspRS) and cause LBSL (Leukoencephalopathy with Brainstem and Spinal cord involvement and Lactate elevation) disease. Intriguingly, pathogenic-related mutations of mt-AspRS have neither the same onset and progression nor the same seriousness on patients, witnessing of mosaic effects [2]. We aim at understanding the differences between mutations impacts at the molecular and cellular levels, by investigating a large panel of mt-AspRS properties.

In the present work, we adapted and validated an engineered mammalian expression system that leans on the controlled-expression of recombinant proteins (developed and published earlier in the lab [3]), combined with mitochondrial purification and fractionation, and other tools in order to study mt-AspRS and define its sub-mitochondrial organization. Altogether, this optimized technique will allow us to place mt-AspRS (and other mt-aaRS) mutants in their cellular environment, and to have a more precise overview of their impacts.

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Keywords: aminoacyl-tRNA synthetase, mitochondria, mutants.

SUN-543
TXNIP increase p53 stability and activity through interaction with hEcd

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The p53 protein plays a central role in cell cycle arrest and apoptosis in response to diverse stress stimuli. Human ecdysoneless (hEcd) is known for its role in stabilizing the p53 protein level and increasing p53-mediated transcription. Here, we report that thioredoxin interacting protein (TXNIP), a member of the tumor suppressor family, interacts with hEcd and decreases murine double minute-2 (MDM2)-mediated p53 ubiquitination, leading to p53 stabilization and an increase in p53 activity. The ectopic overexpression of both TXNIP and Ecd increased actinomycin D-mediated cell death in MCF-7 cells, whereas knockdown of TXNIP and Ecd decreased cell death. These results show that TXNIP is a new regulator of the Ecd-MDM2-p53 loop

Keywords: None.

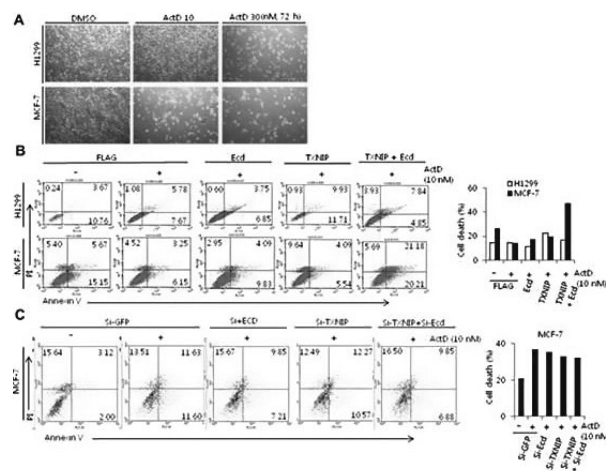

Fig. 1.

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CSII-01 – Chromosomal Structure, Centromeres & Telomeres

MON-001
A view to montelukast drug products in terms of impurity regulations: impurities analysis in drug products and in silico/in vitro genotoxicological assessment of sulfoxide impurity

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During manufacturing and storage of drugs that include montelukast, various impurities -particularly genotoxic impurities- which affect safety, efficacy and quality of drugs, can form. The montelukast from leukotrien antagonists is commonly used for control of asthma on adults and children. Probable chronic impurity exposures should be scrutinized thoroughly to avoid unnecessary exposures, particularly in children. This study aims analysis of impurities in montelukast drug products and their in silico and in vitro toxicological assessments considering regulatory aspects.

Quantitation of impurities (sulfoxide, cis-isomer, Michael adducts I&II, methylketone, methylstyrene) was conducted using reverse phase HPLC analysis on pediatric and adult drug products on market. Validation of analytical method was conducted according to ICH guideline. For sulfoxide impurity that was above qualification limits, prediction analysis in in silico QSAR method and bacterial gene mutation test were performed. The cytotoxicity and genotoxicity were investigated respectively by determining mitotic index and using in vitro chromosomal aberration (CA) test.

Impurities except sulfoxide were under qualification limits in the analysis of different batches of 20 products at 4 mg/10 mg doses for 11 companies. The sulfoxide exceeds limits in 2 companies for pediatric chewable tablets and in 7 companies for adult tablets. Sulfoxide impurity was not mutagenic in Leadscope QSAR analysis and bacterial gene mutation assay (Ames MPF Penta I) in Salmonella typhimurium TA98, TA100, TA1535, TA1537, Escherica coli wp2[pKM101] and wp2uvrA with/without metabolic activation system. Sulfoxide impurity was dose-dependent cytotoxic in human peripheral lymphocytes with/without metabolic activation system, however, it was nongenotoxic in CA test.

These results indicate that sulfoxide impurity should be considered a nonmutagenic/nongenotoxic and to be classified as an ordinary impurity according to guidelines.

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Keywords: drug impurities, genotoxicity, qsar analysis.

MON-002
Dicentric chromosomes breakage is coupled to cytokinesis

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Most eukaryotes contain a single centromere per chromosome ensuring accurate chromosome segregation. Dicentric chromosomes, holding two centromeres, are abnormal structures resulting from erroneous genomic rearrangements (e.g. accidental telomere fusion). Dicentrics are inherently unstable and often undergo breakage when the two centromeres of the same chromatid are pulled towards opposite poles during mitosis. The mechanism by which breakage occurs and the precise moment in the cell cycle when it takes place remain unclear.

Our goal is to elucidate, at the molecular level and using budding yeast as a model system, the mechanism of dicentric chromosome breakage. The mechanism we will decipher will give valuable clues about this issue in all eukaryotes and how cells maintain genome stability. For this purpose, we have optimized a tool to stabilize and select individual dicentrics. A chromosome with a conditional centromere under the control of two galactose-inducible promoters is fused to another native chromosome by telomere fusion or recombination-induced rearrangements. This structural dicentric chromosome behaves as a functional monocentric when the conditional centromere is inactivated. On glucose, the centromere is rapidly reactivated, thus allowing the study of dicentric breakage during a single mitosis in synchronized populations of cells.

Previous work carried out in our laboratory on dicentrics with telomere fusion showed that they often break at the fusion (Pobiega and Marcand 2010, Genes & Dev). Recently, we have found that dicentrics created by recombination-induced rearrangements tend to break at pericentric regions. In both cases, breakage requires anaphase exit indicating that mitotic spindle tension is not enough to account for breakage. In cells released from anaphase, breakage occurs at a time coincident with cytokinesis. In yeast, cytokinesis comprises a set of sequential and interdependent processes: actomyosin ring contraction, plasma membrane ingression, and primary and secondary septa deposition. Actin is a structural component of the ring and is also required for the other steps through cargo vesicles delivery. We have observed that in the presence of Latrunculin A, a drug inhibiting actin polymerization, dicentric breakage is abolished. Depletion of the ring myosin Myo1, via an auxin-inducible degron, has the same effect. Both results indicate that dicentric breakage is coupled to cytokinesis. Breakage could be generated enzymatically through a nuclease activated by cytokinesis. We decided to look for dicentric breakage in cells defective for known nucleases. So far, we have tested eight nucleases and breakage still occurs. Currently, we keep on testing other candidate activities.

Keywords: centromere, cytokinesis, dicentric.

MON-003
Fluorescent probes for imaging repeated DNA sequences: synthesis of fluorescent polyamide minor groove binders and studies of their interaction with double-stranded DNA in vitro and in fixed and living cells

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Hairpin N-methylpyrrole/N-methylimidazole polyamides are able to penetrate into living cells and to bind a specific sequence in the minor groove of the native dsDNA. Nine hairpin polyamides and two antiparallel tandem *bis*-polyamides, targeting mouse pericentromere repeated sequences, were synthesized by manual solid-phase synthesis and click chemistry. After labeling by fluorophores

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these ligands can be used as probes for fluorescence microscopy imaging of repetitive genomic DNA regions (centromeres, telomeres) in living cells. The polyamides were purified and characterized by HPLC, UV-visible spectroscopy and mass-spectrometry. Interaction of polyamides with the murine pericentromere dsDNA fragment has been studied *in vitro* by the gel shift electrophoresis, thermal denaturation, circular dichroism and fluorescent spectroscopy. Our results reveal different binding affinity of various polyamides for the target DNA. These differences depend on the target sequence, position of the ligand on the target sequence, the number and position of N-methylimidazole units, the nature of conjugated fluorophores and the presence of ß-alanine residues replacing N-methylpyrrole carboxamides. Thus, the correspondence of the polyamide structure to recognition/binding rules is necessary but insufficient condition for construction of the probes. Fluorescent labeling of the ligands by fluorescein or cyanine fluorophores also affects their DNA binding. Each probe has to be studied individually in order to determine its suitability for cellular imaging applications.

The probes labeled by cyanine fluorophores demonstrate specific increase of fluorescence intensity upon interacting with the target DNA. FRET is observed when two polyamides (one labeled by Cy3 and second by Cy5) bind to neighboring DNA sequences.

The proof of the concept has been done in the fixed mouse cells where these probes specifically mark chromocenters. They are suitable for the FISH-like DNA imaging. They are able to penetrate inside living mouse and human cells, however, the specific staining of DNA chromocenters in the nucleus is observed only in several cases depending on the polyamide structure and fluorophore or linker nature.

Keywords: DNA imaging, Fluorescent probe, Minor groove binder.

MON-004
Genome size and chromosome number in some Centaurea (Asteraceae) from Croatia

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Croatian Adriatic coast and islands are unique and distinctive area, thanks to its general features, unusual relief features, climate, complicated geological history and the relatively good preservation. Five world areas characterized by a Mediterranean climate, make 2% of the world area which manage 20% of all known plant species, European Mediterranean with 10% of all known species in only 1.6% of the world surface.

The genus *Centaurea* is one of the largest in the Asteraceae family, containing at least 400 species (even 700). East Anatolia and Transcaucasia are the primary center of the origin and diversification of the genus *Centaurea*, while the Mediterranean area, including the Balkan Peninsula, is the second one. In Croatia 79 *Centaurea* taxa have been listed, comprising 27 endemics.

Our preliminary chemical and biological observations, of extracts and volatiles isolated from few endemic species from *Centaurea* genus, from Adriatic region, showed that there are differences in chemical composition and biological activity of volatile substances between different localities of the same species. In order to determine whether these differences were generated from different ecological conditions or because of different genetic structure, cytogenetic analysis of selected plant species of *Centaurea* taxa from Croatia were made.

The plant samples, leaves and seeds, were collected from natural populations of Croatia. The studied species belong to 7 different sections and present four different basic chromosome numbers, x = 8, 9, 10 and 11. Cytogenetic analysis was performed on a sampling covering 28 populations of 9 *Centaurea* species among which 4 endemic for Croatia or Croatia and Italy. Flow cytometry method, according to Marie and Brown (1993) were used to asses the total DNA amount.

The ploidy levels, dyploid and tetraploid, were detected. The nuclear DNA amounts (2C DNA), assessed by flow cytometry, range from 1.63 to 3.61 pg for diploid and to 6.86 pg when including tetraploid species. Intraspecific genome size polymorphism was observed among populations of five out of nine studied species: *C. dalmatica, C. ragusina, C. rupestris, C. spinosociliata and C. salonitana*. The genome size of a tetraploid *C. salonitana* populations were nearly twice that those of the diploids.

Dysploidy, changement of basic chromosome number, and polyploidy are observed as the main evolutionary mechanisms among studied representatives of the genus *Centaurea* in Croatia.

Keywords: flow cytometry, genome size, polyploidy.

MON-005
hTR (hTERC) and Tankyrase-1 mRNA expression in a human primary fibroblast-like stem cell line culture after treating with hydrogen peroxide

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Introduction: hTR is a telomerase RNA component and together with hTERT (protein component of telomerase) maintains telomere ends by addition of the telomere repeat TTAGGG. Telomeres are highly sensitive to damage by oxidative stress due to their high content of guanines, and H_2O_2 shorter telomeres length. Previously it was shown that hTERT overexpression not only reduces the basal cellular reactive oxygen species (ROS) levels but also inhibits endogenous ROS production in response to stimuli that induce intracellular ROS generation. Tankyrase-1 is involved in regulation of DNA damage responses at telomeres and it can act as a positive telomere length regulator in telomerase positive cells.

The aims of this study were to detect if hTR expression is affected by incensement of exogenous H_2O_2 as ROS agent, and if it also influences Tankyrase-1 expression in the cells.

Methods: For the study a human primary fibroblast-like stem cell line (ASD-1) was used. Cells were treated with 100 μM H_2O_2 for 30 min at 37 °C. After treatment a part of a treated (H_2O_2) passage was harvested and the remaining part was incubated with H_2O_2, a half of the last part was used for the next passage. During all experiments controls (cell without H_2O_2 treatment) were included. For gene expression detection and telomere length measuring the real-time qPCR assay was used.

Results: hTR expression profile showed that after the treatment with H_2O_2 the hTR mRNA expression was elevated in comparison to control cells. The treated (H_2O_2) passage line harvested before the treatment of H_2O_2 exhibited less hTR mRNA expression in comparison with the same passage after the treatment with H_2O_2. In contrast, very small gradual decrease of Tankyrase-1 mRNA expression was observed during passaging. The treated (H_2O_2) passage line harvested before treatment of H_2O_2 showed less Tankyrase-1 mRNA expression in comparison with

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the same passage after the treatment with H_2O_2. Changes of telomere length during passaging did not demonstrate any tendency of elongation or shortening.

Discussion: The study shows that H_2O_2 affects hTR expression by increasing it on the response to exogenous ROS but does not increase expression of Tankyrase-1 showing that potential DNA strand breaks do not stimulate Tankyrase-1 expression. ROS rather reduces it for small amount. In conclusion it is possible that hTR is expressed as potential struggle against stem cells death.

Keywords: hTR (hTERC), Tankyrase-1, telomeres, mRNA expression.

MON-006
Identify the topoisomerase II isozyme-specific targeting agents and investigate their biological responses

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Topoisomerase II (TOP2) plays crucial roles in cells and TOP2-targeting drugs are effective anticancer drugs but with side-effects. There are two types of TOP2-targeting agents: (i) Poisons stabilize TOP2 cleavable complex (TOP2 cc) and induce DNA break; (ii) Inhibitors only interfere with catalytic activity and antagonize the poisoning action. In human cells, the alpha (α) and beta (β) isozymes share similar enzymatic action but play differential functions. Etoposide (VP-16), an active TOP2 poison, induces 2nd malignancies. Notably, TOP2β is mainly responsible for the VP-16-induced DNA sequence rearrangement and carcinogenesis as well as the doxorubicin-induced cardiotoxicity. We screened anthracenedione derivatives and identified potential TOP2 isozyme-specific poisons and/or inhibitors. First, we used comet assay to detect DNA breakage and found anthracenedione derivatives didn't significantly induce DNA damage responses. The inhibitory ability of TOP2 activity was assessed by an *in vitro* relaxation assay. We then selected compound CL-14 that preferentially inhibits TOP2β relaxation activity for further study and found that CL-14 only induced few DNA break, but antagonized (~15~20%) TOP2-mediated DNA damage induced by VP-16 as well as doxorubicin. TOP2β-mediated DNA cleavage, but not TOP2α, induced by VP-16 *in vitro* was also antagonized by CL-14. Together, we suggested that CL-14 is a potential TOP2β-specific inhibitor, which is clinically helpful in preventing TOP2-targeting side-effects. In agreement with above notion, our data also revealed that CL-14 still antagonized VP-16-induced DNA break in TOP2α-knockdown cells. Furthermore, we also found CL-14 could preferentially antagonize VP-16-induced TOP2β-cleavable complex formation in both trapping and *In vivo* complex of enzyme assays. CL-12 and CL-13, though sharing a similar structure with CL-14, but didn't show the isozyme specific inhibition. A structure-based study has also been initiated to unravel the molecular mechanism(s) underlying this isozyme-specific action.

Keywords: None.

MON-007
Leo1 regulates heterochromatin spreading by establishing H4K16 acetylation at boundaries

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Eukaryotic chromatin structure is tightly regulated and defines distinct functional domains; the condensed form, heterochromatin, plays important roles in genome regulation and accurate chromosome segregation. Studies in the model organism *Schizosaccharomyces pombe* have given us a good understanding of the mechanisms promoting heterochromatin assembly, however less is known about how this is regulated. A key property of heterochromatin is its ability to spread from an initiation point to coat large portions of DNA. However, expansion of heterochromatin domains needs to be controlled to avoid mis-regulation of gene expression. Boundary elements found between heterochromatin and euchromatin domains control cis-spreading of heterochromatin along the chromosome fiber. Several DNA elements that function as boundaries have been identified, but only a few proteins have been described to regulate cis-spreading of heterochromatin.

In order to identify new negative regulators of heterochromatin, we performed a large-scale genetic screen using a recently described fission yeast deletion library. Silencing of a *ura4*$^+$ marker gene inserted outside a pericentric heterochromatin domain was used as a read-out for heterochromatin spreading across the boundary, indicative of a disruption of barrier functions. Several mutants showed increased *ura4*$^+$ silencing, including a deletion of the previously described negative regulator Epe1. We have focused on Leo1, a member of the Paf1 complex with a previously un-described role in heterochromatin regulation. *Leo1* deletion mutants display a disruption of the boundary function, characterized by an increase in H3K9 methylation on the *ura4*$^+$ gene. Our analyses suggest that Leo1 is involved in the establishment of a specific chromatin environment at the boundary locus to counteract heterochromatin spreading outside its normal domain.

This study uncovers the function of Leo1 in the activity of chromatin boundaries, helping to develop a more comprehensive view of chromatin domains regulation.

Keywords: chromatin boundaries, Fission Yeast, heterochromatin spreading.

MON-008
Molecular cloning and in silico analysis of human ADAMTS-3 (a disintegrin and metalloprotease with thrombospondin motifs) gene promoter

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ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) is a family of extracellular proteases found in both mammals and invertebrates. They have nineteen members. ADAMTSs have significant roles in processing amino terminus of the procollagen molecules, cleavage of the matrix proteoglycans aggrecan, versican and brevican; inhibition of angiogenesis; blood coagulation homoeostasis as the von Willebrand factor cleaving protease. ADAMTS-3 mainly processes amino terminus of the type II fibrillar collagen molecules, specific component of the articular cartilage. Increased *ADAMTS-3* gene expression was also observed in some pathological conditions such as osteoarthritis, myocardial infarction and breast cancer. However, there is no information in the transcriptional regulation of *ADAMTS-3* gene. Thus, in order to identify transcriptional regulation of *ADAMTS-3* gene, the 5'-flanking region (1381 bp) upstream of the translation start site (TSS) of the *ADAMTS-3* gene was amplified from MG-63 genomic DNA by PCR with a pair of specific primers. PCR Enhancers were also introduced into reaction mixture specific to GC rich sequences. Amplified fragment was cloned into pGEMT-Easy vector using T:A cloning strategy for further studies. The (-1341/+40) promoter fragment was analyzed for putative transcriptional factor binding sites, promoter elements

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and CpG islands by matinspector genomatix, EMBOSS CpG plot and TRANSFAC software applications and database. Bioinformatic analysis of this promoter didn't identify a TATA box but detected several SP1/GC and GA boxes, NFKβ, interferon regulatory factors, E2F, STAT and krueppel like transcription factor binding sites. CpG plot analysis identified a putative CpG island around 350 bp to 700 bp upstream of the TSS of the ADAMTS-3 gene.

Keywords: transcriptional regulation, ADAMTS-3, GC rich promoter.

MON-009
Regulatory interaction of human telomerase reverse transcriptase (hTERT) with CST complex

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Telomerase is chromosome end replicating enzyme that lengthens telomere by its reverse transcriptase activity using its RNA component as template. Chromosomal ends get shortened in each replication cycle; thus telomerase is necessarily required in actively dividing cells like cancer cells. On the other hand Shelterin and CST complexes bind to the telomeres and construct a telomere protecting structure without which chromosomal ends are vulnerable to nucleolytic attacks. CST complex consists of three proteins CTC1, STN1 (OBFC1) and TEN1. Loss of function of any of the three components of CST complex results in degradation of C- strand, and as a result G-overhangs get accumulated. It has been found that CST mediates C-strand fill in through interaction with Polymerase α after telomere elongation by telomerase. CST complex regulates telomere length homeostasis by directly interacting with TPP1-POT1 (components of shelterin complex) and competes with telomerase enzyme for binding to the telomere, thereby inhibiting the telomere elongation by telomerase. Although chromosome end elongation is viewed as primary function of telomerase, recent researches have shown that the telomerase is involved in diverse biological activities. Here we demonstrate that unavailability of hTERT (catalytic component of telomerase) affects the expression of telomere binding proteins. We studied the effect of hTERT knockdown using RNA interference in cancer cells on expression level of CST. Upon hTERT knock down we observed drastic retardation in the growth of cancer cells. When the hTERT level is low in the cells, expression of STN1 and TEN1 was increased and that of CTC1 was decreased. CST is a telomere protecting complex so the expression of STN1 and TEN1 increases in the scarcity of hTERT to increase the protection level of telomeres. Probably CTC1 expression goes down because its function is to bring CST complex to telomere and compete with telomerase for binding to telomere. The mechanism by which hTERT regulate CST expression is not clear at this stage; the regulatory relationship of hTERT with CST needs to be further studied. This study indicates that hTERT not only lengthens telomeres but also regulates the availability of telomere binding proteins. As the telomere protection does not depend on single factor, a combinatorial targeting of telomerase along with disrupting the telomere binding protein complexes would be more effective strategy to inhibit cancer cell growth.

Keywords: CST complex, hTERT, Telomere.

MON-010
Control of telomerase action in fission yeast by SUMO

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Telomeres protect chromosome ends through the assembly of a nucleoprotein complex called shelterin that also has the function of regulating the action of telomerase, the enzyme responsible for the synthesis of telomeric (G-rich) DNA repeats. After telomerase action, telomere replication is completed by synthesis of the complementary (C-rich) strand by lagging strand DNA polymerases through the activity of the CST complex, composed of CTC1/Cdc13, Stn1 and Ten1. In budding yeast, the interaction of Stn1 with overhang-binding Cdc13 is increased by SUMOylation of the latter. In humans, instead, CST interacts with overhang-binding shelterin proteins TPP1/POT1, but it remains unclear whether this interaction is regulated. We show that fission yeast Tpz1, an ortholog of TPP1, is SUMOylated at lysine 242 and that this modification is important to limit telomere length by restricting telomerase action. Our analysis suggests that SUMOylation of Tpz1 is the most important event in the control of telomerase by SUMO in this organism. Chromatin immunoprecipitation analysis indicates that the association of telomerase with telomeres is increased in a tpz1-K242R mutant, whereas the association of shelterin components is unchanged. Strikingly, instead, loss of SUMOylation of Tpz1 leads to strongly reduced association of Stn1 and Ten1 with telomeres. In addition, a SUMO-Tpz1 fusion protein displays increased affinity for Stn1, in yeast two-hybrid assays. Our data indicate that SUMOylation down-regulates telomerase in fission yeast by promoting Stn1/Ten1 action at telomeres through Tpz1. Our findings highlight the evolutionary conservation of the regulation of CST function by SUMOylation.

Keywords: SUMOylation, Telomeres, Tpz1

MON-011
Understanding the role of telomerase in skin homeostasis

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Many tissues with high cell turnover, such as skin, maintain tissue homeostasis by continuous replenishment and mobilization of short- and long- lived epidermal stem and progenitor cells. Strong evidence suggests that telomerase contributes and is required for efficient stem cell self-renewal, but in-depth understanding of TERT regulation and expression pattern in normal tissues has been hampered by challenges in detecting telomerase at the single cell level using current *in situ* detection methods. We have successfully identified and isolated TERT expressing epidermal cells using a mouse model generated in our lab where GFP is expressed from the endogenous TERT promoter, and used as reporter of TERT promoter activity. A combinatorial approach of tail skin wholemounts microscopy and flow cytometry enabled quantification and identification of TERT expression in most epidermal stem and progenitor cell compartments. In the interfollicular epidermis (IFE), TERT expression is focal and not uniformly expressed throughout the Krt14^{+} basal layer of the epidermis. In the hair follicle (HF), TERT is expressed in the junctional zone, isthmus, bulge, outer root sheath and hair bulb. Expression appears to be dynamic since in telogen (HF resting

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phase), TERT is mainly detected in the bulge, whereas in anagen (HF growth phase), it is detected along the ORS and hair bulb. In catagen (HF regression phase), TERT expression decreases substantially, except in the HF bulge. We compared telomerase enzymatic activity between isolated TERT-eGFP$^+$ and TERT-eGFP- sorted cells and found increased telomerase activity in TERT-eGFP$^+$ cells. We are pursuing experiments to identify the function of TERT in each of these compartments using a combination of lineage-tracing and gene expression analysis. Understanding the role of TERT in this context might help elucidate the mechanisms underlying the phenotypes in patients with dyskeratosis congenita, where telomerase mutations lead to epidermal defects. These patients show, among other symptoms, hair loss or greying, abnormal skin pigmentation, oral mucosa leukoplakia and an increase incidence of head and neck squamous cell carcinomas.

Keywords: Epidermal stem cells, Telomerase, Tissue homeostasis.

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CSII-02 – Cilia & Disease

MON-013
Evaluation of uncertainty for coagulation parameters based on EURACHEM/CITAC guide

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Background: The blood coagulation process represents the initial phase of the biological repair mechanism designed to respond to injuries to the vasculature that result in leakage of blood into the surrounding tissue. Poor standardization of preanalytic and analytical variables exerts a strong influence on the reliability of coagulation testing, consuming valuable health care resources and compromising patient outcome. In this study, it was aimed to estimate uncertainty of 8 parameters according to the EURACHEM/CITAC guide and to determine the analytical performance of our coagulation laboratory.
Materials and Methods: The parameters which were used to calculate uncertainty are as follows: Activated *partial* thromboplastin time (aPTT), prothrombin time (PT), fibrinogen, antithrombin III (AT III), factor 7, factor 8, protein C and protein S. Siemens BCS® XP System (Siemens Healthcare Diagnostics Products GmbH, Germany) and original calibrators and reagents of manufacturer were used in the study. We evaluated Internal and External Quality Control (IQC and EQC, respectively) results of 8 parameters for last 12 months between period January 2013 and December 2013. The mathematical calculations and uncertainty of measurements are derived from the EURACHEM/CITAC guide. Then we assessed the analytical performances and uncertainty of measurements according to CLIA, Rilibak and Fraiser.
Results: aPTT, PT and fibrinogen, the only available CLIA rules, met the criterias for CLIA. Fibrinogen, protein C and S % TE values were within the appropriate limits defined by Fraser while aPTT, PT, AT III, factor 7 and 8 did not meet criteria.
Discussion: Although there are regulatory standards like CLIA, Fraser and Rilibak offering allowable limits of performance for the majority of routine measurements (Routine biochemistry, complete blood count, etc.), only Fraser determined criterias for each parameter of 8 coagulation tests. As the result of inadequacy of regulatory standards and poor standardization of preanalytic and analytical variables, laboratories are slog to determine their own uncertainty and to increase their standard. More detailed studies are needed to be performed in this regard.
Keywords: None.

MON-014
Native architecture of the centriole proximal region reveals features underlying its 9-fold radial symmetry

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The centriole and the related basal body is a macromolecular structure that is fundamental for the assembly of cilia and flagella across evolution. Moreover, the centriole is required for the assembly of the centrosome, the principal microtubule-organizing center (MTOC) of animal cells and cilia in most of Eukaryotes. Centrioles are evolutionarily conserved microtubule-based cylindrical macromolecular structures that in human cells are ~500 nm long and ~230 nm in outer diameter. Centrioles are characterized by the presence of nine triplets of microtubules surrounding a cartwheel structure at their proximal end, which is critical for imparting the 9-fold symmetry of the entire structure. We recently showed that the evolutionary conserved SAS-6 proteins, essential for cartwheel assembly and forming a 22 nm ring *in vitro*, is likely to form a ring in vivo, as revealed by the 50A resolution 3D map of the cartwheel from *Trichonympha*, a symbiotic flagellate present in the hindgut of termites. Although this work uncovered the cartwheel architecture, how it connects to the peripheral microtubules is not understood. More generally, a comprehensive structural view of the proximal region of the centriole is lacking, thus limiting understanding of the underlying centriolar assembly mechanisms.

Using cryotomography and sub-tomogram averaging, we report the complete architecture of the *Trichonympha* centriole proximal region. The resulting 3D map at 38A resolution reveals several features, including additional densities in the cartwheel that exhibit a 9-fold symmetrical arrangement, as well as the structure of the Pinhead and the A-C linker that connect to microtubules. Moreover, we uncover striking chiral features that might impart directionality to the entire centriole. Furthermore, we identify *Trichonympha* SAS-6 and demonstrate that it localizes to the cartwheel in vivo.

Our work provides unprecedented insight into the architecture of the centriole proximal region, which is key for a thorough understanding of the mechanisms governing centriole assembly.
Keywords: Centriole, cryo-microscopy, microtubule.

MON-015
Porphyrias in Turkey: from Porphyria Turcica to present

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In 1956 an epidemic of PCT occurred between 1956 and 1961 due to hexachlorobenzene (HCB) in southeastern Turkey and it was estimated that over 3000 individuals were affected. The registered history of the porphyrias and the awareness of this disease started at these years for Turkey. But from that epidemia until 1994 researches were performed individually and there was no institutional development.

Although it was a late decision for the country, Gulhane Porphyrin Laboratory was established in 1994. Nowadays 12 types of porphyrin analysis [porphobilinogen scanning and quantitation (urinary), aminolevulinic acid (urinary), total porphyrin (urinary, stool, plasma, whole blood), fluorescence emission scanning (plasma), free/metal protoporphyrin, porphyrin fractionation with HPLC (urinary and stool), erythrocyte porphobilinogen deaminase activity] are performed by using different analytical techniques (fluorimetric measurement and scanning, spectrophotometric, UV-spot test, TLC, HPLC) in our laboratory. Gulhane Porphyria Laboratory, which is a member of European Porphyria Network since December 2011, is participating in The Royal Collage of Pathologists of Australia external quality assurance scheme for porphyrias since 1996. A total of 61 patients were diagnosed in this twenty-year period.

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As in all developing countries, there are some outstanding issues to overcome for our lab and country. For example there is no foundation for rare diseases including porphyrias. The efforts for being the "Reference (Service) Laboratory" and accreditation process have been initiated. In the future, genetic diagnosis and counseling with DNA mutation analysis are aimed. As a result, we should not forget that none of us are protected from rare diseases like porphyrias by statistics.

Keywords: None.

MON-016
Primary cilia incidence after ionizing irradiation and serum starvation stress in a myoblast cell line (C2C12)

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Primary cilia (PC) act as physical-chemical sensors and their biological functions include perception of the extracellular milieu, regulation of organogenesis and cell polarity. PC are dynamically regulated during cell cycle progression, the axoneme's major component is α-tubulin whereas the basal body is composed of γ-tubulin. Its size ranges between 2 and 10 μm long and 200–300 nm wide although these dimensions may change under stress such as starvation, hypoxia or ionizing radiation. In the present study we concentrated on the analysis of primary cilia incidence after ionizing radiation exposure (2, 6, 10, 20 Gy) and serum starvation stress in a myoblast cell line (C2C12).

A significant number of ciliated cells was observed 1 day after the irradiation within the whole dose range (2–20 Gy), with the highest PC incidence three days after the treatment as compared to non-irradiated cells. However, PC incidence (%) observed in cells exposed to doses of 2, 6 and 10 Gy was significantly lower than that observed in cells irradiated by 20 Gy. Further dynamic of primary cilia formation was evaluated only after 20 Gy dose and it was found that the PC incidence remained significantly higher than in non-irradiated cells even at 6 days post-irradiation. Unexpectedly, multi-ciliated cells were also detected in irradiated cells. In 2–4% of irradiated cells two or more cilia were detected 1 day after the irradiation. A further pronounced increase in cells with multiple cilia was observed 3 days after irradiation by the doses of 10 and 20 Gy (15% and 35% of cells, respectively).

Cell proliferation was inhibited after serum starvation stress, decreasing the number of cells in S phase after 24 h of starvation. Cell viability was not affected and apoptotic cells were not observed. The percentage of ciliated myoblasts increased significantly as soon as after 6 hours of serum starvation as compared with the control. The PC incidence increases further during the starvation, peaking at 24 hours and reaching nearly 80%. Thereafter, the percentage of ciliated cells was increased for the remaining duration of the experiment (120 hours of starvation).

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Keywords: Ionizing radiation, Primary cilia, Serum starvation stress.

MON-017
The role of EGF and TGFβ in modulating the expression of stearoyl CoA desaturase 1 in association with metastatic breast cancer development

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Despite the significant scientific progress in the prevention, detection and treatment of cancer in recent decades, cancer remains a leading cause of death in Canada. Moreover, breast cancer is the leading cause of female cancer deaths in the world. Similarly, the percentage of mortality significantly increases when the cancerous cells acquire metastatic potential. We have previously shown that the high expression of stearoyl CoA desaturase 1 (SCD1), which is a key lipogenic enzyme, was associated with an increase in the metastatic potential of breast cancer cells. SCD1 desaturated fatty acids with long chain forming monounsaturated fatty acids. We believe that SCD1 could be a new therapeutic track for finding new active molecules against metastatic breast cancer.

EGF (Epidermal Growth Factor) and TGFβ (Transforming Growth Factor -beta) are associated with the acquisition of metastatic power. In fact, in breast cancer cells, EGF activates signaling pathways JAK / STAT, PI3K, Src kinase, PLCγ, ERK and TGFβ, pathways involving Smad proteins. Preliminary studies have shown that the expression of the SCD1 protein is increased after treatment of breast cancer cells MDA- MB- 231 by these two hormones.

Project's Objectives: The aim of our study is to verify if EGF and TGFβ regulate transcription of SCD1. Furthermore, we want to characterize the signaling pathways induced by the above two hormones in the MDA-MB 231.

Methodology: The experiments were performed in the MDA-MB 231 cells transfected with a construct containing the 3.7 kb SCD1 promoter which is cloned upstream of the luciferase reporter gene.

The promoter activity was measured in the presence or absence of EGF (10 ng/ml) or the presence or absence of TGF β (0.2 nM).

Specific inhibitors of kinases which are involved in signaling pathways induced by conventional EGF and TGFβ, were used to assess the role of each lane on the activation of SCD1. For that reason, the MDA- MB- 231 cells were preincubated for one hour with specific inhibitors and then treated for twenty four hours with EGF or TGF β inhibitors. The effect of each inhibitor on SCD1 expression was evaluated by RT -PCR.

Results and Conclusion: The preliminary results show that following the addition of the two hormones EGF and TFGβ, a three times greater increase in the expression level of SCD1 was detected in MDA-MB-231. In fact, previous studies have shown transcriptional SCD1 increase in epithelium's cells of the retinal pigment. Therefore, EGF and TGFβ appear to regulate the transcription of SCD1. Indeed, SCD1 appears to play a role in the acquisition of metastatic potential through signaling pathways that will be defined later.

Keywords: breast cancer, EGF and TGFβ, stearoyl CoA desaturase 1 (SCD1).

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CSII-03 – Circadian Clocks

MON-019
Circadian CLOCK mediates activation of TGF-β signaling and renal fibrosis through cyclooxygenase 2

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Circadian rhythm regulates blood pressure and maintains the fluid and electrolyte balance with central and peripheral clock. However, the role of circadian rhythm in the pathogenesis of tubulointerstitial fibrosis remains unclear. Here we found that the amplitudes of circadian rhythm oscillation in kidneys significantly increased after unilateral ureteral obstruction. In mice deficient of circadian gene *CLOCK*, renal fibrosis and renal parenchymal damage were significantly worse after ureteral obstruction. CLOCK–/– mice exhibited increased synthesis of type I collagen, enhanced apoptosis, increased activation of fibroblasts, and greater TGF-b–induced PAI−1 expression. TGF-b mRNA expression oscillated with circadian rhythms and were under the control of CLOCK-BMAL1 heterodimers. Renal tubular epithelial cells from CLOCK–/– mice exhibited increased apoptosis and decreased proliferation. Moreover, expressions of *cyclooxygenase 1* and *2* significantly increased in kidneys from CLOCK–/– mice with or without ureteral obstruction. Treatment of cyclooxygenase inhibitor, Celecoxib, improved renal fibrosis in CLOCK–/– mice. Taken together, these data establish an important role of circadian rhythm in tubulointerstitial fibrosis and suggest a novel therapeutic target of CLOCK/TGF-b through cyclooxygenase inhibition.

Keywords: circadian rhythm, cyclooxygenases, Renal fibrosis.

MON-020
Circadian clock regulates c-MYC oncoprotein via rhythmic acetylation

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Circadian clock function has been implicated in cell cycle progression both *in vivo* and *in vitro*. Clock deregulation is associated with increased tumor incidents and expression of core clock components is greatly reduced in several cancers. Among clock controlled genes there are many key regulators of cell proliferation, such as the *c-MYC* proto-oncogene. c-MYC transcription factor regulates the expression of several hundred genes responding to mitogenic signals. Its own regulation of transcription is rather complex and remains largely unknown. One exciting feature of *c-MYC* promoter is that the circadian activators CLOCK and BMAL1 recognize its E-box elements but their binding results in decreased transcriptional activity. In fact, *c-MYC* is the only known gene whose expression is negatively regulated by the circadian dimer BMAL1/CLOCK and this molecular mechanism has not been unraveled.

In an attempt to elucidate this "discrepancy", we studied c-MYC promoter activity driven by BMAL1 and WT or a lysine acetyltransferase (KAT) deficient CLOCK using a luciferase reporter system. Contrary to our expectations, acetylation status of BMAL1 did not affect c-MYC transcription. On the other hand, manipulating acetylation levels via treatment with deacetylase inhibitor Trichostatin A (TSA) repressed oncogene transcription in a circadian manner. This prompted us to analyse protein accumulation in synchronized neuroblastoma (N2A) cells before and after TSA treatment. Rhythmicity in expression levels was confirmed while TSA resulted in a substantial and prolonged reduction of the oncoprotein only when applied at the peak of c-MYC oscillation. This was also accompanied by cell cycle arrest of N2A on G1. Delayed protein accumulation with respect to maximum of transcription indicates that clock regulates c-MYC at post-translational level as well. Indeed, protein synthesis and degradation rates vary according to circadian time. Both rates were affected by TSA treatment at a specific phase of the circadian rhythm resulting in elimination of c-MYC. Finally, acetylation status of endogenous oncoprotein was analysed. Maximum acetylation was observed at the peak of c-MYC rhythm indicating an interplay between this modification and protein stability.

Thus, our results document a complex role of the circadian clock on c-MYC expression. This includes not only transcriptional regulation but also control of oncoprotein levels via acetylation-dependent mechanisms.

Keywords: acetylation, circadian clock, c-MYC oncoprotein.

MON-021
Direct involvement of HIF1αi n the circadian oscillation of Per2 gene

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PAS factors have been named after the discovery of common domains in Per, Arnt and Sim proteins. The factors sense and bind small molecules, such as oxygen, dioxins and cellar metabolites. These factors are known to be involved in the adaptation to external and internal changes of environment, such as circadian, hypoxic, toxic state changes in mammals. In the circadian system, the core feedback loop that account for the generation of about 24 hours rhythm consists of couple of PAS factors, that is, BMAL1, CLOCK, NPAS2, BMAL2 and three PER proteins. The cross-talk between PAS factors in hypoxic responses and circadian systems have been reported. On the way of our finding cross talk among adaptation system involved in PAS factors, we found transcriptional regulation of *Per2* genes by HIF1α. We will discuss the mechanism of the transcriptional regulation of *Per2* gene by HIF1α and role of HIF1α on *Per2* regulation in tumor cells.

Keywords: circadian rhythm, clock genes, HIF1α.

MON-022
Melatonin signalling controls circadian swimming behaviour in marine zooplankton

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Melatonin, the "hormone of darkness", is a key regulator of vertebrate circadian physiology and behavior. Despite its ubiquitous presence in Metazoa as radical scavenger, the ancestral function of melatonin receptor-dependent signaling remains unknown.

We investigated the effect of melatonin signaling on circadian swimming behavior in a zooplankton model, the marine annelid

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Platynereis dumerilii. We found that melatonin is produced at night in the "clock region": a population of brain photoreceptors with a vertebrate-type opsin-based phototransduction and a light-entrained clock. Using microfluidic high-throughput qPCR, we determined that in these cells the expression of several genes is regulated by the light-dark cycle. We also found that the clock region comprises vertebrate-type ciliary photoreceptors, and develops from neuroblasts expressing a battery of vertebrate eye-field transcription factors.

Platynereis larvae swim with locomotor ciliated cells, and the rate of ciliary arrests affects the vertical position in the ocean. At night melatonin signaling causes an increase of ciliary arrests, hence larval sinking. To dissect the underlying neural circuit, we first established two-photon imaging of the calcium indicator GCaMP6 in *Platynereis*. Melatonin changes directly the firing pattern of cholinergic neurons that innervate locomotor ciliated cells. These cholinergic neurons switch from sparse firing to a rhythmic activity, characterised by the regular discharge of bursts of action potentials. The change of firing mode has a facilitatory effect on synaptic transmission; therefore the rate of ciliary arrests is enhanced during the night.

To summarize, in *Platynereis* melatonin is the output of the circadian clock and modulates neuronal activity to establish a nocturnal behavioral state. We propose that this neuromodulatory system controls the rhythmic swimming of zooplankton in the ocean, known as diel vertical migration. Moreover, we hypothesize that melatonin receptors and opsins coevolved from the duplication of the same GPCR to regulate, together with the circadian system, the vertical position of zooplankton in response to light-induced oxidative stress.

Keywords: circadian rhythm, evolution, Neural circuits.

MON-023
Oxidative stress and insulin resistance in policemen working shift

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Shift work is a work schedule involving irregular or unusual hours, compared to those of a normal day time work schedule. In developed countries, night shift work is very common. In several cities of our country, 12/24 shift system is implemented in police organization. While night shift work composes half of the 20 shift in a month, in ergonomic shift system, an alternative shift schedule, shift work can be performed in three shifts in a day. This system has minimal effect on circadian rhytm. In this study we aimed to investigate the effects of 12/24 shift work system on insülin resistance and oxidative stress and systemic inflammation.

204 12/24 shift workers (age, 44.3 ± 5.6) and 193ergonomic shift workers (age, 42.6 ± 5.5 years) were included to study. Serum oxidized LDL (ox-LDL), neutrophyl gelatinase lipocalin-2 (NGAL) as oxidative stress markers, glucose, insülin, ferritin, high sensitive C reactive protein (hsCRP) and erythrocyte sedimentation rate (ESR) values were measured. HOMA was calculated to evaluate insülin resistance.

Serum ox-LDL, HOMA, hsCRP and NGAL levels in 12/24 shift system were found to be significantly higher compared with ergonomic shift workers ($p < 0.0001$, $p = 0.02$, $p = 0.03$, $p = 0.02$, respectively). When evaluated all subjects, weak but significant correlation was found between HOMA with ox-LDL ($r = 0.12$, $p = 0.01$), hsCRP ($r = 0.17$, $p = 0.001$) and ferritin ($r = 0.15$, $r = 0.003$). Also in 12/24 shift work group, there were significant correlations between HOMA with hsCRP ($r = 0.17$, $p = 0.01$) and ferritin ($r = 0.25$, $p = 0.0001$).

It may be concluded that 12/24 shift system may cause insülin resistance and oxidative stress. Additionally, workers in this system may under risk for systemic inflammatory responce. Working hours must be arranged in accordance with the physiological rhythm.

This study was financially supported by a research fund from the Scientific and Technological Research Council of Turkey (TUBITAK).
Keywords: None.

MON-024
Paraspeckles: nuclear mRNA anchorages responsible for circadian gene expression

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Post-transcriptional controls appear increasingly essential to the circadian system functioning. We recently reported that two multifunctional nuclear proteins, NONO and SFPQ, display a circadian expression pattern in pituitary cells. In addition to their function in transcriptional processes, these proteins participate in post-transcriptional mechanisms such as nuclear RNA retention by paraspeckles. The present study aimed to determine whether one of the post-transcriptional mechanisms responsible for circadian gene expression involves circadian nuclear retention by paraspeckles. We showed that all key components of paraspeckles including both the major protein components and the structural long noncoding RNA Neat1 were shown to display a circadian expression pattern. This later component was also rhythmically expressed in other circadian oscillators including the master clock, the suprachiasmatic nucleus. We reported examples of mRNA whose circadian rhythmicity was post-transcriptionally controlled in pituitary GH4C1 cells and that were associated with paraspeckle components. These mRNA lost their circadian expression pattern when paraspeckles were disrupted by treatment with Neat1 siRNA. Paraspeckles appear then responsible for post-transcriptionally controlled circadian gene expression by way of the rhythmic nuclear retention of the corresponding mRNA. Rhythmic mRNA retention by paraspeckles is then proposed as one of the post-transcriptional mechanisms involved in the circadian clock system functioning.

Keywords: Circadian rhythm, Long non coding RNA Neat1, paraspeckles.

MON-025
Peripheral biological clock and its role in age-related decline in function of rat Leydig cells

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Reproductive physiology is profoundly influenced by circadian rhythms. In mammals, growing body of evidence indicates that the circadian clock regulates the serum concentrations of many reproductive hormones including testosterone. Although the association between circadian rhythms and testosterone is a long-established phenomenon, the molecular mechanisms by which the circadian clock regulates testosterone production are still unknown. In this study, we analised the presence of peripheral circadian clock in Leydig cells. Results supported hypothesis

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about peripheral circadian nature of Leydig cell function. First, the secretion of testosterone from Leydig cells has a low-amplitude of diurnal rhythm. In long day model (14 hours day – 10 hours night) the circulating LH and testosterone levels fluctuate in a circadian fashion. LH reach zenith in the morning and have nadir in the late evening while testosterone levels reach zenith at the end of the day. Second, the circadian rhythm in transcription of the main steroidogenic genes (*Star*, *Cyp11a* and *Cyp17*) in Leydig cells of adult rats was also observed. A peak of their expression was registered a few hours before of the peak of testosterone concentration in blood. In addition, *Nur77* (a positive steroidogenic regulator) showed very strong diurnal rhythm, representing the gene regulated by clock. Third, analysis of gene expression of clock elements showed oscillatory pattern of *Bmal-1*, *Per1,2,3*, as well as *Rev-erb a* and *b* expression in Leydig cells of adult rats. Accordingly, it is possible that those genes could be clock candidates involved in diurnal regulation of steroidogenic genes expression. Several preliminary results obtained from 24-month old rats (corresponds to 60 year in humans) support involvement of clock system with functional deficits of Leydig cells during aging. First, the circadian rhythm of circulating LH and androgens was preserved in aged rats. Second, aging attenuated Leydig cell *Star*, *Cyp11a* and *Cyp17* transcription but those genes retained the similar oscillatory pattern as in Leydig cells from adult rats. In addition, the transcription of *Nur77* decreased in old rats, but the rhythm was preserved. In parallel, aging also decreased the expression of the clock genes (*Clock*, *Bmal-1*, *Cry1*, *Per1*, *Npas2*, and *Rev-ebra*) in Leydig cells but again they oscillatory pattern was retained as in Leydig cells from adult rats. Altogether, these observations, support correlation between clock system and functional deficits of Leydig cells during aging.

Keywords: circadian clock, Leydig cells, rat.

MON-026
The circadian rhythm controls telomeres and telomerase activity

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Circadian clocks are fundamental machinery in organisms ranging from archaea to humans. Disruption of the circadian system is associated with premature aging in mice, but the molecular basis underlying this phenomenon is still unclear. In this study, we found that telomerase activity exhibits endogenous circadian rhythmicity in humans and mice. Human and mouse *TERT* mRNA expression oscillates with circadian rhythms that are temperature compensated and are under the control of CLOCK-BMAL1 heterodimers. CLOCK deficiency in mice causes loss of rhythmic telomerase activities, *TERT* mRNA oscillation, and shortened telomere length. Physicians with regular work schedules have circadian oscillation of telomerase activity while emergency physicians working in shifts lose the circadian rhythms of telomerase activity. These findings identify the circadian rhythm as a mechanism underlying telomere and telomerase activity control that serve as interconnections between circadian systems and aging.

Keywords: circadian rhythm, Telomerase, telomerase activity.

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CSII-04 – MicroRNAs in Health & Disease

MON-028

A novel approach towards finding regulatory microRNAs during phagosomal maturation in infection

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Studies have found microRNAs to be involved in various diseases including mycobacterial infections (*M. tuberculosis, M. avium* etc.). In this context, they control mechanisms in the host cell e.g. regulates caspase activity in *M. Avium* infection. But generally bacteria are engulfed and degraded inside host cell via mechanism of phagosomal maturation and there is paucity of knowledge about association of microRNAs in this mechanism. So here we have used a targeted approach to address involvement of microRNAs in phagosomal maturation. After infecting THP-1 cells, three time points (15, 30, 240 min) were identified where microRNAs could possibly be involved in phago-lysosomal inhibition. As *Mycobacterium bovis* BCG was known to inhibit phagosomal maturation it was used for our study as a model for the *M. tuberculosis* complex. In addition, *M. avium* as well as *M. smegmatis* were considered. Differences in LAMP-1 localization on phagosome (present and absent in heat killed and viable bacterial phagosome respectively) were observed at 30 min and 240 min, however Rab7 signal was prominent in all time points. Based on these observations total RNA at given time points post infection were isolated for evaluation of *in silico* predicted target-interactions based on microRNA-specific RT-qPCR. For the *in silico* analysis, we have focused on 15 relevant genes that were found to be mutually targeted by 10 identified microRNAs. Out of these 10, three microRNAs were down regulated upon infection. Ongoing functional experiments will determine their involvement in phagosomal trafficking and maturation.

Keywords: microRNA, Mycobacteria, phagocytosis.

MON-029

A possible regulation of ACTC1 expression by miR-30b in the peripheral blood of dogs with ACVIM stage B heart failure

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It is known that miRNAs are a crucial regulators of a wide range of physiological and pathological processes and they can influence the transcription profile of many genes. miR-30b have been earlier described in literature as being involved in cardiovascular pathology. The aim of the present study was to investigate the level of miR-30b in the plasma of dogs in the early stage of heart disease and screening for potential miR-30b target genes in the peripheral blood nuclear cells.

The miRNA study was conducted on 16 dogs. A clinical examination and echocardiographic examination were carried out. The dogs were divided into groups according to ACVIM (American College of Veterinary Internal Medicine) classification scheme as stage A (healthy – control) – 8 dogs, stage B (asymptomatic) – 8 dogs. Circulating RNA was extracted from the plasma and the level of miR-30b was measured using qPCR method. The results showed that the expression of miR-30b differed significantly study groups. The level of these miRNA was 2.63 times lower in ACVIM stage B than in healthy dogs (2-ΔΔCT method, $p < 0.05$). In order to verify the influence of miR-30b on peripheral blood nuclear cells transcriptomic profile next transcriptomic study was conducted. 24 dogs after clinical and echocardiographic examinations were divided into groups according to the above mentioned classification as stage A – 11 and stage B – 13 dog. Total RNA was extracted from whole blood samples. The transcriptomic profile analysis of peripheral blood nuclear cells was performed using microarray technology (Agilent, USA). The results were analysed with Gene Spring and Pathway Studio software. Microarray experiment revealed 426 differentially expressed transcripts between healthy dogs (ACVIM A) and ACVIM stage B (FDR<0.05). The most significantly regulated signalling pathway was EphrinR -> actin signalling. Among differentially expressed genes involved in this pathway was ACTC1 – actin, alpha, cardiac muscle 1 which was upregulated in ACVIM stage B dogs in comparison to healthy control. Using an online database – miRDB we found out that 6 out of 426 differentially expressed genes are predicted targets of canine miR-30b (cfa-miR-30b). Moreover, one of them is ACTC1. The results of recent studies revealed that circulating miRNAs may be delivered to recipient cells where then they regulate expression of target genes. Having this in mind we can conclude that the downregulation of miR-30b in the plasma may result in upregulation of ACTC1 – its target gene in the peripheral blood nuclear cells.

Keywords: heart failure, microarray, microRNA.

MON-030

A role for miRNAs regulate prostate cancer metastasis and tyrosine kinase inhibitors (TKIs) resistance by targeting EGFR signaling pathway

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Several mechanisms have been suggested to affect prostate cancer progression, including activation of EGFR signaling pathway, either genetically or epigenetically during prostate cancer metastasis. However, therapies targeting EGFR have demonstrated limited effectiveness and acquired drug resistance. miR-203 and miR-34a levels are down-regulated in clinical samples of primary prostate cancer and further reduced in metastases. Here we show that ectopic miR-203 and miR-34a expression displayed reduced bone metastasis and induced sensitivity to tyrosine kinase inhibitors (TKIs) treatment in a xenograft model. Our results demonstrate that the induction of bone metastasis and TKIs resistance are miR-203 and miR-34a down regulation, activation of the EGFR pathway by altered EGFR ligands (AREG, EREG and TGFA), and anti-apoptosis proteins (API5, BIRC2, and TRIAP1) gene expression. Importantly, a sufficient reconstituted invasiveness effect and resistance to TKIs treatment were observed in cells transfected with anti-miR-203 or anti-miR-34a.

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Human AREG, EREG, and TGFA 3'UTR reporters containing miR-203 and/or miR-34a homology sites were analyzed with reporter assays, indicating the directly regulatory role of miR-203 and miR-34a. We observed decreased miR-203 and miR-34a and increased AREG, EREG and TGFA correlated to metastatic prostate cancer with analysis of clinical gene expression databases and patients. Our results support the existence of a miRNA, EGFR, TKIs resistance regulatory network in prostate cancer progression. We propose that loss of miR-203 and miR-34a is a molecular link in the progression of prostate cancer metastasis and TKIs resistance characterized by high EGFR ligands output and anti-apoptosis proteins activation.

Keywords: bone metastasis, prostate cancer, tyrosine kinase inhibitors (TKIs) resistance.

MON-031

Analyses of microRNA-34b and c-myc expression profile in papillary thyroid carcinoma

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Background: Papillary thyroid cancer (PTC) is the most common type of thyroid cancer, exibiting 75-85% of all thyroid malignancies. Thyroid cancer initiation and progression occurs through gradual accumulation of various genetic and epigenetic alterations, including activating and inactivating somatic mutations, alteration in gene expression patterns, microRNA (miRNA) deregulation and aberrant gene methylation. Recent studies have shown a difference in expression of certain miRNAs between normal thyroid tissue and tumour tissue and have highlighted their potential as diagnostic markers. Study aims to examine and establish the relationship between miR-34b expression and one of its predictive target for PTC cases (c-myc).

Methods: Total RNA was extracted from histologically confirmed PTC and non-cancer thyroid tissue from patients (n = 16). miRNA was isolated from 32 samples (16 matched PTC and their adjacent normal tissues as controls) using mirVana kit. miR-34b expression levels were estimated in qRT-PCR with RNU43 as reference. Since it is known that miRNAs regulate gene expression by promoting the degradation of targeted mRNAs, we performed *in silico* analysis (miRTarBase) in order to identify the possible targets for miR-34b. One potential target was chosen c-myc oncogene, and its expression levels were determined in RT-PCR. Statistical analysis was performed with GraphPad Prism.

Results: Compared with corresponding normal tissue, miR-34b was found to be downregulated (>2 fold, $P < 0.003$) in PTC samples, as for its target an inverse corelation between miR-34b and c-myc expression levels was noted. Results indicates a significantly increased c-myc expression in PTC subjects ($P < 0.001$). For PTC patients, c-myc could be a target of miR-34b and the incresed levels of c-myc oncogene, underlying tumorigenic changes in thyroid cancer, may be due to miR-34b downregulation. Further studies are needed to establish the relationship between miR-34b and its target in papillary thyroid carcinoma.

Keywords: c-myc, miR-34b, Papillary thyroid cancer.

MON-032

Analysis of miRNA expression in canine mammary cancer stem-like cells shows epigenetic regulation of TGF-Beta signalling

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Cancer stem cells (CSCs) posse the unique ability to self-renewal and differentiate into all kind of cancer cells. That is why they are responsible for cancer initiation, recurrence and drug resistance [1]. Despite a variety of methods are currently employed to target them, just a little is known about miRNA regulation of CSCs. However, miRNA may constitute a new element of combined therapy targeting these cells [2].

The aim of our study was to assess miRNA expression in canine mammary cancer stem cells. Using FACS Aria II we sorted out subpopulation of cells expressing Stem cell antigen 1 (Sca1; these cells also expressed CD44 and epCAM) from canine mammary tumour cell lines (CMT-U27, CMT-309 and P114). Next, we conducted colony formation assay, which confirmed that only stem-like cells were able to form colonies from single cell in all the examined cell lines. In these cells we examined miRNA expression profiles using Agilent custom-designed miRNA microarrays. These results were validated by Real-time rt-PCR analysis of expression of randomly selected miRNAs.

The results revealed 33 significantly deregulated miRNAs in Sca1-positive cells comparing with differentiated tumour cells. Twenty-four of them were down- regulated whereas nine were up-regulated. Further, we graded them by number of miRNAs in which they occurred. We selected 240 target genes of down-regulated miRNAs and analysed over-represented pathways using Gene Set Enrichment Analysis (GSEA). According to KEGG and BioCarta databases these target genes were involved in MAPK signalling pathway, TGF-Beta signalling, ALK, and PGC1A pathways. Genes targeted by up-regulated miRNAs have not been significantly involved in particular pathways. However, analysis of single-gene overlapping with different pathways showed that the most important were: TGFBR1, TGFBR2, SOS1, CHUK, PDGFRA, MEF2C, TGFBR1, MEF2D and MEF2A. All of them are involved in TGF-Beta signaling showing its important role in cancer stem cells biology.

The results of our study showed significant epigenetic regulation of cancer stem cells transition to differentiated cancer cells and are important not only for veterinary medicine but also for comparative oncology and cancer research in general.

This work was supported by the grant no. N N308574940.

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Keywords: cancer stem cells, microRNA, TGF-beta signalling.

MON-033

Analysis of miRNA expression in prostate tumors and urine samples

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Prostate cancer (PCa) is one of the most prevalent malignancies characterized by high mortality rates among males in Lithuania

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and world-wide. PCa, compared to non-cancerous prostate tissue (NPT), contains various molecular alterations including aberrant expression of small non-coding RNAs. MicroRNAs (miRNAs) are 21–22 nt RNAs responsible for gene expression regulation and coordination of multiple physiological processes. In cancer, expression of various human miRNAs is markedly deregulated which causes subsequent changes in multiple cellular pathways and impacts disease outcome. The aim of this study was to identify differentially expressed miRNAs specific to PCa and suitable for early and non-invasive disease detection and prognosis.

Expression profile of 754 mature miRNAs was assessed using TaqMan Low Density Array (TLDA) cards A and B v3.0. Selected miRNAs were further analyzed with Custom Design TLDA cards to confirm the expression changes in an expanded set of samples. To evaluate miRNAs circulating in urine of PCa patients qPCR assays were applied.

In PCa tissues (N = 42), expression of 95 miRNAs significantly differed as compared to NPT samples (N = 12), 68 of them were up- and 27 downregulated. Comparison of miRNA profile in PCa cases with and without biochemical disease progression (BCR) revealed a marked upregulation of expression of 61 miRNAs. Based on the most significant differences between PCa and NPT and correlations with several clinicopathologic characteristics, 19 miRNAs were selected for further validation in the expanded set of samples. Significant upregulation of miR-19a, miR-21, miR-148a, and miR-375 was confirmed in PCa (N = 52) as compared to NPT (N = 12) samples, while expression of miR-340 was significantly increased in BCR-positive in comparison to BCR-negative cases. In further analysis, miR-19a and miR-21 were successfully quantified in urine collected from PCa (N = 137) and benign prostatic hyperplasia (BPH, N = 25) patients. Higher expression level of miR-19a was observed in PCa than in BPH, while in BCR-positive PCa cases both miR-19a and miR-21 were more abundant as compared to BCR-negative cases.

In conclusion, targeted inactivation of tumorigenic miRNAs might be considered as a novel treatment strategy for PCa. Non-invasive detection of selected miRNAs in urine might serve as a molecular tool for early identification of PCa.

Keywords: Prostate cancer, miRNA.

MON-034
Characterization of the cellular factors involved in microRNA destabilization by mouse cytomegalovirus

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miRNA biogenesis and their functions have been extensively studied during the last decade, however less is known about the regulation of their stability. Nonetheless, an increasing number of examples illustrating the need to control mature miRNA stability (i.e. adaptation to biotic and abiotic stress) has emerged recently in the literature.

Our laboratory has previously discovered the destabilization of the cellular miR-27 upon mouse cytomegalovirus (MCMV) infection. A viral transcript (called m169), harbouring a functional miR-27 binding site, was found to be solely responsible of directing miR-27 for degradation [(1)]. High-resolution northern blot analyses and small RNA sequencing, demonstrated that the interaction between miR-27 and m169 induces the tailing and trimming of miR-27, a mechanism previously described in Drosophila and human cells [(2)].

While an extensive miRNA-target mRNA pairing seems to be a prerequisite to induce miRNA tailing and trimming, how this mechanism works or which factors are involved in this process is currently unknown. By using both MCMV infection and miR-27/m169 interaction as model systems, a biochemical approach based on the transfection of biotinylated-antisense oligonucleotides was used to induce the tailing-trimming of specific miRNAs and to pull down protein complexes for their analysis by mass spectrometry.

We identified novel factors that could be involved in the tailing-trimming of miRNAs, of which the roles of Terminal Uridylyl Transferase-1 (TUT1) and 3′-5′ exoribonuclease DIS3 like 2 (DIS3L2) proteins in the miRNA decay pathway will be discussed.

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Keywords: Degradation, microRNAs, Turnover.

MON-035
Clinical relevance of VKORC1 genotype and response to warfarin therapy

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Background: The management of warfarin therapy is challenging because it shows large inter and intra individual variability. The aim of current study was to characterize the effects of the *VKORC1* (−1639G>A) polymorphism and other personal characteristics on warfarin dose requirements in Turkish patients.

Methods: Unrelated 183 subjects with (n = 90 cases) and without (n = 93 controls) hemorrhagic complications during warfarin therapy were consecutively enrolled. MALDI-TOF based Sequenom MassARRAY platform was used for genotyping process. Multiple statistical analyses were performed to define the relation of VKORC1 variants with warfarin dose requirement, INR level, hemorrhage points, hemorrhage risk and severity, comorbidity and medications.

Results: The cases and controls did not have a significant difference in terms of VKORC1 (−1639 G>A) genotype distribution (p = 0.084). Frequencies were determined as 30.1%, 52,5% and 17.5% for AA, GA, GG genotype respectively. Allele distribution was 56.2% for (A) and 43.7% for (G) (HWE, p = 0.001) in the screened cohort. A multiple linear regression model revealed a strong relation between warfarin dose and age (p = 0.001), INR level (p = 0.001), hemorrhage risk (p = 0.02). The VKORC1 AA variant have significant association with higher INR (p = 0.01) and lower warfarin dose (mean dose was 3.37 ± 1.43 mg/day vs 5.75 ± 2.63 mg/day among cases) requirement (p = 0.001). The number of the cases with long term (>1 year) warfarin usage was higher, however, significant relation was not found between VKORC1 variants and treatment period.

Conclusions: VKORC1 −1639AA genotype is associated with lower warfarin dose and higher INR status and its frequency in the screened cohort was higher than it has already been reported for Turkish population. Determining the impact of genetic factors in a given population is important for research, development and implementation of personalized health care models which allows developing individualized pharmacological and medical follow-up advices and more targeted preventative healthcare strategies.

Keywords: drug metabolism, pharmacogenetics.

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MON-036
Detection of microRNAs in hair roots and hair shafts as the novel potent biomarker: evaluation of the usefulness for the diagnosis of systemic sclerosis

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A lot of researches has indicated serum microRNA levels are useful as the biomarkers for various diseases. In this study, we compared several methods to extract hair microRNAs, and evaluated their usefulness for the diagnosis of scleroderma.

A single hair root and 5 pieces of hair shafts (about 5 cm) were obtained from 11 scleroderma patients and 13 normal subjects at the time of serum sampling. microRNA was extracted from sera or hair roots using commercially available kits. Hair shaft microRNAs were purified using four different methods. microRNA expression was determined by PCR array and real-time PCR.

We found microRNAs were detectable and quantitative in hair roots and hair shafts using our method. We demonstrated the difference of microRNA levels in hair roots and hair shafts obtained from different places of head in each individual were within 2-fold, thus indicating the reproducibility of hair microRNA levels by our method. PCR array results indicated microRNAs from sera, hair roots and hair shafts have different expression pattern, and can be independent biomarkers.

Serum and hair root miR-196a levels were not significantly altered in scleroderma patients, whereas miR-196a levels in hair shafts were significantly down-regulated in scleroderma patients compared to those in normal subjects.

Because hairs are more accessible than sera, microRNAs levels in hair roots and hair shafts may become effective and independent biomarkers.

Keywords: hair, microRNA.

MON-037
Early differential miRNA expression following in vitro fertilization of mouse eggs incubated in two standard culture media

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Background: At fertilization, the incoming sperm triggers the increase in egg metabolism by causing a series of repetitive Ca^{2+} signals that stimulate mitochondrial oxidative phosphorylation. Several reports have shown that the culture media impacts egg functioning and may affect the long-term developmental processes either in animal or in human. According to the recent discovery of mitochondrial function in microRNA pathway, we assumed that early miRNA expression in the egg should be functionally linked with mitochondrial activity during the period of egg activation. The aim of the study was to measure whether the miRNA expressions are differentially regulated by culture media during the initial period of fertilization.

Material and Method: Four groups of 30 freshly ovulated oocytes were used as control. Eight groups of 30 mouse oocytes were fertilized by Intra Cytoplasmic Sperm Injection (ICSI) and incubated for 4 hours in standard M16 or KSOM medium which have the same 10 compounds but at lower concentrations in KSOM (inorganic ions, energy substrates and protein). All the groups were used to isolate total cells and mitochondria, cytosolic and nucleus fractions by differential centrifugations in specific buffers. RNA extraction kit adapted for small amount of materials was used to extract total RNA from the egg or cell fractions and perform a multiplex RT-qPCR (742 mouse and rodent miRNA).

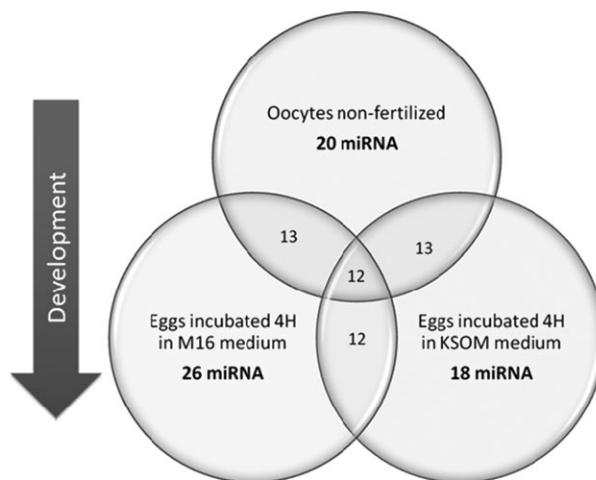

Fig. 1. Number of miRNA expressed in oocytes and eggs after 4 hours of incubation in two standard culture.

Results: Significant changes of miRNA expressions were recorded 4 hours following ICSI. The total number of miRNA expressed per group was highly significantly different after fertilization in KSOM (18 miR) *versus* M16 (26 miR) or in non-fertilized oocytes (20 miR) (figure). Moreover, differential miRNA expressions were observed between cytosolic (15 *vs* 32 miR), mitochondrial (15 *vs* 18 miR) and nuclear (17 *vs* 17 miR) fractions in eggs according to the medium (KSOM *vs* M16). Among the predicted genes targeted by the more expressed miRNA, the following pathways were significantly identified by miRPath: MAPK signaling, PI3K-Akt, metabolic pathway, calcium signaling, Jak-STAT and dorso-ventral axis regulation.

Conclusion: We reveal a functional linkage between the culture media formula and the initial miRNA expressions immediately after fertilization. Such miRNA activity could regulate transcription of thousands of maternal mRNA which are further involved in zygotic genome activation. Such functional genomic analysis provides a potential lever to identify the molecular mechanisms involved in phenotype programming following in-vitro fertilization.

Keywords: mitochondria, Posttranscriptional regulation, RNA interference.

MON-038
EBV-Epstein Barr virus miR-BART20-5p regulates cell proliferation and apoptosis by targeting BAD

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Although Epstein-Barr virus (EBV) BART microRNAs (miRNAs) are ubiquitously expressed in EBV-associated tumors, the role of most BART miRNAs is unclear. In this study we showed that Bcl-2–associated death promoter (BAD) expression was significantly lower in EBV-infected AGS-EBV cells than in EBV-negative AGS cells and investigated whether BART miRNAs target BAD. Using bioinformatics analysis, five BART miRNAs showing seed match with the 3' untranslated region (3'-UTR) of

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BAD were selected. Of these, only miR-BART20-5p reduced BAD expression when individually transfected into AGS cells. A luciferase assay revealed that miR-BART20-5p directly targets BAD. The expression of BAD mRNA and protein was decreased by miR-BART20-5p and increased by an inhibitor of miR-BART20-5p. Annexin V staining and cell proliferation assays showed that miR-BART20-5p promoted cell proliferation and reduced apoptosis. Furthermore, miR-BART20-5p increased chemoresistance to 5-fluorouracil. Our data suggest that miR-BART20-5p contributes to tumorigenesis of EBV by directly targeting the 3'-UTR of BAD.

Keywords: BAD, BART miRNA, Epstein-Barr virus.

MON-040

Expression profile of MAGI2 as a novel biomarker in combination with major deregulated genes in prostate cancer

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Complex molecular changes that occur during prostate cancer (PCa) progression have been described recently. Specifically, whole genome sequencing of PCa tissue identified gene rearrangements which disrupt the gene loci of PTEN and MAGI2 (PTEN scaffolding protein). In the present study, we analyzed the expression profile of *MAGI2* gene in a cohort of clinical PCa ($n = 45$) and benign prostatic hyperplasia (BPH) samples ($n = 36$) as well as three PCa cell lines. We also studied the expression of PCa-related genes including *PTEN, NKX3.1, SPINK1, DD3, AMACR, ERG,* and *TMPRSS2-ERG* gene fusion in these samples. The expression of *MAGI2* mRNA was significantly down regulated in PC3, LNCaP and DU-145 PCa cell lines ($p = 0.000$) and also in clinical tumor samples (*Relative expression*=0.307, $p = 0.002$, 95% C.I., 0.002–12.08). The expression of *PTEN, NKX3.1, SPINK1, DD3,* and *AMACR* genes was significantly deregulated in prostate tumor samples (*p* range: 0.000–0.044). A significant correlation was observed between *MAGI2* and *NKX3.1* expression in tumor samples ($p = 0.006$). Furthermore, the inclusion of *MAGI2* in the gene panel improved the accuracy for discrimination between PCa and BPH samples with the sensitivity and specificity of 0.88 (CI: 0.76 to 0.95) and 0.83 (CI: 0.68 to 0.92), respectively. The data presented here suggest *MAGI2* gene as a novel component of the gene signatures for the detection of PCa .

Keywords: MAGI2, prostate cancer.

MON-041

Fenofibrate prevention of increased mitochondrial fatty acid oxidation capacity in skeletal muscle of DIO mice is associated with muscle-specific microRNA

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Mitochondrial disfunction is claimed to be a risk factor for insulin resistance, and consequently type 2 diabetes (T2D). MiRNAs are small non-coding RNAs that negatively regulate gene expression by promoting degradation or repressing translation of target mRNAs. The aim of this study was to investigate in skeletal muscle of diet-induced obesity mice, treated or not with fenofibrate, the expression of muscle-specific miRNAs and their correlation with expression of genes involved in mitochondrial metabolism. Male C57bl/6 mice (n = 20) were fed for 6 wk with a standard lab diet (SD) or with a high-fat diet (HFD). Subsequently, the mice were maintained on SD/HFD for 2 more wk, but half of the mice were treated with fenofibrate (50 mg/Kg/day) via oral gavage. At the end of the treatment, soleus muscles were isolated and subjected to insulin-induced glucose uptake and CO2 production or flash-frozen in liquid nitrogen immediately for total RNA extraction. Muscle specific microRNAs (miR-1, miR-206 and miR-133a and -133b) and expression of genes involved in mitochondrial fatty oxidation were measured by TaqMan[â] Real-time PCR assay, using snoRNA202 or Rpl, for normalization, respectively. Soleus muscles from HFD-fed mice were unresponsive to insulin, and fenofibrate treatment restored insulin mediated glucose uptake and glycogen synthesis, but had no effect on glucose oxidation. HFD significantly down regulated muscle-specific miRNAs compared with SD group levels in muscle. Fenofibrate treatment restored only miR-1 expression to levels identical of the SD-fed group. Using targetscan software, we found that miRs-1, 206, 133a/b were predicted to target carnitine palmitoyltransferase 1 (Cpt1b), uncoupling protein (*UC*P)3, peroxisome proliferator-activated receptor ϒ coactivator (Pgc1-α), and estrogen-related receptor gamma (Esrrg); all genes involved in mitochondrial metabolism. HFD up-regulate these genes, except Ucp3, compared with SD in muscle, but fenofibrate treatment restored mRNA levels of these genes to SD-fed mice levels. Fenofibrate induced a down regulation of *UC*P3 expression compared to HFD-fed mice. In HFD-fenofibrate group, a significantly negative correlation was observed between miR-1 and Esrrg ($r^2$0.81), Pgc1a ($r^2$0.87) and Ucp3 ($r^2$0.70). Our results demonstrate that muscle specific-miRs may be involved in pathogenesis of T2D, given that they were down regulated in mice on HFD. Fenofibrate improves glucose metabolism in muscle from diabetic mice, and treats increased mitochondrial fatty acid oxidation, probably through up-regulation of miR-1.

Keywords: diabetes mellitus, microRNAs, Mitochondrial dysfunction.

MON-042

From mice to men: fine tuning of cholinergic signaling by non-coding RNAs

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Continuous communication between the nervous and the immune system is essential both for maintaining homeostasis and for ensuring rapid and efficient response to stressful and infection insults. Non-coding and microRNA (miRNA) regulators provide exciting and challenging models for studying this communication in anxiety and inflammation. Global genomic analyses show that miRNAs co-evolved with their target transcripts to efficiently control neuronal signaling pathways and enable contribution to the development of higher brain functions while avoiding damaging evolutionary impact. Specifically, miRNA controllers of acetylcholine signaling modulate both anxiety and inflammation reactions to external insults through physiologically relevant bidirectional competition on interaction with their targets. We found rapid increases of the evolutionarily conserved neuro-modulator acetylcholinesterase (AChE)-targeted CholinomiR-132 in acute stress, intestinal inflammation and post-ischemic stroke, inversely to its drastic reduction in the Alzheimer's disease brain. Furthermore, single nucleotide polymorphisms interfering with the AChE-silencing capacities of the primate-specific CholinomiR-608 associate with elevated trait

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anxiety, inflammation and diverse aging-related diseases in human volunteers, whereas long non-coding RNAs complementary to such miRNAs are modulated in Parkinson's disease and by deep brain stimulation. Deepened understanding of the evolution and complexity of neuronal non-coding RNAs may highlight their role in the emergence of human brain functions while enhancing the ability to intervene with diseases involving cholinergic signaling impairments.

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Keywords: non-coding RNA, Inflammation, microRNA.

MON-043
Functional high-throughput screening identifies microRNAs regulating *Salmonella* infection

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In recent years it has become clear that microRNAs, in addition to their pervasive and well-established functions in physiological and pathological processes, also play crucial roles during infection by different pathogens. However, apart from a small number of microRNAs involved in the host inflammatory response, no microRNAs have been shown to directly modulate infection by bacterial pathogens.

Salmonella enterica serovar Typhimurium, a common and highly investigated facultative intracellular bacterium, is one of the most important causes of lethal food-borne diseases.

To systematically identify microRNAs able to regulate *Salmonella* infection, we performed a high-content, fluorescence microscopy-based screening using a genome-wide library of microRNA mimics. Using this unbiased approach, we identified 17 microRNAs able to decrease *Salmonella* infection by at least 2-fold, as well as microRNAs able to increase *Salmonella* infection (11 microRNAs by at least 2-fold). Detailed time-course infection experiments showed that the identified microRNAs affect *Salmonella* infection at different stages of the infection cycle (e.g. invasion, maturation of the *Salmonella* containing vacuole, replication). Interestingly, many of the identified microRNAs do not affect infection by *Shigella flexneri*, a closely related bacterial pathogen, demonstrating their specificity towards *Salmonella*.

By performing deep-sequencing analysis of the small RNA population of *Salmonella* infected cells, we also determined that some of the microRNAs that strongly inhibited infection in our functional screening are downregulated during infection. Using a combination of experimental and bioinformatic approaches, we have identified and characterized the targets of these microRNAs that play a relevant role in the context of infection.

Overall, these findings uncover a novel mechanism whereby *Salmonella* promotes its own survival and replication by modulating the levels of host cell microRNAs, targeting pathways not previously shown to be relevant for infection.

Keywords: high content screening microscopy, host-pathogen interaction, microRNAs.

MON-044
Functional muscle microRNAs in type 2 diabetes mellitus

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Maintenance of appropriate levels of circulatory glucose levels results from a balance between normal insulin secretion and action.

Diabetes mellitus (DM) is a complex, multisystem disease that represents the most common metabolic disorder. Type 1 DM (T1DM) results from insulin deficiency, usually secondary to autoimmune beta-cell destruction; and type 2 diabetes mellitus (T2DM) is a progressive metabolic disorder characterized by reduced insulin sensitivity, insulin resistance in tissues such as skeletal muscle, liver and adipose tissue, combined with pancreatic β-cell dysfunction, resulting in systemic hyperglycaemia. Improper treatment of T2DM can lead to severe complications such as heart disease, stroke, kidney failure, blindness and nerve damage.

The discovery in mammalian cells of hundreds of small RNA molecules, called microRNAs, with the potential to modulate the expression of the majority of the protein-coding genes has revolutionized many areas of biomedical research, including the diabetes field. MicroRNAs function as translational repressors and are emerging as key regulators of most, if not all, physiological processes.

microRNAs also control insulin signalling in target tissues, including the liver, skeletal muscle, and adipose tissues.

microRNAs circulating in the blood can potentially serve as novel noninvasive biomarkers of diseases including diabetes.

The potential role of microRNAs as biomarkers in diabetes and how aberrant pathways could be corrected therapeutically.

Target recognition relies on rules that are relatively easily satisfied within the transcriptome, each microRNA can potentially regulate translation of a large number of different mRNAs. Each mRNA can possess multiple binding sites for a single or for many different microRNAs. One microRNA can potentially target several genes. These discrepancies and facts reflect the challenges of microRNA-based therapeutics.

Increase and decrease of microRNA levels are important in the pathophysiology.

Understanding of the relationship between functional analysis of microRNAs and insulin resistance is important.

Molecular therapeutic approach, should include interactions of physiological and pathological events with treatment.

Identifying the role and regulation of skeletal muscle miRNAs during various phases of muscle development, as well as in healthy and diseased conditions,will significantly enhance our understanding of skeletal muscle biology and may result in new therapies to target muscle diseases or chronic diseases associated with impaired muscle growth, regeneration or function. Understanding miRNA biology and function will enhance our understanding and application of current therapies.

Keywords: functional microRNA, Muscle microRNA, Type 2 Diabetes Mellitus.

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MON-045
GSEA for the circuitry genes NF-κB/Snail/YY1/RKIP in multiple myeloma

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Background: The presence of a dysregulated NF-κB/Snail/YY1/RKIP loop was recently established in metastatic prostate cancer cells and non-Hodgkin's lymphoma; however, its involvement in multiple myeloma (MM) has yet to be investigated.
Objectives: Aim of the study was to investigate the role of the NF-κB/Snail/YY1/RKIP circuitry in MM and how each gene is correlated with the remaining genes of the loop.
Methods: Using GSEA and Gene Neighbors Analysis in data received from four datasets included in the Multiple Myeloma Genomics Portal (MMGP) of the Multiple Myeloma Research Consortium, we identified various enriched gene sets associated with each member of the NF-κB/Snail/YY1/RKIP circuitry.
Results: In each dataset, the 20 most co-expressed genes with the circuitry genes were isolated subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment. The FOXO4, GATA binding factor, Sp1 and AP4 were the transcription factors that most likely affect the expression of the NF-κB/Snail/YY1/RKIP circuitry genes. GEO datasets computational analysis revealed elevated YY1 and RKIP levels in MM vs. the normal plasma cells, as well as elevated RKIP levels in MM vs. normal B lymphocytes.
Conclusion: The present study highlights the relationships of the NF-κB/Snail/YY1/RKIP circuitry genes with specific cancer-related gene sets among four MM datasets.

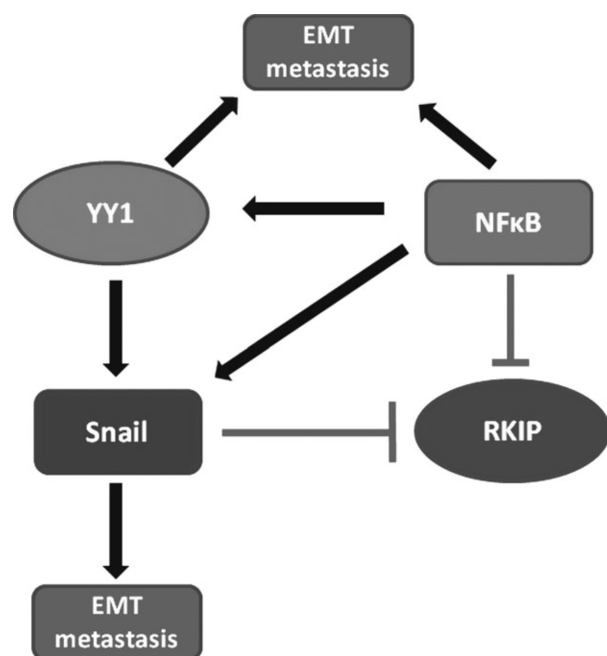

Fig. 1.

Keywords: NF-κB/Snail/YY1/RKIP circuitry; multiple myeloma; Gene Set Enrichment Analysis; Gene Neighbors Analysis; gene co-expression; gene sets.

MON-046
Identification of full-length 3′UTR of ITSN1-L and possible regulation of ITSN1-S by miR-19 and miR-30

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MicroRNAs appear to be important posttranscriptional regulators of many cellular processes through targeting of 3'UTRs of different genes participated in cell life. Human intersectin 1 gene (*ITSN1)* encodes two isoforms (ITSN1-S and ITSN1-L) of multidomain adapter protein involved in clathrin-mediated endocytosis, cell signaling and cytoskeleton reorganization. The aim of our work was to identify whether microRNAs could regulate ITSN1-S and define full-length 3'UTR of ITSN1-L.

To find full-length 3'UTR of ITSN1-L we performed computational analysis of GenBank EST database and identified 11 ESTs which showed that the most probable end of 3'UTR of ITSN1-L mRNA could be located approximately 11600 downstream from the characterized 3' end of exon 41. Then we performed 3'RACE, RT-PCR, cloning of RT-PCR products in pGEM-T-easy vector, and sequencing and confirmed this prediction.

Using web servers TargetScan and miRanda for microRNA target prediction we identified 12 conserved target sites for different microRNAs. To confirm these results we performed luciferase assay using the construct based on pTKluc vector with insertion of full-length ITSN1-S mRNA 3'UTR on HEK 293 cells. Results of the essay showed the 5-fold inhibiting of pTKluc-3′UTR ITSN1-S construct luciferase activity in 293 compared to the intact pTKluc vector. Among the high-ranked target sites we found sites for miR-30 and miR-19. Mir-30 is associated with antioncogenic effect in different tissues while miR-19 is considered to be prooncogenic microRNA. As far as ITSN1 participates in clathrin-associated endocytosis, MAPK-pathway and reorganization of actin cytoskeleton and disorders in these processes are associated with cancer we decided to investigate if these microRNAs could regulate ITSN1-S. We cultivated 293 cells 72 h and transfected them every 24 h with pre-miR-30a and pre-miR-19a. Levels of endogenous ITSN1-S were estimated by Western-blot analysis. Obtained results showed inhibition of ITSN1-S expression by both microRNAs. Opposite, we found no effect from transfection by miR-181a – microRNA with two high-ranked predicted target sites. This suggests that both, miR-30 and miR-19 could negatively regulate ITSN1-S in processes ITSN1-S takes part.

To further investigate ITSN1-S posttranscriptional regulation more precisely we will perform luciferase assays using ITSN1-S mutants with fully or partially deleted 3'UTR and qRT-PCR to estimate microRNA impact on ITSN1 expression on RNA level.
Keywords: ITSN1, microRNAs, Posttranscriptional regulation.

MON-047
Implication of Epstein–Barr Virus-encoded microRNAs in immune evasion of infected B cells

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Epstein-Barr virus (EBV) is a prevalent, persistent human pathogen that preferentially infects primary B cells. EBV resides in its host for a lifetime and is a major cause of lymphomas in immunocompromised patients. In order to achieve viral persistence,

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EBV employs several strategies that have an impact on cellular gene expression and immune surveillance. The first stage of infection is key for EBV's success and requires mechanisms of immune escape to allow establishment of its latent cycle (Jochum et al., 2012). Apart from viral proteins, certain viruses including EBV resort to RNA interference (RNAi) to dampen the host immune response. RNAi is a posttranscriptional regulation of gene expression used by metazoans, plants and certain DNA viruses. RNAi requires non-coding RNAs of ~23 nucleotides in length called microRNAs (miRNAs). They specifically interact with target messenger RNAs (mRNAs) and direct either the degradation of the mRNA or the inhibition of its translation (Bartel, 2009). EBV encodes 44 miRNAs that are widely represented in infected cells at early time post-infection. Functions of these viral miRNAs are poorly understood but few examples indicate a role in controlling cellular immune responses of the host. Published cellular targets of EBV-encoded miRNAs include the T-cell attractive chemokine CXCL-11 (Xia et al., 2008), the NK cell ligand MICB (Nachmani et al., 2009), and the NRLP3 inflammasome subunit (Haneklaus et al., 2012). Each miRNA may regulate a large number of target genes suggesting that the presumed functions of EBV's many miRNAs are mostly unknown. We have preliminary evidence that some cellular genes, which are involved in immune response, are among the targets of EBV's miRNAs. We developed a set of tools to uncover these genes. These tools include mutant viruses that encode all, none or only subsets of viral miRNAs, bioinformatics prediction tools, miRNA reporter assays, and cellular immune assays in order to identify cellular targets of EBV-encoded miRNAs and to decipher the implication of EBV-encoded miRNAs in viral immune evasion of infected human B cells.

Keywords: Epstein-Barr virus, Immune escape, microRNAs.

MON-049
Metabolic pathways are deregulated in kidney cancer

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Clear cell renal cell carcinoma (ccRCC) is the predominant subtype of renal cell carcinoma (RCC). It is one of the most therapy-resistant carcinomas, responding very poorly or not at all to radiotherapy, hormonal therapy and chemotherapy. A more comprehensive understanding of the deregulated pathways in ccRCC can lead to the development of new therapies and prognostic markers. We performed a meta-analysis of 5 publicly available gene expression datasets and identified a list of co-deregulated genes, for which we performed extensive bioinformatic analysis coupled with experimental validation on the mRNA level. Gene ontology enrichment showed that many proteins are involved in response to hypoxia/oxygen levels and positive regulation of the VEGFR signaling pathway. KEGG analysis revealed that metabolic pathways are mostly altered in ccRCC. Similarly, Ingenuity Pathway Analysis showed that the antigen presentation, inositol metabolism, pentose phosphate, glycolysis/gluconeogenesis and fructose/mannose metabolism pathways are altered in the disease. Cellular growth, proliferation and carbohydrate metabolism, were among the top molecular and cellular functions of the co-deregulated genes. qRT-PCR validated the deregulated expression of several genes in Caki-2 and ACHN cell lines and in a cohort of ccRCC tissues. *NNMT* and *NR3C1* increased expression was evident in ccRCC biopsies from patients using immunohistochemistry. ROC curves evaluated the diagnostic performance of the top deregulated genes in each dataset. We show that metabolic pathways are mostly deregulated in ccRCC and we highlight those being most responsible in its formation. We suggest that these genes are candidate predictive markers of the disease.

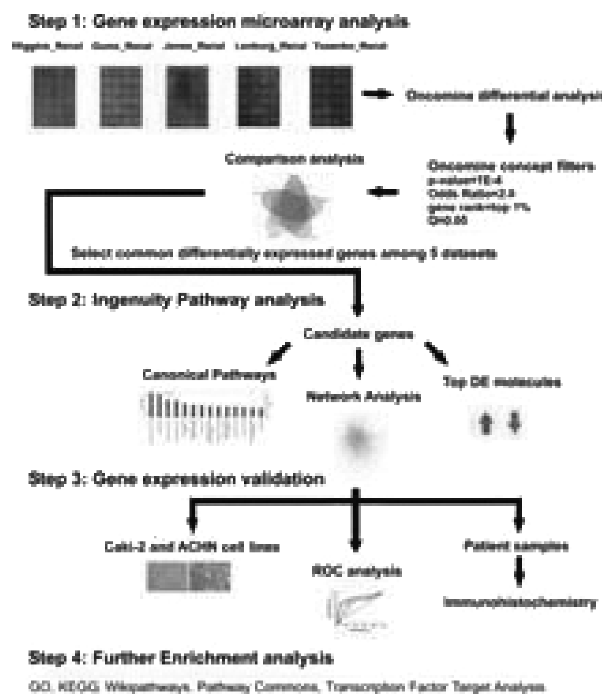

Fig. 1.

Keywords: clear-cell renal cell carcinoma; gene networks; metabolism.

MON-050
Micro RNA'S in cancer: is their a potential for targetting strategies

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Over the past few years, scientists have discovered that a new class of genetic regulators called "microRNAs" influence normal human growth and development. There is a growing evidence that microRNAs may also play an important role in human cancer and its etiology. Cancer is a complex and dynamic disease, involving a variety of changes in gene expression and structure. Traditionally, the study of cancer has focused on protein-coding genes, considering these as the principal effectors and regulators of tumorigenesis. Recent advances, however, have brought non-protein-coding RNA into the spotlight. MicroRNAs (mi RNAs), one such class of non-coding RNAs, have been implicated in the regulation of cell growth, differentiation, and apoptosis. While their study is still at an early stage, and their mechanism of action along with their importance in cancer is not yet fully understood, they may provide an important layer of genetic regulation in tumorigenesis, and ultimately become valuable therapeutic tools. Targeting strategies for these genes and m RNA which are supposed to control and regulate more than one target, estimates indicate they may be able to regulate up to 30 percent of the protein-coding genes in the human genome. Therefore, by using novel carriers such as liposomes, appended liposomes, fusosomes etc. and targeting to the regulated sites for therapeutic benefits.

Keywords: Cancer, micro RNA, targetting.

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MON-051
MicroRNA biomarkers for detection of lung ischemia reperfusion injury

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Background: Ischemia reperfusion injury (IRI) is a leading cause of acute lung injury, a common problem in various clinical settings associated with significant morbidity and mortality. MicroRNAs (miRs) are a class of small single stranded noncoding RNAs that function as post-translational regulators of specific target mRNAs. Changes of miRs expression have been associated with different diseases and pathophysiological conditions. Accumulating evidence underlines a critical function for miRNAs in the modulation of innate and adaptive immune responses. In the last years they have emerged as regulators of IRI and it has been suggested that the miRs are potentially involved in the pathogenesis of solid organ rejection, including renal, intestinal and hepatic rejection. However the role of miRs on lung IRI has not been completely understood yet. Lidocaine (lido), a commonly used local anesthetic agent, has proved its anti-inflammatory activity in several tissues including lung but its possible modulation of miRs has not been investigated.
Aim: To investigate a potential involvement of miRNAs in lung IRI in a lung auto-transplant model. In addition the effect of lidocaine was investigated.
Animals and Methods: 3 groups (Sham-operated, control and lido) of 6 large-white pigs each were submitted to a left lung auto-transplant. All groups received the same anesthesia. In addition animals of lidocaine group received a continuous IV administration of lidocaine (1.5 mg/Kg/h) during surgery. In order to measure the expression of miRs, lung tissue samples were taken at: 1) 5 minutes before pulmonary artery clamp (PPn), 2) 5 min before reperfusion (PRp), 3) 30 min post-reperfusion (PR30) and 4) 60 min post-reperfusion (PR60). Lung tissue samples were analyzed for miRs (miR-122, miR-145, miR-146a, miR-182, miR-107, miR-192, miR-16, miR-21, miR-126, miR-127, miR142-5p, miR152, miR155, miR-223 and let7) using RT-QPCR. Results were normalized using miR-103.
Results: The expression of miR-127 and mir-16 did not increase after IRI. All the other miRs investigated exhibited more than two-fold differences at the PR60 time point and this effect was positively correlated with the severity of IRI. This increase in miRs levels was significantly down-regulated by lido administration.
Conclusions: Our results support the notion that IRI causes changes in miRs expression that can be used as markers of injury. In addition, lidocaine administration modifies miRs expression.
Keywords: ischemia reperfusion injury, lung, miRNAs.

MON-052
MicroRNA expression profiling predicts melanoma metastatic potential

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Melanoma is aggressive skin, uveal or mucosal tumor with early metastatic onset. Even small tumors are capable of producing distant metastases. Uncertainties in the detection of metastases complicate the choice of a suitable treatment strategy.

The aim of our work is to develop a set of microRNA capable of discrimination between non-invasive and metastatic melanoma. We performed miRNA-seq analysis of 14 benign skin tumors, 18 primary melanoma neoplasms including 7 metastatic ones. We identified the set of 23 microRNA with statistically significant expression alterations between metastatic and non-metastatic melanomas ($p < 0.05$) and 17 microRNA – between benign tumors and malignant melanomas. Additionally we performed a screening of literature results obtained previously by other groups and finally collected a set of 31 microRNA with presumably differential expression patterns between metastatic and non-invasive melanomas, and a set of 25 microRNA – between melanomas and benign tumors. RT-qPCR validation allowed to identify a subset of miR-145, miR-150, miR-155, miR-193a/b, miR-196a, miR-211, miR-214, miR-221/222, miR-342-3p, miR-455-3p and miR-497 capable of discrimination between melanoma skin cancer and benign tumors (with overall sensitivity of approx. 90% and specificity of 85%), and subset of miR-30b miR-145, miR-149, miR-155, miR-182, miR-200a/b/c, miR-221/222 and miR-497 for the evaluation of the presence of metastases (sensitivity 87% and specificity 79%). These biomarker sets includes well-known miR-182 targeting *FOXO3* and miR-30b targeting *GALNT7*.

Further validation of these microRNA biomarker candidates on an extended cohort of patients would allow the creation of test-systems capable of detecting both malignant melanomas and discriminate tumors with high metastatic potential to facilitate the choice of appropriate therapy strategy.

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Keywords: melanoma, metastases, microRNA.

MON-053
MicroRNA-15b/16 enhances the induction of regulatory T cells by regulating the expression of Rictor and mTOR

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Background: Regulatory T cells (Tregs) are required for maintenance of immune tolerance and protect from unwanted immune

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responses. The development of Tregs requires microRNA (miRNA) regulation of gene expression. Previous work has identified microRNAs (miRNAs) as important regulators of Tregs. Conditional knockouts of the genes encoding Drosha and Dicer, which are RNases required for miRNA synthesis, significantly decrease Tregs development and function. However, the question of how individual miRNAs regulate the gene expression program controlling the development and function of Tregs remains to be elucidated.

Methods: *In vitro* induction of Tregs, over-expression and inhibition of miRNAs using retroviral based vectors, Protein estimation by Western Blot, Flow-cytomix for cytokine measurement, Flow cytometry and *in vivo* model of inflammatory bowel disease.

Findings: To understand miRNA function in Treg development, we set about to identify important miRNAs and their relevant target genes. Of the more abundantly expressed miRNAs in Tregs, only miR-15b/16, miR-24, and miR-29a impacted the *in vitro* induction of Tregs (iTregs) in overexpression and blocking experiments. miRNA mimics for these significantly enhanced the induction of iTregs in *Dicer*$^{-/-}$ CD4^{+} T cells. Furthermore, the overexpression of miR-15b/16 in conventional CD4^{+} T cells reconstituted into *Rag2*$^{-/-}$ mice increased the *in vivo* development of peripheral Tregs and diminished the autoimmune response. In looking for targets of miR-15b/16, it was observed that the mTOR signaling pathway was enhanced in *Dicer*$^{-/-}$ CD4^{+} T cells, and its pharmacological inhibition restored induction of iTregs. The mTORC2 component Rictor contained a functional target site for miR-15b/16. Rictor was more abundantly expressed in *Dicer*$^{-/-}$ T cells as was mTOR, and their expression was downregulated by the overexpression of miR-15b/16. Finally, knockdown of Rictor by siRNAs enhanced Treg induction in *Dicer*$^{-/-}$ CD4^{+} T cells.

Conclusion: Our data provide an important mechanism of miRNA regulation of Tregs development is through regulation of the mTOR signaling pathway and its novel role in the development of inflammatory bowel disease.

Keywords: Inflammation, miRNAs, Tregs.

MON-054
microRNA-9 – in ALS pathogenesis and therapy

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Amyotrophic lateral scleroses (ALS) is a progressive neurodegenerative disorder of the motor system, which is characterized by the loss of upper and lower motor neurons. ALS patients rapidly deteriorate after the onset of symptoms and the expected survival time is of only a few years. The development of therapies is halted by limited knowledge about the patho–mechanisms underlying the disease.

Pioneering studies from recent years discovered ALS-causing mutations in several genes including SOD1, TDP-43, FUS, hnRNPA1 and C9ORF72. Many of these genes encode for RNA-binding proteins, thus raising the hypothesis that dysregulation of RNA activity is involved in the pathogenesis of ALS.

microRNAs (miRNAs) are small non-coding RNAs that silence gene expression post transcriptionally, in a sequence-dependent manner, and have been suggested by our group and others to play a role in ALS pathology. One particular miRNA gene that gathers significant attention is miR-9, one of the most highly abundant miRNAs in the brain. miR-9 was previously suggested to play important roles in brain development, post-mitotic neural development and neurite morphogenesis. Interestingly, dysregulation of miR-9 expression was demonstrated in neurodegenerative disorders including Alzheimer's disease and Huntington's disease. Importantly, miR-9 was recently found to be upregulated in the spinal cord from ALS mice (SOD1 G93A). We therefore hypothesize that miR-9 upregulation contributes to pathogenesis and investigate if inhibition of miR-9 is beneficial in ALS.

We utilize a unique method for manipulation of miRNA expression in vivo. The method is based on delivery of recombinant adeno-associated virus, pseudotype 9 (rAAV9), which effectively transduces motor neurons after stereotactic intraventricular (ICV) injection. We utilize rAAV9 to overexpress miR-9 or inhibit miR-9 activity, (sing a tough decoy (TuD) in SOD1 G93A mice and assess the clinical impact of miR-9 on overall survival, body weight, neurological score and locomotion.

Our data demonstrate that reduction of miR-9 expression in spinal cord and brain of SOD1 G93Amice increases survival and improves neuromuscular function. Accordingly, miR-9 overexpression resulted in notable decreases SOD1G93A mice survival. We further identified specific targets of miR-9 and investigate novel pathways downstream of miR-9 in ALS pathogenesis.

miRNA research has broad implications for understanding mechanisms of brain integrity, and the pathogenesis of ALS and other neurodegenerative disorders. miRNA research may lead in addition to the development of a novel RNA-based therapeutic approaches.

Keywords: ALS, microRNA, RNA therapy.

MON-055
Micro-RNA-mediated targeting of Runx2 reduces breast cancer metastasis and progression of osteolytic bone disease

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Progression of breast cancer to metastatic bone disease is associated with an aberrantly elevated expression of Runx2, which promotes the disease progression by activating many genes involved in the 'vicious cycle' of cancer-induced bone disease. Because transcription factors are not readily targetable for therapeutic intervention, our goal was to evaluate the potential clinical use of Runx2-targeting miRNAs to reduce tumor growth and bone metastatic burden. Expression analysis of a panel of miRNAs regulating Runx2 revealed a reciprocal relationship between the abundance of Runx2 protein and two miRNAs, miR-135 and miR-203. These miRNAs are highly expressed in normal breast epithelial cells where Runx2 is not detected, and conversely are absent in metastatic breast cancer cell lines and tissue biopsies that express Runx2. Reconstituting metastatic MDA-MB-231-Luc cells with miR-135 and miR-203 reduced the abundance of Runx2 and the expression of the metastasis-promoting Runx2 target genes IL-11, MMP-13, and PTHrP. Additionally, tumor cell viability decreased and cell migration was suppressed *in vitro*. *In vivo* implantation of MDA-MB-231-luc cells stably expressing miR-135 or miR-203 into the mammary gland, followed by additional intratumoral administration of the synthetic miRNAs reduced tumor growth and importantly, spontaneous metastasis to bone. Furthermore, intratibial injection of these miRNA-expressing cells impaired

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tumor growth in the bone environment, inhibited bone resorption and secondary metastasis to lung. We conclude that miRNAs targeting Runx2 are protective against metastasis, while deregulated expression of Runx2 in aggressive tumor cells is related to the loss of specific Runx2-targeting miRNAs. Our studies have also demonstrated for the first time that delivery of synthetic miRNAs is a viable therapeutic strategy to target transcription factors for the prevention of metastatic bone disease.

Keywords: bone metastasis, breast cancer, micro rna.

MON-056
miR-199a-5p regulates urothelial permeability and bladder smooth muscle cell morphology and plays a role in bladder dysfunction

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Chronic lower urinary tract diseases, including bladder pain syndrome / interstitial cystitis (BPS/IC) and bladder outlet obstruction-mediated overactive bladder, pose a recurrent problem in urological practice. Epithelial dysfunction resulting in a leaky urothelium has been suggested as a possible cause of BPS. Some of the changes in the expression levels of signalling and adhesion molecules in the bladder can be attributed to the alterations in the levels of regulatory microRNAs, which we have identified in the biopsies of BPS patients. Recently we showed that microRNA miR-199a-5p, which was elevated in BPS, is an important regulator of intercellular junctions. Upon overexpression in urothelial cells it directly targeted mRNAs encoding LIN7C, ARHGAP12, PALS1, RND1 and PVRL1 and impaired correct tight junction formation leading to increased permeability. MiR-199a-5p is predominantly expressed in the bladder smooth muscle, but also detected in the mature bladder urothelium and primary urothelial cultures. In the urothelium its expression can be up-regulated following activation of cAMP signaling pathways. Ectopic expression of miR-199a-5p in TEU-2 urothelial cells and subsequent mRNA-sequencing analysis revealed the activation of cytoskeleton remodelling, TGF, WNT and cell adhesion signalling pathways.

We sought to elucidate the function of mir-199a-5p in the bladder smooth muscle cells (SMC), and investigated its levels in the biopsies of patients with bladder outlet obstruction-induced fibrotic changes in the detrusor. In contrast to BPS, the miR-199a-5p levels were significantly down-regulated in the patients with end-stage fibrotic acontractile bladders. Using primary cultures of bladder smooth muscle cells we show that, concomitant with the smooth muscle cell de-differentiation, miR-199a-5p expression was decreasing, and the levels of its target mRNAs increased. Anti-miR-199a-5p expressed in SMC using lentiviral vectors selectively inhibited miR-199a-5p function. It caused an increase of cell proliferation, a significant cell size reduction and an up-regulation of WNT2 and other miR-199a-5p targets. Concomitant down-regulation of WNT2 in antimiR-transduced SMCs using WNT2 shRNA-expressing lentiviruses normalized WNT2 mRNA levels, and restored the cell phenotype and proliferation rates.

Our results point to a crucial role of miR-199a-5p in the WNT-mediated regulation of proliferative, developmental and fibrotic processes in the bladder smooth muscle. MiR-199a-5p thus may behave as a key modulator of the smooth muscle hypertrophy and fibrosis, relevant for bladder obstruction-induced organ remodelling.

Keywords: bladder smooth muscle, microRNA, WNT signalling.

MON-057
miR-449 controls apical actin network formation during multiciliogenesis of *Xenopus laevis* embryos

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Multiciliated cells (MCCs), found throughout the metazoan kingdom, contribute to multiple biological processes. Recently, we demonstrated that microRNAs of the miR-449 family control vertebrate MCCs differentiation by repressing the Notch pathway (REF). Here, we report that the apical actin cytoskeleton reorganization, a prerequisite for basal bodies anchoring and cilia elongation, is also controlled by miR-449. Using *Xenopus* embryonic epidermis as a model system, we show that miR-449 silencing inhibits apical actin web formation in MCCs. We identify transcripts coding for the small GTPase R-Ras as miR-449 validated targets. Apical actin reorganization and multiciliogenesis were impaired when the *RRAS* mRNA was protected from miR-449 binding. Multiciliogenesis was rescued when the translation of protected *RRAS* transcripts was prevented. Altogether, our data demonstrate that miR-449 acts at several distinct steps of multiciliogenesis in vertebrates, and identify R-Ras as a new player in apical actin reorganization.

Keywords: cilia, microRNA, R-Ras.

MON-058
miRNA profiles in the mammary gland of dairy and beef cattle breeds

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Micro RNA (miRNAs) are small non-coding RNAs which participate in gene regulation and various metabolic processes. Its function during mammary gland development and activity of mammary gland stem cell niche is still unknown. In the present study, we performed high-throughput microarray analysis of miRNAs expression in mammary gland of 2 years-old non-pregnant Holstein–Friesian (HF) and Limousin (LM) heifers to identify miRNAs associated with high dairy potential. Statistical analysis was based on unpaired t-test with Benjamini-Hochberg multiple testing correction ($p \leq 0.05$). Ontological analyses of miRNA targets were carried out using binomial overrepresentation test with Bonferroni correction ($p \leq 0.05$). We identified 52 miRNAs differing significantly between mammary gland of dairy and beef cattle. The highest interbreed differences in expression changes were observed in the following miRNAs: bta-miR-375, bta-miR-218, bta-miR-24, bta-miR-183, bta-miR-147, bta-miR-204, bta-miR-421, bta-miR-155, bta-miR-146b, bta-miR-218_v13.0, bta-miR-29b, bta-miR-154c, bta-miR-101, bta-miR-194, bta-miR-1434-3p, bta-miR-2285t. Results obtained showed higher levels of miRNA expression in the mammary glands of LM heifers. Enrichment analyses performed for targeted genes revealed that major differences between miRNA activity in mammary glands of dairy and beef heifers are associated with regulation of signalling pathways crucial for mammary gland development. Among highly significantly targeted signalling pathways were: inflammatory pathways, TGF-beta, EGFR, insulin and WNT signalling pathways. Based on performed ontological and interactions network analyses we identified more than 20 genes associated with stem cells activity

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significantly targeted by differentially expressed miRNAs. These results indicate that high mammogenic potential of dairy cattle is not only dependent of genetic and central neuro-endocrine regulation, but also epigenetic control by miRNA from local intramammary factors. We suppose that this release from miRNAs inhibitory influence on genes whose products are associated with stem cell renewal and constitute the microenvironment for stem cells niche, may be an important mechanism promoting mammary gland development and high milk productivity.

Keywords: mammary gland, Microarrays, miRNAs.

MON-059
Modulation of PTEN expression by SET/TAF-Ibeta

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PTEN (phosphatase and tensin homolog) protein controls the levels of the phospholipid phosphatidylinositol (3,4,5) -trisphosphate (PIP3), and consequently, the PI3K-Akt signals to cell survival, proliferation and growth. SET/template-activating factor-Ibeta (TAF-Ibeta) participates in histone acetylation control, as a subunit of the inhibitor of the histone acetyltransferases (INHAT) complex; recently, it has been reported that SET inhibits p53 acetylation resulting in transcription repression of the p53 target genes. Although it is known that p53 positively regulates PTEN expression, we previously observed that SET protein accumulation increases phosphorylated PTEN (inactive form) levels and this coincided with cell survival and Akt activation in head and neck squamous cell carcinoma (HNSCC). This observation suggests that when SET is accumulated, PTEN expression can be regulated by mechanisms other than p53. Then, in this study we hypothesized that SET can regulate PTEN by modulating the levels of microRNAs associated with PTEN expression, such as mir-19a, mir-21 and mir-214. For this, we analyzed by quantitative real time PCR (Syber Green®) the levels of PTEN mRNA in the HNSCC cell lines Cal27, HN12 and HN13, as well as in HEK293 cells overexpressing SET (HEK293/SET). The levels of mir-19a, mir-21 and mir-214 were assessed by using the TaqMan® Assay in HEK293-SET and HNSCC cells transfected with small interfering RNA (siRNA) for SET (Qiagen). The levels of PTEN were altered in HEK293/SET, as well as in HN12, HN13 and Cal27 cells with siRNA for SET. Also, the levels of the microRNAs studied were modulated in the presence of SET silencing. Therefore, we can propose that SET regulates the PTEN mRNA levels through modulation of the microRNAs mir-19a, mir-21 and mir-214. This new SET action in PTEN is in line with our previous evidence that SET accumulation induces PTEN inactivation, considering that both the responses to SET accumulation contribute to activate Akt signaling and promote cell survival.

Keywords: head and neck carcinoma, micro rna, SET/TAF-Ibeta.

MON-060
Pathophysiological role of inflammation-related microRNA in murine skin wound healing

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MicroRNAs (miRNAs) belong to the small, non-coding RNA family; they consist of approximately 20–25 nucleotides of single stranded RNA and regulate translational processing of target mRNAs. In this study, we screened for known and novel miRNAs that showed altered expression dynamics during repair of murine skin wounds, using next generation sequencing. We identified inflammation-related miRNAs by comparing WT and PU.1 KO mice, which possess no neutrophils, macrophages, mast cells, or T-cells, and thus cannot raise a standard inflammatory response. These mice exhibit rapid repair and scarless healing in contrast to their WT littermates. The candidate miRNAs identified may be involved in various level regulation of the inflammatory response at sites of tissue repair. We are currently analyzing the functions of these inflammation-related miRNAs in skin wound healing using knockout mice.

Keywords: inflammation, micro rna, wound healing.

MON-061
Ponatinib inhibits breast cancer cell proliferation by regulating miRNA expressions

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Breast cancer remains the most common malignancy in women. Dysregulated FGFR is a significant risk factor in breast cancer. Ponatinib is a multi-targeted, ATP-competitive tyrosine kinase inhibitor that inhibits BCR-ABL and also several kinases such as SRC family kinases, FGFR, KIT, VEGFR, PDGFR. MicroRNAs (miRNAs) are single strand, non-coding RNA molecules with the length of 18–25 nucleotides which have role in the epigenetic regulation of gene expressions.

The aim of the study was to evaluate the effect of ponatinib on the miRNA expression levels associated with breast cancer in MCF-7 cells. Also we aimed to investigate cytotoxic and apoptotic effects of ponatinib.

Cytotoxic effects of ponatinib in MCF-7 cells were measured online with xCELLigence system. Apoptotic effects of the IC_{50} dose of ponatinib were evaluated by ApoDIRECT In Situ DNA Fragmentation Assay with flow cytometry. For this study, custom design 96-well plates consist of miRNA primers which are important in breast cancer progression were used for miRNA expression analysis by using Light Cycler 480 real time online qRT-PCR. $_{\Delta\Delta}$CT method was used for data analysis. Statistical analysis was performed by web based RT² Profiler PCR Array Data Analysis program.

IC_{50} dose of ponatinib was calculated as. It was shown that IC_{50} dose of ponatinib induced apoptosis approximately 3 fold according to the control cells. The tumor suppressor hsa-let-7a-5p that is down-regulated in breast cancer is up-regulated 24.99 fold with ponatinib treatment (4.59 μM). Two of the members of oncogenic miR-17-92 cluster hsa-miR-19b-3p expression down-regulated 4.37 fold change and hsa-miR-19a-3p expression completely suppressed. In breast cancer hsa-miR-210-3p overexpression is associated with tumor growth and proliferation, migration and invasion and this oncomiR is also down-regulated 5.16 fold with ponatinib treatment.

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In conclusion, ponatinib can be an attractive drug for inhibiting breast cancer cell proliferation via deregulating expressions of miRNAs which are related with breast cancer.

Keywords: breast cancer, miRNAs, Ponatinib.

MON-062
Profiling of circulating miRNAs in response to toxic or traumatic skeletal muscles damages in rats

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Skeletal muscle damages can occur in a wide range of situations such as genetic myopathies, physical exercise, or toxic and traumatic injuries. Classical blood markers display high variability. Recently, circulating microRNAs (miRNAs) have been identified as biomarkers of various pathologies or tissue damage. Muscle-specific miRNAs could be biomarkers of muscle dystrophies. However, whether miRNAs can be used as markers of acute and limited muscle damage, the most frequent, is unknown. Our aim was to study plasma miRNAs profiles and kinetics in response to muscle injury in rat, to identify new markers.

Muscle injury was induced in the right soleus muscle of male rats by either notexin injection (NTX) or by crushing (CRUSH). Blood samples were drawn 6 h, 12 h, 24 h, and 48 h post-injury in NTX, CRUSH or sham operated (SHAM) rats and in a control group (CTRL) to measure creatine kinase (CK) and circulating miRNAs. First, a RT-PCR profiling of 752 miRNAs was performed in plasma pools of NTX/CRUSH and CTRL 6 h, 12 h and 24 h after injury. Then, 79 miRNAs were selected on the basis of their detectability, tissue specificity or literature description and analyzed in each sample. Results were normalized with the seven most stables miRNAs.

Plasma CK increased in NTX at 6 h and 12 h post-injury with a return to the baseline at 24 h, no changes were found in other groups. Plasma levels of muscle-specific miRNAs miR-1-3p, miR-133a, miR-133b, miR-206-3p and miR-499-5p were increased in NTX group with a peak value at 12 h compare to CRUSH, SHAM and CTRL. Similar profiles were observed for non muscle-related miR-378a-3p, miR-434-3p and miR-409-3p. These results could be explained by the fact that notexin injection is known to be a more drastic model and is associated with extended muscles damages compared to crush model. MiR-208b was increased in NTX and to a lower extent in CRUSH at 6 h and 12 h. miR-497-5p and miR-93-5p progressively decreased from 12 h to the minimum at 48 h in NTX. Interestingly, miR-122-5p which has been proposed as a markers of liver injury, transiently increased at 6 h in NTX and CRUSH injuries. Whether miR-122-5p was released by injured muscle or by liver is unknown. MiR-126a-3p, highly expressed in endothelial cells, remained stable and cardiac-specific miR-208a, proposed as a marker of cardiac injury, remained undetectable.

In conclusion, we describe new circulating miRNA profiles associated with skeletal muscle injuries. Further analysis will determine how to combine miRNAs measurements to optimize diagnosis.

Keywords: Biomarkers, Circulating miRNAs, Muscle damage.

MON-063
Reduction of Hepatitis C viral replication through targeting HCV-genotype-4 p7 ion channel by mir-29a in cell replicon

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P7 is a hepatitis C virus (HCV) protein that is essential for assembly and release of HCV. The crucial role which the P7 ion channel plays in HCV life cycle renders it an attractive therapeutic target. Recent data suggest that effectiveness of P7 inhibitors depends mainly on the amino acid sequence of P7 ion channel which shows significant heterogeneity among the different HCV genotypes. γT827A,T977S vector harboring P7 sequence of genotype 4 HCV. The designed cell replicon was transfected with mimics and antagomirs of mir-29a. Interestingly, It was found that mir29a mimics reduced viral titer by 49% (p = 0.04).

In the present study we endeavored to search for microRNAs that bind to a sequence specific to the P7 protein of genotype 4, in an attempt to investigate a novel therapeutic approach for HCV-genotype 4 infection. We thus performed a bioinformatics analysis, which revealed that mir-29a has 2 binding sites on the P7 ion channel. Using cell culture system, we transfected Huh7 cell lines with in-vitro transcribed pED43/JFH1-

In conclusion, decreasing viral load after forcing expression of mir29a suggests a potential role for this microRNA in targeting HCV P7 ion channel.

Keywords: Hepatitis C Virus, micro RNA.

MON-064
Serum micro RNA122 as a prognostic marker in patients with liver cirrhosis

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Chronic liver diseases and cirrhosis are now being recognized as an important cause of morbidity and mortality worldwide. Established cirrhosis has a 10-year mortality of 34–66%. Hepatorenal syndrome and spontaneous bacterial peritonitis are important cause of mortality in cirrhosis. Patients with liver cirrhosis are mostly asymptomatic until decompensation occurs, so it is very difficult to assess the real prevalence and incidence of cirrhosis in the general population. In the liver miR-122 accounts for approximately 70% of all miRs, whereas other organs express much lower amounts of this miR. miR-122 regulates many genes in the liver that control the cell cycle, differentiation, proliferation and apoptosis. In contrast loss of miR-122 in the liver leads to hepatic differentiation with malignant phenotype. The aim of the study was to evaluate miR-122 as a prognostic marker in patients with liver cirrhosis. The study included 100 patients with liver cirrhosis All were subjected to clinical evaluation, abdominal ultrasonography, a group of laboratory investigations and serum miR-122 level by real time PCR. Serum miR-122 level among the studied groups showed significant statistical difference between group I "compensated" and both groups II "ascites" and III "SBP" (P < 0.001), while there was *high* statistical significanct difference between group I "compensated" and group IV "HRS" (P < 0.001). *S*trong negative correlation between serum miR-122 level and MELD score (p = 0.001), and very strong negative correlation between serum miR-122 level and Child score (p < 0.001). Lower serum miR-122 levels are associated with ascites, spontaneous bacterial peritonitis and hepatorenal syndrome. Therefore, serum miR-122 could be considered as a new

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potential parameter for liver function and a prognostic parameter in patients with liver cirrhosis.
Keywords: None.

MON-065
Silencing of HSP90 affects miRNA profile in monocyte-like cells

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Heat shock protein 90 (HSP90), a highly conserved chaperone, is crucial for the stability and function of many proteins, including oncogenic proteins involved in tumorigenesis (*e.g.* antiapoptotic proteins, transcription factors, signal-transduction proteins, Tyr-kinase receptors, *etc*.). Inhibition of HSP90 has been shown to be a promising therapy approach with clinical relevance for treatment of specific tumour types. On the other hand, microRNAs (miRNAs), small, non-coding RNAs (20–23 nucleotides) involved in the control of gene expression, are recognised as key players in the pathogenesis of human malignancies, but also as potential biomarkers and therapeutic targets. Yet, there is no data on the effects of the suppression of HSP90 expression on miRNA profile. This is why is this study we investigated the miRNA profile in monocyte-like cells after the exposure to the specific small interference RNA (siRNA) directed to HSP90 mRNA.

The reduction of Hsp90 mRNA expression by 70% and 65% was accomplished following 24 h and 48 h of transfection of human acute monocytic leukemia THP-1 cells with 10 nM siRNA, respectively, as determined by the TaqMan real-time PCR quantification. ApoTox Glo assay revealed that, at the same time, silencing did not affect cell viability, neither showed cytotoxic nor pro-apoptotic effect. The expression patterns of microRNAs were determined using Agilent microRNA arrays on biological duplicate time-courses. Cells transfected with scrambled RNA served as controls.

The obtained data suggest profound changes of miRNA profile in the cells with significantly reduced HSP90 expression; namely, after 24 h, the expression of 3 miRNAs were reduced (miR-195, miR-221, miR-224) while the expression of 20 miRNAs were significantly increased, especially of let-7b and let-7f, miR-15a, miR-4270, miR-769, miR-933. After 48 h, the reduced expression of 14 miRNAs (*e.g.* miR-1, miR-125b, miR133b, miR145, miR-451, miR-517a and b), and increased expression of 10 miRNAs, the most significantly of miR-1234, miR-135a, miR-146a, miR-374, and miR-4298 were observed.

The understanding of biological meaning of the observed changes of miRNA profile after supersession of HSP90 expression as well as molecular mechanisms underlying these events remain to be elucidated. These data could contribute to the better understanding of mode of action of the therapeutic HSP90 inhibitors but also reveal potential new therapeutic targets.
Keywords: HSP90, miRNA.

MON-066
siRNA loaded chitosomes as an adjuvant therapy in cancer treatment

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The aim of the present study was to study the effect of siRNA loaded chitosomes as an adjuvant in chemotherapy with doxorubicin. siRNA was designed to target anti-apoptotic *Bcl-2* gene. Chitosomes were synthesized by coating liposomes prepared by reverse phase evaporation method using phosphatidylcholine and cholesterol with water soluble chitosan. The effect of process variables on mean particle size, polydispersity index, zeta potential and encapsulation efficiency of prepared chitosomes were studied using factorial design. siRNA loaded chitosomes were evaluated for serum and short term stability studies. Cytotoxicity of siRNA loaded chitosomes and doxorubicin was evaluated by MTT assay on normal and cancerous cells. Expression studies were performed after transfecting siRNA loaded chitosomes to MCF-7 and MDA-MB-453 cells. *In vivo* efficacy of siRNA loaded chitosomes with doxorubicin were tested in Ehrlich Ascites Carcinoma (EAC) tumor bearing Swiss albino mice. Chitosomes were having mean particle size of 220 nm, surface charge +30 mV, PDI 0.18 and encapsulation efficiency of siRNA was 40%. Chitosomes were found to be stable up to 12 weeks at 4 °C. siRNA degradation by serum nucleases was prevented up to 24 h when incubated with 10% FBS. Blank chitosomes were found to be non-toxic. mRNA and protein level of *Bcl-2* was significantly reduced by chitosomes. Chitosomes with half the dose of doxorubicin were found to be effective in improving increase in mean survival time and % increase in life span of tumor bearing mice. Chitosomes loaded with siRNA can be promoted as an adjuvant therapy in cancer treatment to reduce the dose of the chemotherapeutic agent and reduce the toxicity associated with it.

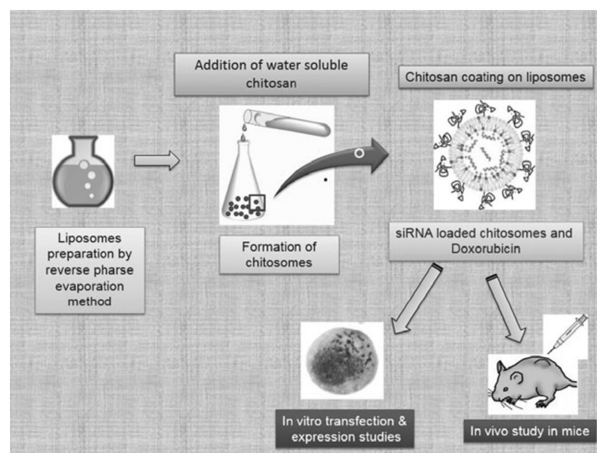

Fig. 1.

Keywords: cancer, Chitosomes, siRNA.

MON-068
Targeting cancer cell specific by siRNA-peptide complex

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Despite the clinical response of anticancer treatments, including conventional chemotherapeutic treatments, tumorigenic B-cell lymphomas show an incomplete response to clinical treatments that result in a minimal residual disease (MRD) where few residual neoplastic cells undetected *in vivo*, replenish the cancer cell

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reservoir. This scenario, which is also shared with other cancer diseases, requires the development of strategies to advance in novel, selective targeting toward the tumorigenic cells that survive to the anticancer agents. Here, our experimental system takes advantage of the therapeutic properties of an idiotype specific peptide (A20-36) that binds specifically the B-cell receptor (BCR) of murine lymphoma cells (A20 cell line) [1] in the setting of an anticancer strategy, based on the selected delivery of electrostatic-based complex, peptide-siRNA [2]. To this purpose, two engineered, arginine rich, peptides that included the A20-36 targeting sequence (EYVNCDNLVGNCVI) were designed to bind fluorescent-labeled siRNA. One peptide was engineered at C- terminal of A20-36 with 9 Arg (9R-A2036) whereas the other included 5 Arg at the N- and C-terminus, respectively (5R-A2036-5R). A random peptide (SSAYGSCKGPCSSGVHSI) arginine modified has been used as control. Compared to the random peptide-siRNA complexes, both A20-36-siRNA complexes were able to selective deliver of fluorescent-labeled siRNA into A20 cells, as evaluated by cytofluorimetry and confocal microscopy, whereas fluorescent-labeled siRNA alone was not internalized. For potential delivery *in vitro*, the capability of peptides to deliver an anti-GAPDH siRNA in the cells was investigated by evaluating the expression levels of the enzyme. The results suggest that both A2036-9R and 5R-A2036-5R peptides may enable the delivery of siRNA to B cells. Even though we observed a relative modest silencing of GAPDH, with respect to classical transfection procedure, this method should have the great advantage of being based on the specific ligand-receptor interaction delivery of the siRNA [3]. In this setting, the improvement of this strategy is expected to provide a safe and non-invasive approach for the delivery of therapeutic molecules.

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Keywords: B lymphoma cell, drug delivery, siRNA-peptide complex.

MON-069
The intestinal microbiota interferes with the microRNA response upon oral *Listeria* infection

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The intestinal tract is the largest reservoir of microbes in the human body. The intestinal microbiota is thought to be able to modulate alterations of the gut induced by enteropathogens, thereby maintaining homeostasis. *Listeria monocytogenes* is the agent of listeriosis, an infection transmitted to humans upon ingestion of contaminated food. Crossing of the intestinal barrier is a critical step of the infection before dissemination into deeper organs. Upon infection, the setting up of a contentious dialogue between the bacterium and its host translates into a drastic remodelling of both prokaryotic and eukaryotic gene expression programmes.

Here, we have investigated the role of the intestinal microbiota in the post-transcriptional regulation of the host transcriptome during infection [1]. We highlight a complex interplay between the host cell, the bacterial invader and the microbiota on the regulation of both host genes and microRNAs during intestinal response to listeriosis. We provide a regulatory network defined by the host microRNAs and their target genes during infection, and show that the microbiota plays an important role in modulating microRNA-based regulations. The influence of the microbiota on host gene expression correlates with its property to dampen the infectious process. Our work provides an unprecedented insight into the impact of the intestinal microbiota on host transcriptional reprogramming during infection by a human pathogen.

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Keywords: Listeria monocytogenes, Microbiota, microRNA.

MON-070
The role of miRNAs in zebrafish cardiac regeneration

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Zebrafish exhibit a tremendous ability to regenerate their organs, i.e brain, fin and heart, after injury. miRNAs are small non coding RNAs regulating genes at the post-transcriptional level and they have been implicated in virtually every cellular process so far. It is thus rational to hypothesise that miRNAs play a role during the regeneration process, where the precise regulation of wound closure, necrotic tissue removal as well as production and patterning of new cells into functional tissue is of paramount importance. Complete regeneration is missing from mammals, despite being much needed after cases like heart infarction. We used zebrafish to identify miRNAs that are differentially regulated during heart regeneration after cryoinjury. This miRNA signature could represent functional features missing or failing to be activated in mammals. One of the upregulated miRNAs at 4 days post cryoinjury is miR-15c. Using TALEN technology, we induced a genomic lesion resulting in an 11nt deletion in the mir-15c transcript, removing the seed region and thus theoretically disrupting miR-15c function. The mutant fish do not show an obvious phenotype during development and are viable. Their response to heart injury remains to be tested.

Keywords: miRNAs, regeneration, zebrafish.

MON-071
Uncovering the dual clinical value of miR-143/145 cluster in bladder cancer epithelium and patients survival outcome

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Forming a cluster, miR-143 and miR-145 are colocalized at 5q32 and expressed by a bicistronic primary transcript. A well-documented tumor suppressor role has been attributed to miR-143/145 cluster, targeting HRas, KRas, c-Myc, MDM2, IGF1R and

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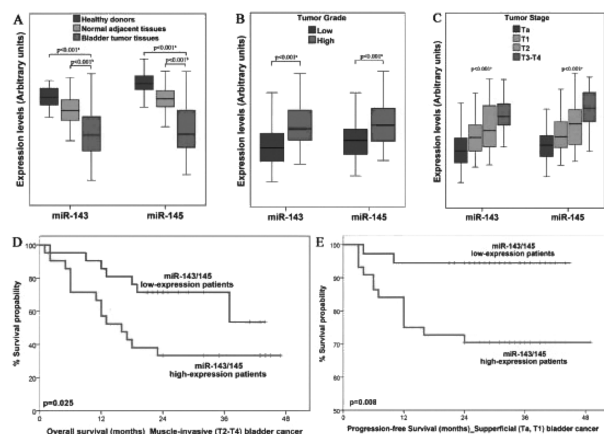

Fig. 1. Evaluation of miR-143/145 cluster clinical significance in bladder cancer. Expression analysis of miR-143/145 in bladder tumors and normal tissues (A), according to tumor grade (B) and stage (C). Kaplan-Meier analysis of the OS of the muscle-invasive (T2-T4; D) and the PFS of the superficial (Ta, T1; E) BlCa patients.

EGFR, in human malignancies, including bladder cancer (BlCa). However, its clinical significance for disease progression and the survival of the patients has been only partially investigated. Total RNA was isolated from separate specimens obtained from bladder tumors and the adjacent normal bladder wall of 140 BlCa patients, as well as from 40 healthy donors. Thereafter, total RNA was polyadenylated and reverse transcribed to cDNA, which was subjected to qPCR analysis. The expression of miR-143/145 was strongly downregulated in BlCa patients compared to healthy donors ($p < 0.001$), while reduced expression revealed in approximately the 80% of the tumors compared to their normal adjacent tissues ($p < 0.001$). An opposite profile was observed in tumor specimens, as elevated miR-143/145 levels were correlated with high-grade tumors and muscle-invasive (T2-T4) BlCa compared to low-grade or superficial (Ta, T1) tumors ($p < 0.001$). A strong unfavorable value of miR-143/145 for BlCa was thereafter revealed as its higher expression was correlated with poor patients' survival. Muscle-invasive BlCa (T2-T4) patients with higher miR-143/145 levels showed significantly shorter overall survival (OS; Kaplan-Meier analysis: $p = 0.025$) compared to those with reduced expression. Additionally, higher risk for disease progression to muscle-invasive BlCa (Cox multivariate analysis: HR= 4.756; $p = 0.016$) and significantly shorter progression-free survival (PFS; Kaplan-Meier analysis: $p = 0.008$) was observed for the superficial BlCa (Ta, T1) patients with elevated miR-143/145 levels, independently from tumor grade and stage. These data clearly highlight the deregulation of miR-143/145 expression in BlCa epithelium and their clinical significance for the prediction of superficial tumors progression to muscle-invasive stage and the survival expectancy of the patients.

Acknowledgments: This research was partially funded by the University of Athens Special Account of Research Grants no 10812.

Keywords: bladder cancer, miR-143, miR-145.

MON-072
Uncovering the interconnectivity between infertility-associated woman diseases

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Background: Infertility became a major problem worldwide. Nowadays, approximately 50 million couples worldwide are affected from infertility. Infertility is defined as a disease of the reproductive system that impairs the body's ability to perform the basic function of reproduction. The goal of this study is to integrate transcriptomics datasets with biological networks to (i) identify candidate genes, proteins and metabolites that have the potential for being biomarker for infertility-associated woman diseases, and (ii) map the interconnectivities between genetic mechanisms of these diseases.

Method: Nineteen transcriptomics datasets for infertility-associated woman diseases (polycystic ovary syndrome, endometriosis, ovarian, cervical cancer and uterine leiomyomas) were analysed statistically (via RMA and LIMMA methods) to identify differentially expressed genes (DEGs) for each disease. Proteins encoded by DEGs were determined and data was integrated with reconstructed protein-protein interaction network and human Recon2 metabolic model for further analyses (via BioMet toolbox) to identify reporter genes, proteins and metabolites. Enrichment analyses were performed (via DAVID bioinformatics tool) to map the interconnectivities between diseases and biological pathways.

Results:Integrated analyses indicated that (i) samples of same disease obtained from different donors were representing variable gene expression profiles (i.e., 1–25% of the genome was differentially expressed); (ii) the inspected diseases were representing high interconnectivities at protein-protein interaction level; and (iii) several genes and proteins were representing potential for being biomarker for these infertility-associated women diseases.

Conclusion: Analysis of whole genome expression profiles provides a good opportunity for systems level investigation to characterize the interconnectivity between infertility-associated woman diseases and provides hypotheses for further *in vivo* studies to characterize novel biomarker proteins.

Keywords: Biomarker, Infertility, Transcriptomics.

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CSII-05 – Neural Circuits

MON-075
Activation of NMDA receptors causes disruption of the cerebral endothelial cell-constructing tight junction barrier

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Background: The N-methyl-D-aspartate (NMDA) receptor is a ligand-gated calcium (Ca^{2+}) channel. Recently, the NMDA receptor (NMDAR) was implicated in blood-brain barrier (BBB) dysfunction. The present study was designed to evaluate the effects of the NMDAR on the cerebral endothelial cell (CEC)-constructed barrier and its possible signal-transducing mechanisms.
Methods: Cultured mouse CECs were used as an experimental model. We performed immunofluorescence, immunoblotting, RT-PCR assays, and an intracellular Ca^{2+} analysis to determine the existence of the NMDAR. After treatment with NMDA, the permeability extents of transendothelial electrical resistance (TEER) and FITC-dextrans were measured. And the occludin structure was immunostained. Levels of occludin, matrix metalloproteinase (MMP) 2 and 9, extracellular signal-regulated kinase (ERK) 1/2, and mitogen-activated/ERK kinase (MEK) 1 were detected and quantified.
Results: The presence of NMDAR subunits was proven at the mRNA and protein levels. Analysis of intracellular Ca^{2+} mobilization demonstrated the functionality of the NMDAR. After exposure to NMDA for 24 h, the TEER value decreased and the structure of occludin became disorganized. Investigations also revealed the occludin protein level significantly decreased. As to the mechanism, NMDA enhanced MMP2 and MMP9 levels in time-dependent manners. Meanwhile, levels of phosphorylated (p) MEK1 and pERK1/2 were augmented following NMDA treatment.
Conclusions: In this study we show that activation of the NMDAR caused dysfunction of the BBB by decreasing the occludin protein level and possibly through a MMP expression and MEK/ERK signaling pathway.
Keywords: cerebral endothelial cells, NMDA receptor, tight junction.

MON-076
Anxiolytic-like effect of α-asarone from *Acorus gramineus* in a rat model using chronic corticosterone injections

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We investigated the anxiolytic-like activity of α-asarone from *Acorus gramineus* (AAS) in an experimental rat model of anxiety induced by repeated administration of the exogenous stress hormone corticosterone (CORT). The putative anxiolytic effect of AAS was studied in behavioral tests of anxiety, such as the elevated plus maze (EPM) test and the hole-board test (HBT) in rats. For 21 consecutive days, male rats received 50, 100, or 200 mg/kg AAS (i.p.) 30 min prior to a daily injection of CORT. Dysregulation of the HPA axis in response to the repeated CORT injections was confirmed by measuring serum levels of CORT and the expression of corticotrophin-releasing factor (CRF) in the hypothalamus. Daily AAS (200 mg/kg) administration increased open-arm exploration significantly in the EPM test, and it increased the duration of head dipping activity in the HBT. It also blocked the increase in tyrosine hydroxylase (TH) expression in the locus coeruleus (LC) and decreased mRNA expression of brain-derived neurotrophic factor (BDNF) and its receptor, TrkB, in the hippocampus. These results indicated that the administration of AAS prior to high-dose exogenous CORT significantly improved anxiety-like behaviors, which are associated with modification of the central noradrenergic system and with BDNF function in rats. The current finding may improve understanding of the neurobiological mechanisms responsible for changes in emotions induced by repeated administration of high doses of CORT or by elevated levels of hormones associated with chronic stress. Thus, AAS did exhibit an anxiolytic-like effects in animal models of anxiety.
Keywords: Anxiety.

MON-077
Behavioral and brain-region specific biochemical changes in streptozotocin-induced diabetes

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Type I diabetes (T1D) is characterised by lack of insulin (Ins) secreting pancreatic β cells while type II diabetes (T2D) by reduced sensitivity of peripheral tissues to Ins. Previous work in experimental animals indicated that T1D may show an association with depression-like behavior, while T2D has been linked to dementia. The aim of the present study was to investigate the effects of streptozotocin-induced T1D on the cholinergic system and on behavioral indices including fear-anxiety, memory-learning, social dominance and depression. We also sought to determine whether Ins administration can reverse these effects in T1D adult male mice. Mice (n = 42) were divided into 3 groups (n = 14). The first 2 groups became diabetic after intraperitoneal (IP) administration of streptozotocin (50 mg/kg body weight/per day), for 5 consecutive days. After 21 days the second diabetic group was given long-acting Ins glargine (6 IU/kg) IP for 6 consecutive days. A euglycemic control animal group was also included in the study. All 3 groups underwent behavioral analysis 2 days following Ins administration in the Ins-treated mice. Fear-anxiety was assessed by using the thigmotaxis test and the elevated plus-maze test. Memory-learning was evaluated by the step-through passive avoidance test. Social dominance was assessed by the social dominance tube test. Depression-like behavior was evaluated by the forced swimming test. In addition to the behavioral tests biochemical analyses, including acetylcholine (ACh) levels, the acetylcholinesterase (AChE) activity in different brain regions (cerebral cortex, midbrain, hippocampus, striatum, diencephalon and cerebellum), plasma glucose, total cholesterol and triglycerides levels, were performed. Correlation coefficients between behavioral indices and glucose levels were also determined. The results showed that T1D engendered anxiogenesis, memory loss,

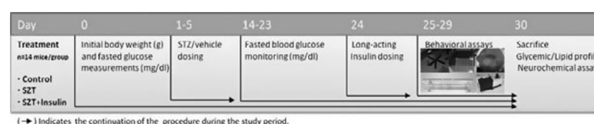

Fig. 1.

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social defeat and depression-like behavior in mice. Spearman correlation analysis revealed that the coefficients between the particular behaviors and plasma glucose levels were linearly correlated. In T1D mice, we observed a statistically significant decrease in the ACh levels of the brain regions studied. The AChE activity in both salt-soluble and detergent-soluble fractions was significantly increased in the same brain regions. In the Ins-treated T1D mice both ACh levels and AChE activity were efficiently reversed in a brain region-dependent manner. Additional biochemical and molecular mechanisms involved in cholinergic system adaptation in diabetes are currently under investigation.

Keywords: Behavior, Brain, Type 1 diabetes.

MON-079

Diabetes induces changes in motor proteins distribution in the rat retina: implications for axonal transport

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Diabetic retinopathy is a leading cause of vision loss and blindness in working-age adults. It has been considered a microvascular disease, but increasing evidence has shown that the neural components are also affected, even before the detection of vascular changes. However, the mechanisms underlying neural dysfunction are not elucidated. Impairment of axonal transport is associated with several neurodegenerative diseases and might also play a role in diabetes-associated disorders affecting nervous system. In this study, we investigated the impact of type 1 diabetes (2 and 8 weeks duration) on KIF1A, KIF5B and dynein motor proteins in the retina. Additionally, since hyperglycemia is considered the main trigger of diabetic complications, we investigated whether prolonged exposure to elevated glucose could affect the content and distribution of motor proteins in retinal cultures.

The immunoreactivity of motor proteins was evaluated by immunohistochemistry in retinal sections and by immunoblotting in total retinal extracts from streptozotocin-induced diabetic and age-matched control animals. Primary retinal cultures were exposed to high glucose (30 mM) or mannitol (osmotic control; 24.5 mM plus 5.5 mM glucose), for seven days.

Diabetes decreased the content of KIF1A at 8 weeks of diabetes as well as KIF1A immunoreactivity in the majority of retinal layers, except for the photoreceptor and outer nuclear layer (ONL). Changes in KIF5B immunoreactivity were also detected by immunohistochemistry in the retina at 8 weeks of diabetes, being increased at the photoreceptor and ONL, and decreased in the ganglion cell layer (GCL). Regarding dynein immunoreactivity there was an increase in the GCL after 8 weeks of diabetes. No changes in the immunoreactivity of motor protein were found in retinal cultures.

The alterations in motor proteins detected in the retinas of diabetic animals suggest that axonal transport may be impaired under diabetes, which might contribute to early signs of neural dysfunction in the retina of diabetic patients and animal models.

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Keywords: diabetes mellitus, kinesin trafficking, Retrograde transport.

MON-080

Early cranial irradiation alters epigenetics parallel to reduced hippocampal neurogenesis in adult mice

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Radiotherapy is an effective tool in the treatment of pediatric malignancies but it is associated with adverse side effects, both short and long term. One of the cardinal late onset effects is cognitive deficits. Exposure of cranial irradiation to the early brain in rodents has been shown to potentially change the hippocampal neurogenesis levels in adulthood. Here, we demonstrated that epigenetics is also altered accompanying with the reduced hippocampal neurogenesis in the adult brain of C57BL6/J mice long after the early cranial irradiation.

For the experimental design, a single dose of 8 Gray (Gy) whole cranial irradiation at postnatal day 14 (P14) (Rad^+ Group) or double doses (Rad^{++} Group) of 8 Gy both at P14 and P21 (total of 16 Gy) were administered to the pups. Additionally, a group of age and body weight matched mice were assigned as sham (anesthetic) or naive controls. Seven months after the cranial irradiation, three main groups of mice (Control, Sham and Rad) were first assigned for Open Field (OF) test to measure the locomotor activity, and afterwards for Morris Water Maze (MWM) paradigm to test the hippocampal dependent spatial learning and long term memory. No significant difference was observed between the groups in OF test. In the MWM paradigm, Rad^+ and Rad^{++} groups displayed significantly weaker cognitive abilities as compared to the controls. Lastly, a significant dose-dependent difference of irradiation was also detected.

Following MWM experiments, we employed immunohistochemical stainings (im) with phenotypic neuronal and epigenetic markers to test the ongoing neurogenesis and epigenetic events in the P230 hippocampi. We found a significant decrease of Doublecortin-im (immature neuron marker) and Neuronal Nuclei-im (mature neuron marker) in the subgranular layer of the dentate gyrus of irradiated mice as compared to the controls. In the same hippocampal regions, there was also significant reduction of DNA methylation determinants (DNA methyl-transferase (DNMT) DNMT1-im, DNMT3a-im and Methyl-CpG Binding Protein 2-im positive cells). Our overall data suggests that exposure of cranial irradiation to the young brain alters not only the neurogenesis but also the epigenetic profile in adult hippocampus which may reflect the cellular base of the weakened cognitive abilities observed in the MWM experiments. All data was analyzed via parametric tests, using SPSS statistical software (V17).

Understanding the mechanism by which ionizing radiation affects epigenetic programming will provide insight into how to develop protection against the potentially harmful risks associated with radiation exposure.

Keywords: Adult Hippocampal Neurogenesis, Cranial Irradiation, DNA methylation.

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MON-081
Endogenous human neuromodulators Lynx1 and Slurp1: structure, function and mechanism of action

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Discovery of endogenous neuromodulators Lynx and SLURP having homology both to Ly6 proteins and three-finger snake α-neurotoxins expressed in the various human tissues provoked much interest to their role in the organism. In contrast to α-neurotoxins, highly specific inhibitors of nicotinic acetylcholine receptors (nAChRs), Lynx and SLURP proteins exhibit their activity only in the presence of a receptor agonist and characterized as allosteric modulators. Lynx proteins are membrane-tethered by a GPI anchor and co-localized near nAChRs in the brain, while SLURPs are secreted proteins and probably play the role of autocrine/paracrine hormones in different non-neuronal tissues.

We developed the effective bacterial expression systems, which allowed us to produce the set of mutant and isotope labeled variants of water-soluble domain of human Lynx1 (ws-Lynx1) and human SLURP1. For the first time ws-Lynx1 and SLURP1 activity against the number of pharmacologically relevant receptors was characterized, and new targets were revealed. We characterized the distribution of Lynx1 at the organ, cellular, and sub-cellular level, and for the first time detected Lynx1 in the cerebrospinal fluid. Spatial structure and conformational plasticity of neuromodulators were studied by NMR-spectroscopy. Contrary to α-neurotoxins having rigid structures, NMR analysis revealed high mobility of the loop regions of ws-Lynx1 and SLURP1. Site-directed mutagenesis identified amino acid residues of ws-Lynx1 important for interaction with muscle-type and α7 nAChR. Computer modeling, based on NMR structures of ws-Lynx1 and SLURP1 and the model of extracellular domain of α7 nAChR, together with mutagenesis data, revealed a possible modes of interaction between neuromodulators and receptor. It was shown that in spite of the high structural homology, Lynx1, SLUPR1, and α-neurotoxins interact with the receptor in different manners and their binding sites overlapped only partially.

Keywords: Lynx1, nAChR, SLURP1.

MON-082
ETS proteins Elk-1 and Pea3 and their transcriptional targets in neuronal survival and differentiation

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ETS proteins are transcription factors that are targeted by the MAPK pathway, and are associated with a wide range of processes including proliferation, survival, inflammation, and differentiation. Although they are known to bind a typical GGAA/T motif, the domain structure, interaction partners, and DNA recognition motirs of the subfamilies of ETS proteins can vary immensely, resulting in a wide range of responses in different tissues. In neurons, Elk-1, a member of the Ternary Complex Factor (TCF) subfamily, was reported in axonal and dendritic structures and to correlate with survival, with *survival of motor neuron (SMN)* as a novel target promoter. On the other hand Pea3 subfamily of ETS proteins, namely Pea3, Erm and ER81, was reported to function in establishing functional motor neuron circuits, although primary transcriptional targets were not thoroughly analyzed. In this study, we have carried out microarray analysis in neuronal model cells transfected with Elk-1, Pea3, Erm and Er81 in an effort to distinguish transcriptional targets of these proteins. Interestingly, quite a large percentage of the genes were found to be repressed in Pea3-transfected cells, including Elk-1; in the case of Pea3 subfamily main groups of genes found to be upregulated were found to be either immune system-related or neuron-related genes, in particular cytoskeletal dynamics-related genes, whereas Elk-1 was found to regulate channel or transport protein coding genes, hypoxia-related genes, putative Pea3 targets, as well as genes related to apoptosis and proliferation. The putative neuronal targets of Elk-1 and Pea3 proteins, as well as potential cross-regulatory loop between them, will be discussed in relation to neuronal survival and differentiation.

Keywords: ETS, Neuronal cell death, Neuronal differentiation.

MON-083
Evaluation of three novel hydroxypicolinaldehyde oximes as efficient uncharged reactivators of VX-inhibited human acetylcholinesterase

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Organophosphates (OP) inhibit acetylcholinesterase (AChE, EC 3.1.1.7), both in peripheral tissues and the central nervous system, causing adverse and sometimes fatal effects due to the accumulation of the neurotransmitter acetylcholine. The currently used therapy, which focuses on the reactivation of inhibited AChE, is limited to peripheral tissues because the commonly used quaternary pyridinium oxime reactivators do not cross the blood brain barrier at therapeutically relevant levels. Three new uncharged oximes **1** [E)-3-hydroxy-6-(4-morpholinobutyl)picolinaldehyde oxime], **2** [(E)-6-(5-(diethylamino)pentyl)-3-hydroxypicolinaldehyde oxime], and **3**[(Z)-3-hydroxy-6-(5-(piperidin-1-yl) pentyl)picolinaldehyde oxime] have been synthesized. These novel compounds exhibited *in vitro* reactivation potencies toward VX-phosphorylated human acetylcholinesterase equal or superior to those of pyridinium oximes (HI-6, 2PAM, etc.), which are currently used as antidotes against nerve agents. The evaluation of individual reactivation constants revealed that the potent reactivation exhibited by oxime **1** referred to its efficient interaction with the phosphonate group of the VX-conjugated AChE through forming a transition state reflected in an up to 2-times faster k_{+2} than HI-6. Interestingly, the binding affinity of the other two oximes was very similar to that of HI-6, meaning that the structural requirements of novel compounds to react with phosphorylated AChE were fulfilled. In conclusion, we obtained potential antidotes for OP-poisoning that could act centrally, reactivating brain OP-phosphorylated AChE.

Keywords: acetylcholinesterase, enzyme kinetics, reactivation.

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MON-084
Excitatory and depressor synaptic processes in neurons of spinal cord and substantia nigra in conditions of Parkinson's disease combined with bilateral ovariectomy and protection by synestrol

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In intact female Albino rats, subjected to bilateral ovaryectomy (OVX) in conjoining with Parkinson's disease (PD) and with protection by Sinestrole (S) after 4-5 weeks the activity registration of spinal cord (SC) L4-L5 segments‘ motoneurons (MNs) to high frequency stimulation (HFS) of hind limbs extensor (P), flexor (G) nerves and subnstantia nigra (SN), as well as SN to HFS of Caudate Putamen (CPu) by means of on-line selection and software mathematical analysis based on depressor and excitatory tetanic and post tetanic (unilateral and mixed) effects revealed the following: In norm noted the prevalence of excitatory tetanic effects, except of those in SN neurons to HFS of CPu, in which along with powerful depressor reaction took place reasonably expressed excitatory, not lower than the above-mentioned. In conditions of OVX combined with PD was found comparatively worse expression of depressor effects in all examined structures, except the SC MNs to HFS of G, while such excitatory effects were increased, especially to HFS of SN and also except SC MNs to HFS of G. However, only in conditions of OVX, compared with norm, in SC MNs to HFS of P and G depression was declined, especially in poststimulus depressor succession and increase of excitability to HFS of P, particularly in the same mixed condition; to HFS of SN depression increased only in unilateral succession, but twice elevated the excitation in such excitatory reactions and finally in SN neurons to HFS of CPu a depression declined or was equal the norm, was not excitatory sequence and slightly increased tetanic potentiation in a mixed sequence. In conditions of S protection on the model of PD with OVX in SC MNs to HFS of P, G and SN it is obvious the significant increase of tetanic depression, except it abrupt decrease in SN neurons. Also fell sharply the tetanic excitation in SC MNs and SN neurons, but with increase the background activity. Thus tetanic excitation in conditions of OVX with PD has experienced an increase and with S – decrease, except SN neurons.

Keywords: Parkinson's disease, SPINAL CORD, substantia nigra.

MON-085
Microarray identification of potential novel targets of Ets-domain transcription factor Pea3

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PEA3 subfamily of ETS domain transcription factors act as nuclear targets of signal-transduction pathways, regulating variety of responses including cell proliferation, differentiation, development and apoptosis. Pea3 subfamily, including Pea3, Erm and Er81, has been reported to regulate functional motor neuron circuitry, as well as identify specific motor neuron pools. Pea3's transactivation capacity is known to be regulated by MAPK pathways in response to stimuli, and a number of target promoters involved in breast cancer progression and metastasis have been identified. However, the specific downstream targets of Pea3 in neuronal circuitry are still not clear. In this study, we have used gene expression microarray analysis to determine potential novel target genes in SH-SY5Y model system. To that end, cells were transfected with either Pea3VP16 (constituvely active) expression vector or pCDNA3 (empty vector) and microarray analysis was performed. Microarray data were analyzed by bioinformatics tools and database such as KEGG pathway analysis program and DAVID. Subsequently, promoters of nervous system-related genes were analyzed for presence of a consensus Pea3-binding ets motif. Furthermore, mRNA expression levels of a selected subset of genes were confirmed by using Real-time PCR or RT-PCR.

Keywords: Pea3 transcription factor, Microarray, Identification.

MON-086
Parasympathetic ganglia derive from Schwann Cell Precursors

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Neural crest cells migrate extensively and give rise to most of the peripheral nervous system, including the three types of autonomic ganglia: sympathetic, parasympathetic and enteric. The migration pathways of sympathetic and enteric precursors towards the dorsal aorta or in the walls of the gut, repectively, are well charted, but surprisingly little is known about the way in which parasympathetic ganglia form, at many locations, close to their target organs.

Here, we show that parasympathetic precursors first colonize cranial nerves, where they co-express the pan-visceral transcriptional determinant Phox2b and markers of Schwann Cell Precursors (SCPs). Some of these cells down-regulate Phox2b and give rise to Schwann cells, while others maintain Phox2b expression and go on to form parasympathetic ganglia. Thus, cranial SCPs have a dual neuronal/glial fate and are the source of parasympathetic neurons during normal development. Unexpectedly, cells with a history of Phox2b expression are also found associated with limb nerves, and are even capable of forming at least one ganglion, of unknown function, in the upper limb, showing that the dual Schwann cell/neuron fate is not unique to cranial nerves.

These data imply that the numerous locations of parasympathetic ganglia throughout the head and trunk are specified by the pattern of cranial nerve outgrowths —which carry preganglionic parasympathetic fibers— a parsimonious way of hooking up synaptic partners in development.

Keywords: Neural Crest Cells, Parasympathetic ganglia, Schwann Cells.

MON-087
Profiling imidazolium and benzimidazolium oximes as antidotes in organophosphorus compound poisoning

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Organophosphorus compounds (OP) used as pesticides account for more than 3 000 000 accidental or deliberate cases of poisoning registered per year worldwide. Furthermore, OPs developed as nerve agents (soman, sarin, tabun, VX) still present a threat in terrorist attacks and conflicts, such as in the recent case of Syria. The main targets of OP compounds are cholines-

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terases (ChEs) of the central and peripheral nervous system: acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8). Upon exposure, OP compounds covalently bind to the specific active site serine after which physiological activity of these enzymes is permanently lost. The antidotal property of the oximes is attributed to their ability to reactivate ChEs inhibited by OPs and thus restore vital functions and reduce the severe consequences for an organism. In the search for more efficient oximes, a better understanding of their interactions with ChEs presents an important step considering the strict criteria set for their *in vivo* applications. For this purpose, we investigated a set of imidazolium and benzimidazolium oximes, presenting a structural shift from the oximes currently used in medical practice. A comprehensive analysis was performed defining all relevant kinetic parameters of reactivation as well as interactions with uninhibited ChEs. Several oximes were pointed out as leads in the development of new antidotes especially in the case of OP-inhibited BChE reactivation. Experimental data were correlated with computational studies including QSAR analysis. We were able to thoroughly describe the characteristic of novel oximes that contribute to favourable interactions with ChEs and should be the basis for further structure refinement. Furthermore, novel oximes were characterised for their cytotoxic profiles on a set of cell lines in order to elucidate all unwanted effects *in vivo* before investigating their further development as potential pharmaceuticals.

Keywords: acetylcholinesterase, butyrylcholinesterase, reactivation.

MON-088
Promising serum protein marker for early detection of Alzheimer's disease

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Sirtuin (SIRT) pathway has a crucial role in Alzheimer's disease (AD). The present study evaluated the alterations in serum sirtuin1 (SIRT1) concentration in healthy individuals (young and old) and patients with AD and mild cognitive impairment (MCI). Blood samples were collected from 40 AD and 9 MCI patients as cases and 22 young healthy adults and 22 healthy elderly individuals as controls. Serum SIRT1 was estimated by Surface Plasmon Resonance (SPR), Western Blot and Enzyme Linked Immunosorbent Assay (ELISA). A significant ($p < 0.0001$) decline in SIRT1 concentration was observed in patients with AD (2.27 ± 0.46 ng/μl) and MCI (3.64 ± 0.15 ng/μl) compared to healthy elderly individuals (4.82 ± 0.4 ng/μl). The serum SIRT1 concentration in healthy elderly was also significantly lower ($p < 0.0001$) compared to young healthy controls (8.16 ± 0.87 ng/μl). This study, first of its kind, has demonstrated, decline in serum concentration of SIRT1 in healthy individuals as they age. In patients with AD and MCI the decline was even more pronounced, which provides an opportunity to develop this protein as a predictive marker of AD in early stages with suitable cut off values.

Keywords: Alzheimer, Mild cognitive Impairment, Sirtuin.

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CSII-06 – Translation and Ribosomes

MON-091

A study on the effects of chaperone fusions on protein folding and activity using firefly luciferase fusions with DnaK and GroEL

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The quantities of proteins required for biotechnological or pharmaceutical applications are often so vast that they cannot be supplied from their natural reservoirs. Modern biotechnology has provided solutions by developing systems for protein overproduction in many organisms, especially the bacterium *Escherichia coli*. However, the potential of *E. coli* as a protein «cell factory» is limited by the inability of many overproduced proteins to fold properly and their subsequent accumulation in the bacterial inclusion bodies. Taking advantage of the normal roles of the major *E. coli* molecular chaperones, DnaK and GroEL, in protein folding, we constructed plasmid vectors that express N-terminal fusions with DnaK or GroEL and showed that such fusions can overcome the solubility problem. To study whether these soluble chaperone fusions have acquired proper conformation and are biologically active, we fused the firefly luciferase (LUC) gene to DnaK or GroEL. LUC overexpression in bacteria, at both 28°C and 37°C, yields little soluble protein, while the vast majority accumulates in inclusion bodies. In contrast, the DnaK-LUC fusion accumulates mainly in the soluble fraction (especially at 28°C) and its solubility increases when co-expressed with DnaJ and GrpE, the two DnaK co-chaperones. GroEL-LUC displays only intermediate solubility. Following IMAC purification of the recombinant LUC and its fused forms under denaturing conditions, we measured the ability of these proteins to remain in the soluble fraction after removal of the denaturant. Following dialysis, the chaperone-luciferase fusions remained predominantly soluble, whereas the majority of the unfused LUC precipitated. *In vitro* protein folding experiments, measuring the regain of luciferase activity after chemical denaturation, showed that DnaK-LUC fusions refold far more efficiently than unfused LUC, even in the absence of ATP. ATP addition to the refolding reactions dramatically improved both the rate and degree of refolding, which was further increased in the presence of DnaJ and GrpE. To verify the specificity of these results, we tested a fusion of LUC to DnaK25, a DnaK mutant with ATP hydrolysis and substrate binding defects and found that its protein folding ability was compromised. These results shed light on the molecular mechanisms involved in the protein folding properties of chaperone fusions. More importantly, they indicate that chaperone fusions can refold with high efficiency *in vitro*, following chemical denaturation and removal of the denaturant. The latter property has significant implications in the production of biotechnologically relevant proteins.

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Keywords: chaperone fusions, protein folding, recombinant protein expression.

MON-093

Crystal structures of the γ subunit of the heterotrimeric translation initiation factor 2 of archaeal aIF2 in GTP-bound and GDP-bound forms

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In eukaryotes and archaea, translation initiation begins with the assembly of the ternary complex (TC), consisting of initiator methionyl-tRNA (Met-tRNAi) anchored to the GTP-bound form of the heterotrimeric initiation factor 2 (e/aIF2). GTP hydrolysis and Pi release from e/aIF2 control the correct selection by the initiation ribosomal complex of the initiator tRNA and of the start codon on the mRNA. In eukaryotes, the GTPase activating protein (GAP), eIF5, participates in the control of this checking step. However, archaea have no equivalent of eIF5. Therefore, GTP hydrolysis on aIF2 is likely to occur without GAP assistance.

The catalytic mechanism of the GTP hydrolysis on e/aIF2 remains unknown. In our study, we determined the crystal structures of the wild-type aIF2 γ subunit from Sulfolobus solfataricus bound to GDPNP, and GDP at high resolution (1.4 Å and 2.1 Å, respectively). The structures revealed conformational changes of the protein upon nucleotide binding, in particular in the P-loop and in the switch I and switch II regions. These regions carry catalytically important and conserved residues. In particular, a histidine residue in the switch II region and an aspartate in the GKT loop are conserved in e/aIF2 sequences but also in the sequence of elongation factors. These residues were modified by site-directed mutagenesis. The crystal structures of the mutant proteins bound to GTP were determined. The data give some clues about the mechanism of GTP-hydrolysis by aIF2. New hypotheses are being tested using molecular dynamics simulations.

Keywords: Archaea, GTP hydrolysis, translation initiation.

MON-094

Effect of Alu RNP and scAlu RNP on transaltion initiation in vitro

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Human mobile genetic elements present in genome predominantly like Alu repeats.

There was found near 1.3 million ones that form 10% of human genome. Most of Alu repeats are transcribed by RNA pol III generating Alu RNA. Alu RNA tends to degrade to scAlu RNA (small cellular Alu RNA). These ncRNAs specifically bind protein heterodimer SRP 9/14. Alu RNP complexes inhibit translation initiation at uncapped mRNA without 5'UTR in cell-free translation systems. We carried out action of Alu/scAlu RNP to understand mechanism of the inhibition. For this purpose we used cell-free system and reconstituted eukaryotic translation system (RETS). We estimated effect of Alu/scAlu RNP on mRNA with different 5'UTRs in rabbit reticulocyte lysate. The influence of Alu/scAlu

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RNP and RNA on initiation complex assembly was detected by toe-print analysis in RETS. We have found that scAlu RNP inhibits mRNA containing IRES's translation in RRL. However Alu/scAlu RNP had no effect on cap-dependent initiation (on mRNA with β-actin and BRCA1 leaders). But uncapped β-actin leader was inhibited by scAlu RNP. Also we have found inhibition of 80S assembling on CrPV leader by Alu RNP in REST but scAlu RNP had no effect. Ribosome assembling on mRNA containing uncapped

β-globin UTR was affected by both Alu and scAlu RNP. We propose that scAlu RNP inhibits any cap-independent loading of mRNA in 40S ribosomal subunit. Human mobile genetic elements present in genome predominantly like Alu repeats. There was found near 1.3 million ones that form 10% of human genome. Most of Alu repeats are transcribed by RNA pol III generating Alu RNA. Alu RNA tends to degrade to scAlu RNA (small cellular Alu RNA). These ncRNAs specifically bind protein heterodimer SRP 9/14. Alu RNP complexes inhibit translation initiation at uncapped mRNA without 5'UTR in cell-freetranslation systems. We carried out action of Alu/scAlu RNP to understand mechanism of the inhibition. For this purpose we used cell-free system and reconstituted eukaryotic translation system (RETS). We estimated effect of Alu/scAlu RNP on mRNA with different 5'UTRs in rabbit reticulocyte lysate. The influence of Alu/scAlu RNP and RNA on initiation complex assembly was detected by toe-print analysis in RETS. We have found that scAlu RNP inhibits mRNA containing IRES's translation in RRL. However Alu/scAlu RNP had no effect on cap-dependent initiation (on mRNA with β-actin and BRCA1 leaders). But uncapped β-actin leader was inhibited by scAlu RNP. Also we have found inhibition of 80S assembling on CrPV leader by Alu RNP in REST but scAlu RNP had no effect. Ribosome assembling on mRNA containing uncapped β-globin UTR was affected by both Alu and scAlu RNP. We propose that scAlu RNP inhibits any cap-independent loading of mRNA in 40S ribosomal subunit.

Keywords: Alu RNP, eukaryotic transalation initiation.

MON-095
eIF6-deficient mice exhibit thrombocytopenia due to a defect in polyploidization and maturation of megakaryocytes

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eIF6 is a translation factor acting downstream of insulin/PMA stimulation. Herein, we investigated the consequences of *eIF6* deletion in mice on platelets biogenesis and hemostatis. We show that eIF6 deficiency leads to thrombocytopenia, resulting in severely impaired hemostasis, as indicated by prolonged bleeding time upon injury. The reduction in platelets count is the consequence of a defect in platelets production owing to a defect in bone marrow (BM) megakaryopoieis. As a matter of fact, BM eIF6$^{+/-}$-derived megakaryocytes (MKs) exhibit a decrease in mean ploidy level and a delay in the expression of terminal MK differentiation markers, as for instance the glycoprotein Ib (GPIb). It is well accepted that MKs cell size is related to the degree of polyploidization. Interestingly, both cycling (2N/4N) and polyploid (16N) eIF6$^{+/-}$-derived MKs are reduced in size, with a size reduction slightly more pronounced in mature polyploid MKs than in immature ones. Consistent with a failure in terminal MKs differentiation and maturation, analysis of platelet production *in vitro* reveals that fraction of proplatelet forming megakaryocytes is significantly lower in cultured eIF6$^{+/-}$ MKs than in WT cells. This is the first report of a translation factor regulating platelet development. Analysis of differentially regulated genes between eIF6$^{+/+}$ and eIF6$^{+/-}$ MKs will shed more light on the mechanism by which eIF6 regulates megakaryopoiesis.

Keywords: None.

MON-096
Function of rare codon

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More 26,000 protein coding sequences (CCDS) have been identified in the human genome. Here, we analyzed the codon usage in all these human CCDS and found that there are preferential usage of minor codons for Ala, Pro and Ser in the initial part of CCDS. The usage of GCG out of four Ala codons, CCG out of four Pro codons, UCG out of six Ser codons and ACG out of four Thr codons are significantly higher in the initial 50 codons of CCDS. These codons are most rarely used among their synonymous codons. The gene expression of luciferase-chimeric protein containing five consecutive GCG (rarest used Ala codon) in the initial part of the gene is significantly higher than that containing five consecutive GCC (most frequently used Ala codon). Our results show that the rare codon slow translation and decrease error rate lead to increased translational efficiency reason of because the rare codon may have similar pattern of Kozak sequence, having a strong affinity for ribosome.

Keywords: protein translation, rare codon, tRNA.

MON-097
Function of the Sec61p Loop 7 in ER protein tranlocation

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The Sec61 complex is a heterotrimeric complex responsible for the translocation of secretory proteins through the endoplasmic reticulum (ER) membrane and also likely for the retrolansloca-tion of misfolded proteins subject to ER-Associated Degradation (ERAD). Sec61p, the pore forming subunit of the channel, is a transmembrane protein with 10 transmembrane domains and a single large luminal loop (L7). This loop is thought to be important for the opening of the channel and due to is position and exposure, a potential point of contact between chaperones and ERAD substrates in the ER lumen and the channel. We are working with a Sec61 mutant lacking L7, which has profound post-translational protein import and soluble protein ERAD defects but no cotranslational protein import defect. Our main objective is to identify possible L7 interacting proteins through chemical crosslinking using isolated ER membranes. Crosslinking with a homobifuctional crosslinker (NH_2-reactive) showed that the absence of the L7 impairs the interaction of Sec61p with the other two subunits of the complex (Sbh1p and Sss1p). In preliminary tests with a heterobifuctional crosslinker (NH_2 and non-specific reactive) we identified several potential interactions with Sec61p that are strongly impaired in the sec61ΔL7 mutant. We are currently trying to identify the unknown interacting proteins by analysis of the complexes with a combination of immunoprecipitation, immunoblotting and mass spectrometry. Our ultimate

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aim is to ascertain the role of these interactions in the bidirectional transport through the Sec61 channel.
Keywords: Endoplasmic Reticulum, Protein Translocation, Sec61p.

MON-098
Function of the Sec61p N-terminus in protein translocation across the ER membrane

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Sec61p is a highly conserved polytopic membrane protein characterized by a compact bundle of 10 transmembrane helices spanning the ER membrane. The two halves of the protein form an aqueous pore in the ER membrane and a lateral gate facing the lipid bilayer. Sec61p is the channel-forming subunit of the heterotrimeric Sec61 complex that mediates co-translational import; two single-spanning membrane proteins, Sss1p and Sbh1p, stabilize the channel and mediate interactions with other proteins. In yeast, proteins can also be post-translationally translocated into the ER by the heptameric Sec complex, composed of the Sec61 complex and the heterotetrameric Sec63 complex. The Sec61 channel is also a candidate for the dislocation channel for ERAD substrates. The N-terminus of Sec61p is oriented towards the cytosol and residues 3–21 have the potential to form an amphipathic α-helix; moreover, Sec61p is N-terminally acetylated and the N-terminal acetylation of Sec61p was shown to be important for its function at high temperature (Soromani *et al.*, 2012). In order to gain insight into the function of the N-terminus of Sec61p, we generated two *sec61* variants: one carrying a deletion of the N-terminal residues 4–22, *sec61ΔH1*, and one lacking both the N-terminal acetylation site and the N-terminal residues 4–22, *sec61ΔN21*. Yeast expressing *sec61ΔH1* and *sec61ΔN21* were viable, but their generation time increased approximately 4-fold in YPD and they were tunicamycin-hypersensitive at 24°C. We found that ppαF, a soluble post-translational import substrate, strongly accumulated in the cytosol of *sec61ΔH1* and *sec61ΔN21* cells. In addition, both mutants were defective in ERAD of CPY*, an established soluble ERAD substrate. We are currently testing the *sec61ΔH1* and *sec61ΔN21* mutants for co-translational import and transmembrane protein ERAD defects.
Keywords: Endoplasmic reticulum, Protein translocation, Sec61p.

MON-099
Functional and structural studies of the bacterial toxin ldrD

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Several Toxin/Anti-Toxin (TA) systems have been described in different prokaryotic species including *Escherichia coli*. Kawano and coworkers described in 2002 a genetic element in *E. coli* which acts as a TA system. They called this element LDR (for Long Direct Repeats) [1]. Four repetitive sequences are part of this element. Three of those, *ldrA*, *ldrB*, *ldrC* are clustered in the same locus and present high homology whereas *ldrD* is located in a different locus and presents reduced homology with the other LDR members. *LdrD* codes for two open reading frames of 35 and 28 codons. *LdrD*-35 is a 35 aminoacid peptide which causes growth inhibition and changes in the cell physiology. On the other hand, *ldrD*-28 codes for a complementary mRNA which counteracts the toxic effects of the *ldrD*-35 in a post-transcriptional mechanism [1]. Until now the mode how *ldrD*-35 exerts its toxic effect is unknown; besides, there is no evidence of the expression of the full length *ldrD* peptide although its mRNA is very stable and constitutively expressed under experimental conditions. This information leads to the hypothesis that the events how *ldrD* exerts toxicity to the cell reside during the translation process and might respond to environmental stimulus.

We therefore decided to study the posttranscriptional events coupled to the expression of the *ldrd*-35 peptide in order to understand the mechanism of action in the cell and to elucidate why the peptide is not expressed, we also performed structural studies over the synthetic peptide to gain further hints about the possible targets of the expressed peptide which could lead to the toxic effects exerted by this gene. In this work, by applying cross linking techniques coupled to MS and fluorescence anisotropy studies, we found that the *ldrD* peptide interacts with several ribosomal proteins including the protein L4 in the ribosomal tunnel; we therefore explored the primary structural motifs which could mediate such interactions by mutational studies. The NMR structure of the peptide and CD spectroscopy studies in Tetrafluorethanol (TFE) showed a high helical propensity in the peptide which is a common feature between the so called stalling peptides [2]. Mapping the results of the mutational studies over the NMR structure supports the idea that the secondary structure formed by this peptide is mainly responsible for its activity. We present here the first functional/structural study of *ldrD* and present evidence for proposing *ldrD* as a new ribosome stalling peptide.
Keywords: Peptide, Ribosome stalling, toxin-antitoxin function.

MON-100
HCV IRES involves 18S rRNA in initiation of translation of the viral RNA on the 40S ribosome

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Initiation of translation of genomic RNA of hepatitis C virus (HCV) is provided by a highly structured fragment in its 5'-untranslated region, the so-called Internal Ribosome Entry Site (IRES), which upon binding to the 40S ribosomal subunit stimulates formation of the 48S initiator complex without assistance of the AUG codon recognition initiation factors. It was generally accepted that this binding is realized through the interaction of the IRES with ribosomal proteins. Using chemical probing, we have found that the HCV IRES bound to the 40S ribosomal subunit protects the backbone and bases of the triplet CCC in the 18S rRNA expansion segment 7 (ES7) apical loop from attack by chemical probes, indicating complementary base-pairing of this loop with the IRES subdomain IIId apical loop. Furthermore, we have shown that the universally conserved nucleotide G1639 displays enhanced accessibility to hydroxyl radicals when the 40S subunits are complexed with the IRES, the IRES domain II and initiator AUG are together necessary for this enhancement. We proposed that contacts of the HCV IRES with the 18S rRNA ES7 stabilize viral RNA binding on the 40S ribosome. These contacts presumably provide interplay between IRES domain II and the AUG codon close to ribosomal protein S5, which causes a rearrangement of 18S rRNA structure in the vicinity of G1639. As a result, G1639 becomes exposed and the corresponding site of the 40S subunit implicated in tRNA discrimination can select Met-$tRNA_i^{Met}$. Thus, our data demonstrate at nucleotide resolution

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direct IRES-rRNA interactions and how they induce conformational transition in the 40S subunit allowing the HCV IRES to function without AUG recognition initiation factors.

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Keywords: None.

MON-101
Host fitness effects of aminoglycoside resistance 16S rRNA G1405 and A1408 methyltransferases from clinical pathogens and natural antibiotic producers

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16S rRNA modification by the G1405 and A1408 methyltransferases (MTases) is a protection mechanism in *Actinomycetes* that naturally produce various aminoglycosides, which prevents newly synthesized antibiotic to bind to the ribosome. Unfortunately, these enzymes are found in a growing number of clinical pathogens where they cause a high-level resistance and pose an imminent threat to the successful use of aminoglycoside antibiotics. G1405 and A1408 make part of evolutionary conserved decoding site in the small ribosomal subunit, which is heavily modified by other housekeeping MTases. We and others have found that G1405 and A1408 MTases interfere with housekeeping MTases that act on the neighbouring nucleotides. The effect on the *E. coli* fitness cost has been examined only for three enzymes found in clinical strains in two independent studies based on different experimental systems and results were divergent.

We extended the fitness cost study to 8 enzymes found in both clinical strains and antibiotic producers. We examined how constitutively expressed MTases affect the growth of *E. coli* by measuring the generation time in exponential phase as well as growth rate in growth competition experiments with the parental strain. We tested the translation accuracy by measuring the CUG initiation, UGA read through and −1 frameshifting in β-galactosidase reporter assay. The complete analysis was extended to the *E. coli* strain with inactivated gene for the housekeeping MTase RsmF, whose action was found to be impaired by aminoglycoside resistance enzymes in previous studies.

Our results show that all aminoglycoside enzymes show negative effect on the growth rate of the host in growth competition experiments, but there is a notable difference between the enzymes from the clinical strains *vs.* the ones from the producers. While bacteria expressing MTases from clinical strains completely disappear from the mixed culture after 5–6 days of growth, bacteria expressing enzymes from antibiotic producers still make 20% of the mixed culture even after 10 days. On the other hand, we observed that translation accuracy results vary between the strains expressing different aminoglycoside resistance MTases and depending on the presence of the housekeeping MTase. This suggests that even though the enzymes belong to the same family and have a very similar crystal structure, their binding to the ribosome and their catalytic mechanisms may show subtle differences responsible for the versatile fitness cost effects. It is therefore of great importance to further analyse these differences and consider them for the successful design of new drugs that would overcome aminoglycoside resistance.

Keywords: aminoglycoside resistance methyltransferase, fitness cost.

MON-102
How many initiator tRNA genes does *E. coli* need?

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Multiple copies of a gene require enhanced investment on the part of the cell, and as such, call for an explanation. The observation that *Escherichia coli* has four copies of initiator tRNA ($tRNA_i$) genes, encoding a special tRNA ($tRNA^{fMet}$) required to start protein synthesis, is puzzling particularly because the cell appears to be unaffected by the removal of one copy. However, the fitness of an organism has both absolute and relative connotations. Reasoning thus, we carried out growth competition experiments between *E. coli* strains that differ in the number of $tRNA_i$ genes that they contain. This has enabled us to uncover an unexpected link between the number of $tRNA_i$ genes and protein synthesis, nutritional status and fitness. Wild-type strains with the canonical four $tRNA_i$ genes are favoured in nutrient rich environments and those carrying fewer are favoured in nutrient poor environments. Auxotrophs behave as if they have a nutritionally poor 'internal environment'. A heuristic model that links $tRNA_i$ gene copy number, genetic stress and the growth rate accounts for the findings. Our observations provide strong evidence that natural selection can work through seemingly minor quantitative variations in gene copy number and thereby impact organismal fitness.

Keywords: Competition, evolution, tRNA.

MON-103
Identification and structural studies of human DNA topoisomerases cellular complexes targeted by cancer drugs

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Type 2 DNA topoisomerases (Top2) are essential proteins that regulate DNA topology during cell division, replication, transcription and chromosome replication. The human Top2 is targeted by anti-cancer agents used in chemotherapy. Most of them induce the formation of ternary complexes between Top2-DNA and the drug. These complexes are then converted into DNA breaks leading to cell death. In human cells, two isoforms are encoded by different genes. The α isoform (Top2α) is overexpressed during cell proliferation and is a hallmark in certain types of cancer. The β isoform (Top2β) was discovered later and several studies suggest that this isoform could be involved in the development of secondary malignancy after treatment by anti-topo compounds such as etoposide. The two isoforms share high sequence similarity and a similar structural organization but mostly diverge on their C terminal domain. The structure and the functional role of this domain are not well understood since no structural information is available on this domain alone, or in the context of the full length enzyme.

In addition, the Top2 enzymes are part of large cellular complexes whose role in disease processes are still to be elucidated. The identification of potential drug targets among proteins associated with the Top2 complexes could help to elaborate efficient multi-therapy and decrease side effects of drugs. In this work, we combine the proteomic analysis of the cellular partner of the Top2 enzyme and the structural analysis of these complexes. We

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use cryo electron microscopy to analyze the full length Top2α enzyme in complex with DNA and drugs, and a chemoproteomic approach to identify the Top2 cellular complexes targeted by drugs.

We will present here the production in yeast and the purification of the two isoforms of this large enzyme (340 KDa) that are prepared for functional and structural studies, and the analysis of their post-translational modifications. Our recombinant enzyme is used to validate the chemical probes derived from drugs prior to pulldown experiments on cancer cell lines. We will also present our preliminary study on the three dimensional reconstruction of the full length enzyme complex with DNA and etoposide using single molecule reconstruction by cryo electron microscopy. This project is the first step to characterize large complexes centered on the Top2 and targeted by drugs.

Keywords: Cancers, cryo-microscopy, human Top2 DNA topoisomerase.

MON-104
Identification of small open reading frames with high coding potential in moss *Physcomitrella patens*

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It has been revealed that small open reading frames (sORFs, up to 100 codons) have the potential to encode biologically active peptides that have regulatory roles in eukaryotic cells (Kastenmayer et al., 2006), (Kondo et al., 2010), (Andrews et al., 2014). In plants, a number of peptides encoded by sORFs play significant roles in various aspects of plant growth and development (Hanada et al., 2012). However, most *ab initio* gene prediction programs are not well suited for identifying sORFs with coding potential. Moreover, existing standard proteomic approaches poorly suited for the identification of proteins less than 10 kDa.

We used prediction program sORFfinder (Hanada et al., 2012) to find intergenic regions with high coding potential in the genome of the model object moss *Physcomitrella patens*. High-throughput RNA-Seq by SOLiD 4 genetic analyzer (Life Technologies, Applied Biosystems) and identification of native peptides by TripleTOF 5600 LC-MS/MS (ABSciex) has been carried out on gametophore, protonema and protoplast cells of moss *Physcomitrella patens*. Optimal procedure for endogenous peptide extraction and identification has been worked out to demonstrate translation of sORFs.

Using sORFfinder we distinguished 241,228 sORFs within intergenic region with high coding potential. RNA-Seq confirmed transcription of 8,450 sORFs from intergenic region and 16,928 previously known genes of *Physcomitrella patens*. Tandem mass-spectrometry analysis resulted in identification of 18 peptides derived from 12 sORFs within intergenic region, 52 peptides derived from 42 sORFs that were previously thought to be untranslated region of mRNAs and more than 100 peptides from about 100 alternative sORFs within previously known ORFs.

Comparative analyses of sORFs sequences distinguished in moss *Physcomitrella patens* with genomes of other plant species revealed high conservation in terms of synonymous/nonsynonymous substitutions. The report will be discussed further steps to validate the results overexpression and knockout mutants of coding sORFs, functional categorization and expression under stress.

Keywords: Proteomics, small Open Reading Frames, Transcriptomics.

MON-105
Implementation of a chaperone fusion system for the production of soluble and biologically active chemokines in *E. coli*

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Escherichia coli is widely employed in the production of recombinant proteins for biotechnological and therapeutic use. However, its usefulness as a recombinant protein "cell factory" is hindered by the inability of many recombinant proteins to fold properly when overexpressed. Such misfolding leads to low protein solubility and subsequent accumulation within bacterial inclusion bodies. Several strategies have been employed to alleviate this problem, including the development, in our laboratory, of a chaperone fusion-protein expression system that yields properly folded, soluble recombinant proteins. Here we apply the chaperone fusion system to the production of two biologically important CXC-chemokines, i.e. IP10 (CXCL10) and SDF-1α (CXCL12). While chemokines are considered valuable research/diagnostic and therapeutic tools, due to their biological functions as signaling molecules in immunity and inflammation, their overproduction in bacteria leads to their aggregation and deposition in inclusion bodies. We find that such bacterially overexpressed and aggregated recombinant SDF1α and IP10 are present as covalently linked protein multimers, which revert to monomers upon exposure to a reducing agent. Thus, the observed multimerization can be attributed to the formation of aberrant intermolecular disulfide bonds, instead of the two intramolecular disulfide bonds that normally stabilize the CXC-chemokine structure. Expression of the two chemokines as fusions with DnaK, the *E. coli* Hsp70, resulted in high levels of soluble DnaK-chemokine production, minimal aggregation and negligible formation of intermolecularly linked chemokine multimers. These results cannot be simply attributed to the fusion of a chemokine to a highly soluble protein fusion partner, since chemokine fusions with GroEL (an Hsp60) and glutathione S-transferase displayed greatly reduced or no solubility, respectively. Thus, both the increased solubility of the DnaK-chemokine fusions and their lack of intermolecular disulfide bonds can be attributed to the presence of the specific chaperone fusion partner, i.e. DnaK. The order of the fusion protein partners does not appear to affect protein folding and solubility. The DnaK-chemokine fusions can be readily purified by IMAC under native or denaturing conditions and, unlike the unfused recombinant chemokines, these fusions refold promptly after removal of the denaturant. Furthermore, they are active in chemotaxis assays (wound-healing and cell migration assays). These results provide good evidence that our chaperone-fusion system can be applied in the biotechnological production of both soluble and biologically active chemokines in *E. coli*.

Keywords: chaperone fusions, recombinant chemokines, recombinant protein expression.

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MON-106
Investigating the effect of hybrid vigor in yeast protein synthesis: role of ribosomal protein L39 as a potential heterosis trait locus

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Heterosis, known also as hybrid vigor, refers to the phenotypic superiority of a heterozygous hybrid compared to its homozygous parents. In plants, hybrid vigor is associated with the rate of growth and protein synthesis as well as advanced environmental adaptability. Since the molecular mechanism of this phenomenon has not yet been fully understood, scientists continue to investigate different genetic models. Recent advances in quantitative trait loci (QTL) mapping in *Saccharomyces cerevisiae* have uncovered several targets associated with heterosis. The gene encoding ribosomal protein L39 (*RPL39*) was confirmed as a potential heterosis trait locus (HTL). L39 is an excess non-essential protein found in eukaryotic cells which affects translational fidelity and the rate of peptide bond formation in the 80S ribosome.

Based on these results, we decided to study the potential effect of heterosis on eukaryotic protein synthesis, focusing on the translational mechanism of yeast. The strains used in this study include two parental wild-type strains (6x6 and BYxBY), two hybrids (6xBY and 6xBY6) and a hemizygous strain (Δ6xBY) lacking one of the two alleles of *RPL39*. Following *in vitro* experiments, we tested the translational fidelity, the peptidyl-transferase activity (k_{cat}/K_M), and the ribosomal profile of each strain on sucrose gradient. According to our data, an increased error frequency (EF) of both hybrid strains was observed, compared to their homozygous parents. Moreover, Δ6xBY strain displayed significantly higher EF compared to 6xBY and 6xBY6, exceeding the expected effect caused by the deletion of one of its two alleles. However, the PTase activity of the studied strains did not show any significant difference between parental and hybrid strains ribosomes, as well as their gradient profiles. In conclusion, heterosis which has been associated with cell growth and protein synthesis in yeast, did not exert any effect on the catalytic activity of the ribosomes, but has only a statistically significant effect on translation accuracy.

Keywords: heterosis, ribosome, yeast.

MON-107
Investigating the role of the poly(A) binding protein (PABP) 1 Lysine-606 modification in regulating post-transcriptional control

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PABP1 is a multifunctional regulator of gene expression which acts as a primary determinant of translation efficiency and stability, regulates the fate of specific mRNAs, and participates in microRNA (miRNA)-mediated regulation and nonsense-mediated mRNA decay (NMD). In relation to this, many PABP1 protein partners have been identified which bind overlapping sites within its C-terminal PABC domain, therefore, it unclear how its different functions are coordinated. Recently, we have found that PABP1 is subject to extensive post-translational modifications (PTMs) including putative lysine acetylation/methylation switches which may affect its interaction with different PAM2 motif-containing proteins. In particular, molecular modelling of the acetylation or methylation of Lys606 within the PABC domain, using available structures of PABC in complex with eRF3a and PAIP2, suggests that modification of this residue, which is critical in PABC-PAM2 interactions, may differentially affect these PABP1 interactions. Thus, we aim to experimentally investigate the role of the Lys606 modification as a molecular switch which dictates PABC-mediated protein-protein interactions thereby co-ordinating different aspects of PABP1 function to control the utilisation/destruction of mRNAs within cells. Novel methods are used for recombinant protein production of site-specifically acetylated/dimethylated PABC domain. This is used to study the interactions of PABC with known PAM2 proteins/peptides in biophysical studies and consequently structural studies. Here, the strategies for the investigation are discussed further and current progress is shown.

Keywords: None.

MON-108
Investigation into Pdc behavior upon 14-3-3 binding using NMR, SAXS and Trp fluorescence quenching techniques

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Phosducin (Pdc), a highly conserved 30 kDa phosphoprotein, regulates visual signal transduction by interacting with the beta and gamma subunits of the retinal G-protein. Pdc was also suggested to be involved in transcriptional control, the regulation of transmission at the photoreceptor-to-ON-bipolar cell synapse, and the regulation of the sympathetic activity and blood pressure. The function of Pdc is regulated by phosphorylation at Ser54 and Ser73 in a process that involves the binding of phosphorylated Pdc to the regulatory 14-3-3 protein. The 14-3-3 proteins are highly conserved dimeric molecules that regulate the function of other proteins through a number of different mechanisms. The exact role of the 14-3-3 protein in regulating Pdc function is still unclear, but it is entirely possible that 14-3-3 either sterically occludes and/or affects the structure of Pdc. Both 14-3-3 binding motifs are located within the N-terminal domain of Pdc, which participates in the binding to the beta and gamma subunits of the retinal G-protein as well as contains the SUMOylation site Lys-33.

Our previous study revealed that phosphorylated Pdc and the 14-3-3 protein form a stable complex with 1:2 molar stoichiometry. Complex formation with 14-3-3 affects the structure and reduces the flexibility of both the N- and C-terminal domains of dpPdc, suggesting that dpPdc undergoes a conformational change when binding to 14-3-3. To further investigate this interaction and mainly the 14-3-3 protein-mediated conformational changes of Pdc, we performed structural studies using NMR, SAXS and tryptophan fluorescence whose results are presented here.

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Keywords: 14-3-3 proteins, Phosducin, SAXS.

MON-109
Large-effect beneficial synonymous mutations overcome deleterious synonymous changes in an enzyme-coding gene

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Synonymous mutations have traditionally been considered to evolve neutrally. However, recent evidence shows that synonymous codon changes may face quite strong selection, although it remains difficult to derive general patterns about the nature and strength of such selection. In previous work with synonymous variants of an enzyme-encoding gene of *Methylobacterium extorquens*, we showed that altering codons could be extremely deleterious. Although the exact physiological mechanism likely depends on the specific sequence, the fitness disadvantage arose from insufficient enzyme production. We now show that during laboratory evolution, these synonymous variants rapidly regain fitness via repeatable and mutant-specific beneficial synonymous mutations in the N-terminal region of the gene. Although none of the mutations caused a reversion to the wild- type codon or altered tRNA genes, all of them increased focal gene and protein expression. Different allele variants found diverse mechanistic solutions during evolution, some alleviating unconventional binding to the anti-SD sequence, and others altering mRNA structure or stability. Our results thus suggest that co-evolutionary dynamics between tRNA copy number and codon use may be unlikely in the short-term, potentially because of the existence of multiple local fitness peaks in the adaptive landscape. Thus, bacteria may find diverse evolutionary solutions to the immediate physiological problems caused by accumulated deleterious synonymous mutations; and tRNA gene copy number changes may gradually fine-tune global protein production in the long term.

Keywords: Bacterial adaptation, Molecular evolution, Synonymous mutations.

MON-110
Mutational analysis of the ribosomal A site reveals the binding pattern for the 16S rRNA A1408 methyltransferases from the clinical pathogen and a natural producer of aminoglycosides

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The aminoglycosides represent a class of antibiotics that are often prescribed for the treatment of various infections caused by both Gram-positive and Gram-negative bacteria. They are natural substances derived from *Streptomyces* spp. or *Micromonospora* spp. or synthesized *in vitro*. Aminoglycoside-producing bacteria have evolved a self-protecting system that inhibits the aminoglycoside binding to their target by methylating specific ribonucleotides in antibiotic-binding sites of the ribosome. Enzymes that are involved in this process are members of 16S rRNA G1405 or A1408 methyltransferase subfamilies and were until recently only been found in the natural producers of aminoglycoside antibiotics. However, a number of occurrences of high-level resistance to aminoglycosides in the clinic has been reported in recent time and a few novel G1405 and A1408 methyltransferases have been isolated from clinical pathogens.

In this work, we studied the ribosomal A site binding pattern of two of the A1408 methyltransferases, KamB from the natural producer of aminoglycosides and NpmA, a novel methyltransferase isolated from a clinical pathogen. Both enzymes confer high-level resistance to aminoglycoside antibiotics by methylating the A1408 nucleotide in the 16S rRNA of the small ribosomal subunit. In our work we used a specialized *E. coli* system, in which all *rrn* operons were inactivated, and ribosomal RNA was transcribed from a vector-based *rrn* operon. We constructed single nucleotide mutations in the part of the operon corresponding to the A site of 16S rRNA. We introduced actively expressing enzymes in these cells and monitored their ability to grow in media supplemented with various concentrations of kanamycin. We determined minimal inhibitory concentration of kanamycin for these cells and analyzed the target nucleotide A1408 methylation with primer extension.

Our results show that some of the point mutations introduced into 16S rRNA reduce the ability of both KamB and NpmA methyltransferases to effectively methylate the target nucleotide, A1408. We also observed a slight difference between the 16S rRNA binding patterns for these enzymes, suggesting that even though they have the same mode of action they might not bind to their substrate in the same manner. Our results presented here will be of great assistance in the development of specific NpmA inhibitors that could restore the potential of aminoglycoside antibiotics.

Keywords: 16S rRNA A1408, Aminoglycoside resistance, NpmA.

MON-111
New insights about S1 binding to the ribosome by NMR

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Ribosomal protein S1 is an essential actor for protein synthesis in *E. coli* since it helps mRNA recruitment by the 30S ribosomal subunit and recognition of the correct start codon during translation initiation. *E. coli* S1 is a modular protein that contains six repeats of an S1 motif, which have distinct functions despite structural homology. Whereas the three central repeats have been shown to be involved in mRNA recognition, the two first repeats that constitute the N-terminal domain of S1 are responsible for binding to the 30S subunit. We solved the structure of these two first repeats by NMR and showed that the first repeat is a degenerate OB-fold whereas the second repeat is associated with two helices that stem from the linkers with the first and third repeats. Based on these structures and on interactions properties with nucleic acids determined by NMR we propose a model for the binding of S1 to the ribosome.

Keywords: NMR, S1 protein, translation initiation.

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MON-112
pERp1/Mzb1 – functional characterization of a novel ER protein

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pERp1/Mzb1 is an endoplasmic reticulum (ER)-resident protein of 18 kDa, exclusively expressed in B cells. It is up-regulated during B cell to plasma cell differentiation and plays a key role in IgM assembly and secretion. pERp1/Mzb1 possesses a thioredoxin-like active site motif CXXC, typical of oxidoreductases. However, until now only a modest oxidoreductase activity of it has been measured in *in vitro* experiments, which is increased by the presence of protein disulfide isomerase (PDI), a well characterized oxidoreductase with chaperone activity. Since, the mechanism of action of pERp1/Mzb1 still remains obscure, we aim to determine its activity *in vivo* and identify if it is affected by other ER oxidoreductases, such as PDI, with which it might act synergistically. Low Density Lipoprotein Receptor (LDLR), a cell-surface receptor that mediates the clearance of LDL particles from blood circulation and regulates plasma cholesterol levels, is used as a model protein, since it has thirty disulfide bonds and a characteristic folding pattern. Using pulse-chase experiments, it was shown that pERp1/Mzb1 increases the homogeneity of disulfide bonds of LDLR, delays its folding and leads to its aggregation. On the other hand, PDI co-expression results in more heterogeneous disulfide bond formation compared to only pERp1 expression. Furthermore, PDI decreases the aggregation of LDLR caused by pERp1/Mzb1 and keeps LDLR mostly in its reduced form. These results shed a light into the activity of pERp1/Mzb1 and show how it affects the folding, aggregation and disulfide bond formation of LDLR.

Keywords: LDL receptor, oxidoreductase activity, pERp1/Mzb1.

MON-114
Proteome of the venom of hump-nosed pit viper (*Hypnale hypnale*) from Sri Lanka

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The Sri Lankan hump-nosed pit viper (Hypnale hypnale) venom composition was investigated using two main proteomic approaches: (1) shotgun-liquid chromatography-mass spectrometry/mass spectrometry (shotgun-LC-MS/MS); (2) sequential use of reverse-phase high performance liquid chromatography (rp-HPLC), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and peptide sequencing. Approach (1) revealed 52 venom proteins, 70% of which are toxinologically related variants confined to several protein families. Protein structures conserved in the venoms of phylogenetically related snakes supported the observed cross-neutralization of H. hypnale venom by the Malayan pit viper (Calloselasma rhodostoma) antivenoms. Approach (2) ascertained the venom key components, i.e. zinc-dependent metalloproteases (30%), phospholipases A2 (22%), L-amino acid oxidase (20%), C-type lectins (9%) and serine proteases (7%). These toxins correlate with the venom's principal hematoxic effects and local tissue destruction, indicating the target toxins for immunoneutralization. Interestingly, the findings also revealed the presence of nerve growth factors, aminopeptidases and three-finger toxin-like peptides in low abundances, the occurrence and functions of which require further characterizations. Both the proteomic approaches complement one another in unmasking the H. hypnale venom proteome and its key toxin constituents. The knowledge is essential for elucidating the pathophysiology of H. hypnale envenomation and for optimizing antivenom formulation in the future.

Keywords: Hypnale, proteome.

MON-115
RACK1 binding to the ribosome is required to regulate the translational efficiency of specific mRNAs

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Translation is a fundamental cellular process performed by ribosomes and is regulated by several different signaling pathways. In particular, RACK1 is a protein originally characterized as a receptor for activated PKCs and it is present on the 40S ribosomal subunit near the mRNA exit channel. RACK1 is proposed to act as an interface between signaling and ribosome machinery.

To address RACK1 role in translation, we performed *in vitro* translation assays using HeLa lysates downregulated for RACK1. We show that RACK1 depletion impairs the translation of cap-, TOP- and stem loop-regulated mRNAs (but not IRES-dependent translation). The addition of wt exogenous RACK1 is able to rescue this translation deficit.

To define if RACK1 role in translation depends by its binding to the ribosome, we generated a R36D K38E mutant RACK1 with low affinity for the ribosome. On *in vitro* translation, the addition of exogenous mutant RACK1 is unable to restore translation to the levels of wt RACK1. We have purified wt and mutant RACK1 from transfected HEK293 cells and interactors of both proteins are identified. Both wt and mutant RACK1 are shown to interact with wide pools of proteins, sharing several ones such as grp78. Among the interactors specific for wt RACK1, we are focusing on 40S rpSA which is a required component for 40S biogenesis.

We are going to test whether rpSA presence on the ribosome is affected by RACK1 depletion, and possibly if rpSA role can contribute to explain the translation deficits in those conditions.

Keywords: RACK1, ribosome, translation regulation.

MON-116
Relocalization of translation factor eIF2D to the centrosome in mammalian cells upon stress

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eIF2D (formerly called ligatin) is a mysterious translation factor that is able to facilitate tRNA binding to the P-site of the ribosome in a GTP-independent manner. Its activity requires an AUG codon of mRNA properly positioned in the P-site before the tRNA binding. In a reconstituted system of eukaryotic translation initiation, eIF2D can replace both eIF2 and eIF5B in reactions of 48S and 80S complex formation, respectively. However, in contrast to eIF2-mediated initiation which is limited to Met-tRNAi, this alternative pathway may operate with elongator tRNAs.

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The physiological function of eIF2D is yet unknown. In yeast, eIF2D ortholog TMA64 has genetic interactions and expression profile that predict its involvement in control of the cell cycle and adaptation to stress. In addition to translation-related domains (N-terminal PUA and C-terminal SUI1), eIF2D possesses a much less conserved central part with similarity to Kin17_mid and SWIB/MDM2 domains. This feature suggests that eIF2D may be a component of protein complexes that coordinate cell cycle checkpoints in response to stress. To investigate a putative role of eIF2D in such events, we analyzed its localization within cultured mammalian cells under variety of conditions.

We found that under normal conditions eIF2D was diffusely distributed in the cytoplasm. However, upon induction of severe oxidative stress by sodium arsenite, both endogenous eIF2D and transiently expressed GFP-eIF2D formed two clearly defined foci in the cytoplasm in close proximity to the nucleus. These foci were clearly distinct from stress granules that were extensively formed under these conditions and contained several components of translation machinery. They were positively stained with antibodies against gamma-tubulin, the marker of centrosome. Similar localization pattern was observed under a number of stress conditions. The centrosomal localization of eIF2D was determined by the middle portion of the protein. We concluded that upon induction of stress response, eIF2D is relocated to the centrosome to participate in some specific translation events. This spatially restricted activity of eIF2D may be related to control of the cell cycle progression.

The study was partially supported by RFBR (grant 13-04-01121-a).
Keywords: centrosome, eIF2D, translation initiation.

MON-117
Ribosomal protein S1 unfolds structured mRNAs on the ribosome for translation initiation in *Escherichia coli*

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Structures of mRNA have for a long time been described as key elements in the regulation of gene expression. Many bacterial mRNAs adopt structures in their 5' untranslated regions that modulate the accessibility of the 30S ribosomal subunit. Structured mRNAs interact with the 30S in a two-step pathway where the docking of a folded mRNA precedes an accommodation step. Recently, we demonstrated (1) that ribosomal protein S1 endows the 30S with an RNA chaperone activity that is essential for the docking and unfolding of structured mRNAs, and the correct positioning of the initiation codon inside the decoding channel. The rate of the S1-induced RNA melting is slow, suggesting that this step is ratelimiting in the initiation process of structured mRNAs. The first three OB-fold domains of S1 retain all the activities of the protein on the 30S subunit, while the function of the last two remains to be addressed. However, S1 is not required for all mRNAs and acts differently on mRNAs according to the signals present at their 5' ends. Interestingly, the action of S1 on the ribosome is countered by repressor proteins to prevent translation. All in all, S1 confers activities to the ribosome in such a way that the initiation of translation is selectively adapted for unstructured and structured mRNAs.

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Keywords: Initiation of translation, Ribosomal protein S1, Structured mRNA.

MON-118
RNA mimicry by the Fap7 adenylate kinase in ribosome biogenesis

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Over 200 pre-ribosomal factors are involved in the maturation of ribosomes. Most of these factors are essential to cell survival, but their precise function remains elusive. One of the last steps of maturation of the ribosome small subunit is the cleavage of 20S pre-rRNA in 18S rRNA in the cytoplasm. This cleavage is carried out by the endonuclease Nob1 and also requires the presence of other factors such as the methyltransferase Dim1, and a plethora of NTPases including the Rio protein kinases, Prp43 and its cofactor Pfa1, the Ltv1 GTPase and the Fap7 NTPase.

The function of Fap7 is especially intriguing since the human homologue bears adenylate activity, an enzymatic activity not usually found during ribonucleoprotein biogenesis. In addition, Fap7 regulates Cajal body assembly and cell cycle progression via the p53-MDM2 pathway. Finally, the function of Fap7 is intimately linked to its interaction with the Rps14 ribosomal protein. The Rps14 C-terminal domain is essential for D-site cleavage and is located in proximity to the 18S rRNA 3'-extremity in the mature ribosome. The deletion of this protein causes the 5q syndrome that is phenotypically close to Diamond Blackfan anemia. The link between the enzymatic activity of Fap7 and its role in ribosome biogenesis remains enigmatic.

This work presents the functional and structural characterization of the Fap7-Rps14 complex. We report that Fap7 association blocks the RNA binding surface of Rps14 and, conversely, Rps14 binding inhibits adenylate kinase activity of Fap7. In addition, the affinity of Fap7 for Rps14 is higher with bound ADP whereas ATP hydrolysis dissociates the complex. These results suggest that Fap7 chaperones Rps14 assembly into pre-40S particles via RNA mimicry in an ATP-dependent manner. Incorporation of Rps14 by Fap7 leads to a structural rearrangement of the platform domain necessary for the pre-rRNA to acquire a cleavage competent conformation.

Keywords: Fap7-Rps14, ribosome biogenesis, Structure Determination.

MON-119
S6 kinases interplay with new partner – adaptor protein TDRD7

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Ribosomal protein S6 kinases (S6Ks) are belong to the AGC family of Ser/Thr kinases and are important players in cellular PI3K/mTOR signalling network, implicated in the regulation of cell size, growth and metabolism. Our recent yeast two hybrid screening using S6K1 as bait allowed us to identify a novel bind-

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ing partner of S6K1 – TDRD7, a scaffold protein involved in regulation of cytoskeleton dynamics, mRNA transport, protein translation, piRNAs processing. The aim of current project was to study S6Ks interplay with TDRD7.

First of all we have performed the bioinformatical analysis of TDRD7 primary structure and possible S6K1 sites of phosphorylation on this protein. At the next step six different fragments of TDRD7 were cloned, ovexpresed and purified from bacteria cells. These recombinant proteins were used in a set of pull-down experiments with full-length S6K1. Direct interaction between C-terminal tudor domain of TDRD7 and S6K1 has been shown. This interaction were further confirmed in Far-Western blot on recombinant S6K1 and TDRD7 fragments. Moreover, purified domains of TDRD7 were used as antigens for mouse immunizations and generation of monoclonal antibodies.

The generated antibodies were used for studying S6K1/TDRD7 interaction in mammalian cells *in vivo*. We succeed to detect an interaction between TDRD7 and S6K1 in HEK293 and rat brain lysates. Than, the application anti-TDRD7 and anti-S6K2 antibodies generated previously, allowed us to detect the existence of complexes S6K2-TDRD7 in HEK293. Moreover, the generated anti-TDRD7 antibodies allow us to find several new TDRD7 isoforms in HEK293.

And finally, we have found that S6K1 like S6K2 phosphorylate 3 from 6 fragments of TDRD7 in the *in vitro* kinase reaction.

Confocal microscopy studies suggest possible co-localization of S6K1/TDRD7 and S6K2/TDRD7 within perinuclear region in HEK293, HEPG2 cells and in soma of primary rat hippocampal neurons. And finally, we have detected that C-terminal synthetic peptides of S6K2 with methylated Arg interfere with TDRD7 from HEPG2 lysates. The physiological characteristics of S6K2-TDRD7 interaction and the role of this complex formation need further investigation.

Keywords: TDRD7, S6 kinase, methylation.

MON-120
Selenoprotein mRNA cap hypermethylation and translation initiation

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Mammalian mRNAs are produced from complex and coordinated biogenesis pathways and acquire 5' terminal 7-methylguanosine cap structures that are essential to every aspect of mRNA metabolism, including localization, translation and decay. Selenoprotein mRNAs contain an in-frame UGA selenocystein (Sec) codon, usually sign of the termination of translation, and thus undergo particular translation recoding. Consequently, these mRNAs have to follow dedicated biogenesis pathways involving conserved assembly proteins and chaperon complexes. Our work reveals for the first time in vertebrates that selenoprotein mRNAs bear hypermethylated caps. We show that the acquisition pathway of this cap is common with that of small nuclear and nucleolar ribonucleoprotein particles, involved in RNA maturation processes. Our data also provide evidence that the hypermethylated-capped selenoprotein mRNAs localize to the cytoplasm and are translated but not efficiently recognized by the translation initiation factor eIF4E. These findings suggest that selenoprotein mRNAs may be subjected to a distinctive mode of regulation.

Keywords: mRNA cap hypermethylation, selenoproteins, translation initiation.

MON-121
Structural and functional studies of the archaeal translation initiation complex

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Eukaryotic and archaeal translation initiation complexes have in common a functional core containing mRNA, the ternary initiation complex (e/aIF2, GTP, Met-tRNA$_i^{Met}$), e/aIF1 and e/aIF1A bound to the small ribosomal subunit. In eukaryotes, the functional core is extended by many additional factors, most of them being involved in a long-range scanning of mRNA, necessary to decipher the initiation codon. In archaea, long-range scanning does not occur thanks to the occurrence of Shine-Dalgarno sequences or of very short 5'UTR on mRNA. Concomitantly, archaeal translation initiation only requires the core complexes.

We have determined two cryo-EM structures of archaeal translation initiation complexes containing mRNA and initiator tRNA, with either the full set of initiation factors or in the absence of aIF1. Together with toe-printing experiments, the structures reveal how the initiator tRNA in a P_{out} state cooperates with aIF1 to decipher the initiation codon. aIF1 departure allows concerted motions of the head of the 30S subunit and of the tRNA, leading to a complex with the initiator tRNA fully accommodated in the P site resulting in a P_{in} state. These motions induce a close conformation of the 30S subunit, which is stabilized by the C-terminal helical domain of aIF1A. The results provide insights into translation initiation steps that are common to archaea and eukaryotes.

Keywords: Cryo-EM, Eukaryotes and Archaea, translation initiation.

MON-122
Structural approaches on YIH1: a protein involved in protein synthesis control under starvation conditions

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Protein synthesis is an essential and complex process that is susceptible to different environmental situations. One of them might be the accumulation of uncharged tRNAs due to starvation, leading to the activation of a cell response that affects general protein synthesis.

The proposed mechanism requires GCN2 binding to GCN1, being this interaction mediated by the GCN2 RWD domain. Once these two proteins have interacted, the complex binds to the ribosome, what is essential for the detection of empty tRNAs; since GCN1 is thought to facilitate the transfer of uncharged tRNAs from the ribosome to the histidyl tRNA synthetase like domain of Gcn2. This signal activates the kinase domain of GCN2, leading EIF2α phosphorylation, which promotes the translation of some determinant transcription activators of amino acid biosynthetic genes and also represses general protein synthesis.

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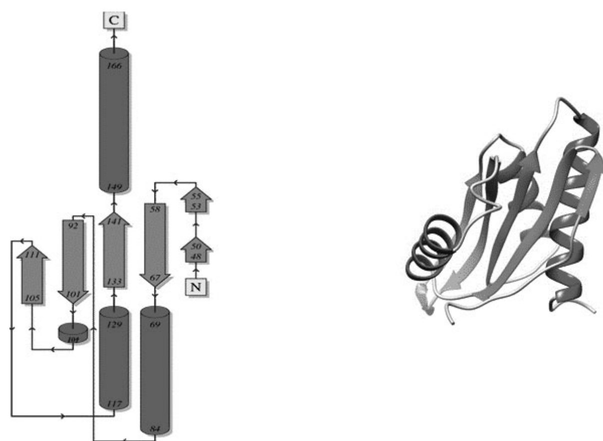

Fig. 1. On the left side we can see the C-terminal topology diagram of Y1H1 fungi homolog obtained from PDBsum. On the right side we show the C-terminal crystal structure of Y1H1 fungi homolog, obtained at 2Å resolution.

YIH1 (yeast IMPACT homolog 1) also contains a RWD domain that interacts with GCN1 through the same region as GCN2, thus competing with GCN2 for GCN1 binding and down-regulating GCN2 activation.

Our aim is to elucidate the crystal structure of the YIH1-GCN1 complex. Here we present the crystallization trials of this complex and also, the C-terminal structure of a fungi YIH1 homolog. This C-terminal belongs to the UPF 0029 group in Pfam with unknown functions described. It is conserved in both procariotes and eucariotes. This domain is organized as beta-alpha-beta-beta-alpha-beta, similar to a ferredoxin like domain (Figure 1)

Keywords: GCN1, Protein structure, YIH1.

MON-123
Structural characterization of the β-propeller domain of Erb1, an essential protein in eukaryotic ribosome biogenesis

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Erb1 (Bop1 in mammals) is a eukaryotic protein essential in ribosome biogenesis. Altogether with Nop7 and Ytm1 it forms a stable complex that participates in early steps of rRNA processing and is necessary for the proper maturation of the 60S ribosomal subunit although its exact implication in the process remains unknown. The amino terminal part of Erb1 harbors a poorly characterized domain (BopNt) which is directly responsible for its function and binding to Nop7 and Ytm1, whereas the carboxy-terminus of the protein contains a big β-propeller domain that is formed by seven WD repeats. It has been postulated that this C-terminal region is not directly involved in the main function of the protein during 60S synthesis; nevertheless it is well conserved within the Erb1/Bop1 family and provides an extensive surface for possible protein-protein and protein-RNA interactions. In total, 22 pre-ribosomal factors contain a β-propeller domain in their structure indicating that it is one of the most common folds that maintain the complex network of interactions required for the 60S assembly.

Here we present the crystal structure of the β-propeller domain of Erb1 from *Saccharomyces cerevisiae* at 1.6Å (residues 417–807) that was obtained as a result of random proteolysis event during crystallization trials of Erb1/Nop7 dimer. The structural information allowed us to exactly define the boundaries of the domain and to describe its particular features, being the presence of a long insertion within the second WD repeat the most distinctive characteristic. This additional segment forms a protrusion on the surface of the propeller and may play an important role in peptide binding. We conclude that the proper folding of the insertion is determined by the neighboring blades of the propeller. The analysis of the electrostatic surface and conserved hot-spot residues allows us to predict the patches that might be involved in protein-protein interactions. At last, we carry out a comparison of the conservation of the WD domain of Erb1 with other known seven-bladed propellers that gives an idea of how this extremely variable sequence produces such a similar fold.

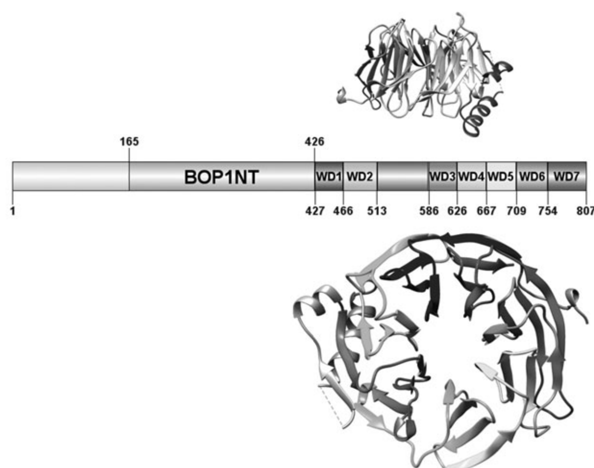

Fig. Diagram showing the domain organization of Erb1 from *Saccharomyces cerevisiae* and the structure of the carboxy-terminal β-propeller (residues 427-807). The coloring of the top-view cartoon representation in the bottom panel corresponds to the seven WD repeats represented on the bar. The position of the insertion on the bar and in the upper panel is highlighted in magenta.

Keywords: Beta propeller, ribosome biogenesis, WD40 Domain.

MON-125
Structure of a thiolase like protein from *Mycobacterium smegmatis*

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Thiolases are enzymes involved in fatty acid metabolism.They occur as either dimers or tetramers. Analysis of the *Mycobacterium smegmatis* genome suggests that it codes for several thiolases and thiolase-like proteins, most of which have not been well studied. We have determined the crystal structure of a thiolase-like protein (type 1) (*Ms*TLP1) from *M. smegmatis* at 2.7Å resolution by the single wavelength anomalous dispersion method. The structure revealed that the N-terminal domain has the thiolase fold. An extra C-terminal domain is also observed. Interestingly, it consists of six β-strands forming an anti-parallel β-barrel. Sequence and structural comparisons with classical thiolases revealed that the residues known to be essential for catalysis in thiolases are not conserved in *Ms*TLP1. Consistent with this observation, activity measurements showed that *Ms*TLP1 does not catalyze the thiolase reaction. Unlike classical thiolases, *Ms*TLP1 is a monomer in solution. This is the first structural report of a monomeric thiolase-like protein. These studies show that *Ms*TLP1 belongs to a new group of thiolase-like proteins of unknown function.

Keywords: Crystal structure, Mycobacteria, Thiolase.

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MON-126

Systematic search for stress-response factors influencing the 100S ribosome formation

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In proteobacteria group gamma including *Escherichia coli*, protein synthesis is suppressed by the formation of 100S ribosomes under stress conditions such as nutrient starvation. The 100S ribosome, a dimer of 70S ribosomes, is formed after association of 70S ribosome monomer with "Ribosome Modulation Factor" (RMF). Upon entry into the stationary growth phase, the 70S ribosomes are converted into 100S dimers, which are functionally inactive in translation. An *E.coli* strain deficient in the *rmf* gene cannot form 100S ribosomes and its lifetime is shorter than that of the wild-type strain, indicating that the transformation of ribosomes is an important strategy for *E.coli* survival under stress conditions. This ribosomal resting state is called the hibernation stage [1]. At present, however, little is known regarding the regulation of stationary-phase-coupled RMF expression.

Recently, we elucidated that cAMP-CRP is involved in transcription regulation of the *rmf* gene and the formation of 100S ribosome [2]. CRP (cAMP receptor protein) is the global regulator of genes for carbon source utilization. In parallel, we also identified that transcription of the *rmf* gene is regulated by ppGpp, an alamone produced under nitrogen starved conditions [3]. These findings indicate that transcription of the *rmf* gene is regulated through several pathways under various stress conditions including carbon and nitrogen starvation. In order to elucidate the pathways, we performed a systematic search for the stress-response factors that influence the 100S ribosome formation. On the basis of these results, we would like to discuss the relationship between the stress response and the 100S ribosome formation.

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Keywords: 100S ribosome.

MON-127

The collapse of ribosome biogenesis promotes terminal erythroid differentiation

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Ribosome biogenesis (RB) is a key event allowing cell growth before division. Defective RB recognized in inherited Diamond-Blackfan anemia, emphasizes the functional importance of ribosome in erythropoiesis. In this study, we aimed at investigating the regulatory role of RB during the erythroid precursor maturation which is characterized by a cell size reduction during 2 to 3 rapid cell divisions (Figure).

We used two *in vitro* systems of expansion and differentiation of erythroblasts (E.) derived of immature hematopoietic progenitors from human mobilized peripheral blood or mouse fetal liver. The expansion step is supported by the Stem Cell Factor (SCF) and the second step depends on erythropoietin (EPO).The structure of the nucleolus was studied by electron microscopy. Compared to immature proerythroblasts (proE), a dramatic size reduction and change in nucleolar structure (*ie*. the disappearance of fibrillar and dense fibrillar components) is observed at the stage of mature polychromatophilic E. suggesting a loss of functionality.

RB was measured by a pulsed SILAC (Stable Isotopic Labeling by Amino acids in Culture cell) proteomic assay that quantified the incorporation of newly synthesized ribosomal proteins in the ribosome. Both in mouse and human models, immature proE expanded upon SCF and EPO demonstrate a maximal RB with a renewal rate of 60% and 50% every 14 h and 24 h, respectively. By contrast, RB rapidly interrupted with the disappearance of proE and basophilic E after the switch to EPO alone. Consistently, the quantities of ribosomal RNA (rRNA) 45S precursor estimated by qPCR are maximal in proE and almost null in orthochromatophilic E. Inhibition of RB at proE stage by RNApol I specific inhibitor (CX-5461) accelerates the onset of terminal erythroid differentiation suggesting that RB is a rate limiting factor for final maturation.

We then hypothesize that degree of signaling intensity in response to SCF and EPO may control the level of RB. To address this question, we investigated the mTORC1 (mechanistic Target Of Rapamycin Complex 1) pathway which is directly involved in RB through its substrate p70S6Kinase. Activation of P-p70S6Kinase and P-Rps6, as well as ribosome renewal, are twice more elevated in response to SCF and EPO than to EPO alone. Furthermore, inhibition of mTORC1/p70S6K/Rps6 pathway by rapamycin disrupts RB and leads to an acceleration of terminal erythroid differentiation.

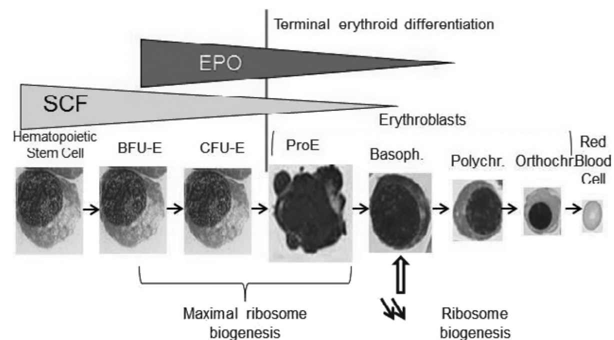

Fig. 1.

This study demonstrates that the collapse of RB promotes erythroid cell terminal maturation and shows the regulatory role of mTORC1 pathway on RB during erythropoiesis.

Keywords: erythroid differentiation, mTORC1, ribosome biogenesis.

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MON-128
The heat shock protein TRAP1/HSP75 regulates protein synthesis in cancer cells

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TNF receptor-associated protein 1 (TRAP1) is an HSP90 chaperone involved in mechanisms of stress protection and resistance to apoptosis in mitochondrial and extramitochondrial compartments. Remarkably, aberrant deregulation of TRAP1 function has been observed in several cancer types with potential new opportunities for therapeutic intervention in humans. Recent studies by our group identified a previously undescribed localization of TRAP1 in the endoplasmic reticulum and ascribed to this localization novel roles of TRAP1 in quality control of mitochondria-destined proteins through the attenuation of their synthesis and degradation. However, the molecular mechanisms involved in such regulation are still largely unknown. To shed light on the signaling pathway of TRAP1-dependent attenuation of mRNA translation, we analysed protein synthesis regulation in HCT116 and HEK293 and found that in both cell lines TRAP1 silencing enhances cap-mediated translation and increases ratio between cap- and IRES-mediated translation. Consistently, expression levels of p70S6K and RSK1, two translation activating kinases, are increased upon TRAP1 knock down. Furthermore, we show that these regulatory functions affect the response to translational stress and cell migration in wound healing assays, processes involving these kinases. Notably, the regulatory mechanisms controlled by TRAP1 are conserved in colorectal cancer tissues, since an inverse correlation between TRAP1 and p70S6K expression is found in tumor samples, thereby supporting the relevant role of TRAP1 in translational regulation *in vivo*. Taken as a whole, these new findings candidate TRAP1 network for new anti-cancer strategies aimed at targeting the translational/quality control machinery of tumor cells.

Keywords: Colorectal carcinoma, Translation control, TRAP1.

MON-129
The SBP2 protein central to selenoprotein synthesis contacts the human ribosome at expansion segment 7L of the 28S rRNA

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The amino acid selenocysteine is encoded by UGA, usually a stop codon, thus requiring a specialized machinery to enable its incorporation into selenoproteins. The machinery comprises the $tRNA^{Sec}$, a 3'UTR mRNA stem-loop termed SElenoCysteine Insertion Sequence (SECIS), which is mandatory for recoding UGA as a Sec codon, specialized elongation factor eEFSec, the SECIS Binding Protein 2 (SBP2) and other proteins. SBP2 is a pivotal protein component in selenoprotein synthesis. It binds the SECIS stem-loop in the 3'UTR of selenoprotein mRNA and interacts with both eEFSec and the ribosome at the 60S subunit. Here, SBP2 binding site on the human ribosome was identified. Cross-linking experiments with bifunctional reagents demonstrated that the SBP2 binding site is mainly formed by the 28S rRNA. Direct hydroxyl radical probing of the entire 28S rRNA in the complex of SBP2 with the ribosome revealed that SBP2 protects helix ES7L-E in expansion segment 7. Diepoxybutane cross-linking confirmed the interaction of SBP2 with helix ES7L-E. Additionally, chemical probing showed that the binding of SBP2 to the ribosome led to increased reactivity of a few bases in ES7L-E and in the universally conserved helix H89, indicative of conformational changes in the 28S rRNA in response to SBP2 binding. Altogether, the findings obtained led us to bring new insight into the selenocysteine insertion mechanism in mammals.

The work was carried out in the frame of the Laboratoire International Associé LIA NUCPROT and supported by the Russian Foundation for Basic Research (grant 12-04-93111-CNRSL_a to G.K.).
Keywords: ribosome, selenoproteins.

MON-130
Visualization of ribosomes in the nucleus of *Drosophila* cells

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Translation requires correct joining of the small and large ribosomal subunits on the mRNA. Although the 40S and 60S subunits are assembled in the nucleolus in eukaryotes, it is believed that there are mechanisms preventing 80S formation and translation initiation in the nucleus. Contrary to this view, with a novel technique we have recently reported that functional 80S ribosomes are also present in the nucleolus and other nuclear sites (1). Notably, the signal was drastically reduced by Pol II inhibition, suggesting that nuclear 80S are preferentially associated with mRNA or their precursors. Flow cytometric analysis reveals that nuclear 80S is present at all stages of the cell cycle which peaks at the S-Phase. Serum starvation and other forms of cellular stress were found to increase nuclear 80S. At present we are using our 80S visualization technique to analyze the sub-cellular distribution of ribosome in different tissues such as photoreceptor neurons with the aim of understanding whether 80S are present along axons and synapses of active neurons. We will present our latest results at the meeting.

Reference

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Keywords: Nuclear 80S ribosome, Ribosomal subunit joining, Translation.

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CSIII-01 – Chromatin & Epigenetics

MON-132
1A2 insulator can interact with two promoters in one model system

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Insulators are regulatory DNA elements that participate in the modulation of the interactions between enhancers and promoters. An insulator located between enhancer and promoter disrupts enhancer-promoter interactions. One possible mechanism of this is the establishment of direct insulator-promoter interactions that prevent physical contacts between promoter and enhancer. Insulators have the ability to neutralize enhancer action with respect to several promoters in one model system. In the present study it was shown that the 1A2 insulator can interact with promoters of the *hsp70* and *yellow* genes in one model system in *Drosophila melanogaster*. Interactions between insulators and promoters were demonstrated using the assay based on the yeast GAL4 activator. The GAL4 activator fails to stimulate the promoter that is located at the long distance from the corresponding gene; however interacting element (insulator) brings it closer to the promoter allowing such stimulation. Additionally the insulator-promoter interaction was confirmed by chromatin immunoprecipitation (ChIP) assay. It was demonstrated that the insulator protein CP190 is detected on the hsp70 promoter only in the presence of the insulator in the transgene. Thus the insulator-promoter interaction can play an important role in the modulation of the enhancer action.

Keywords: insulator, insulator-promoter interactions.

MON-133
5-aza-2′-deoxycytidine up-regulates expression of MGAT3 gene in HepG2 cells and causes significant changes in N-glycome of secreted proteins

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Protein N-glycosylation is an important posttranslational modification which affects protein structure and function. Majority of plasma proteins are synthesized in liver and changes in their glycosylation are often associated with different types of liver diseases, including hepatocellular carcinoma (HCC). HepG2 cell line is a hepatocellular carcinoma cell line which secretome is comparable with secretomes of HCC patients. This cell line can serve as a model to study epigenetically induced changes in glycosylation of secreted proteins. Using epigenetic inhibitor 5-aza-2'-deoxycytidine we have induced global hypomethylation in HepG2 cells. A panel of 84 glyco-genes was analysed and around 20% of the genes changed the expression level following the epigenetic treatment. Change in glyco-gene expression level was correlated with preferential appearance of particular glycan structures in the HepG2 secretome. The overexpression of *MGAT3* gene explained the majority of the changes observed in the glycosylation profile. GNT-III, enzyme coded by *MGAT3* gene, is responsible for the addition of bisecting GlcNAc, which prevents further core fucosylation and branching. When methylation and the expression level of *MGAT3* was specifically analysed and correlated with glycome composition from secretome of HepG2 cells following 5-aza-2'-deoxycytidine treatment we were able to detect decrease of glycan structures with core fucose and highly branched structures. Many epigenetic inhibitors are currently explored as part of a therapeutic strategy or are already used in cancer treatment. The present work contributes to our understanding of their efficiency in altering the N-glycan profiles of secreted proteins.

Keywords: glycome, HepG2, MGAT3.

MON-134
A mechanism for sensing chromatin alterations in mammalian cells

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Detection of genomic changes represents a critical step in the cellular response to DNA damage. Here, we show that the protein acetyltransferase KAT5 (Tip60) becomes tyrosine phosphorylated upon DNA damage, which enhances its binding to the histone mark H3K9me3. This in turn triggers KAT5-mediated acetylation of the ATM kinase, promoting checkpoint activation and cell survival. Notably, we also establish that chromatin alterations *per se* can induce KAT5 tyrosine phosphorylation and ATM-dependent signaling, and identify the proto-oncogene c-Abl as the mediator of this modification. These findings thereby define KAT5 as a key sensor for genomic and chromatin perturbations, and highlight a prime role for c-Abl in such events.

Keywords: checkpoint control, chromatin, genome stability.

MON-135
Aberrant methylation of HOXA10 in eutopic and ectopic endometrial tissues of women with endometriosis

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Endometriosis is a benign gynecological disorder which is associated with both infertility and pelvic pain and it is characterized by the presence of endometrium outside the uterine cavity. Although it has been proposed that endometriosis is a genetic disease, but recent studies revealed that endometriosis can be considered as an epigenetic disorder. In this study, we analyzed mRNA expression of *HOXA10* gene parallel to methylation level of its promoter, as a critical gene responsible for uterine organogenesis, endometrial differentiation and cell proliferation through the regulation of hundreds of genes. To achieve this aim, eutopic and ectopic endometrium samples were collected using laparoscopy from 28 women with documented endometriosis, and also endometrial tissues were collected from 18 healthy fertile women

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undergoing laparoscopy for tubal ligation surgery as a control group. Ethical approval and informed patient consent was gained for the use of tissue samples. Quantitative expression analysis was performed by real-time PCR technique and methylation detection analysis was performed by Chromatin Immunoprecipitation (ChIP), using anti-MeCP2 antibody. Data showed a harmonious pattern between mRNA expression of *HOXA10* and methylation level of its promoter region. In the way that, *HOXA10* gene expression in eutopic endometrium decreased in secretory phase in comparison to control group, parallel to hypermethylation of the promoter. In contrast, hypomethylation of *HOXA10* in ectopic tissue samples caused a significant increase in mRNA level of the gene, in both proliferative and secretory phase, comparing to control group. Our data suggest that epigenetic aberration of *HOXA10* gene may be associated with the etiology of endometriosis.

Keywords: Endometriosis, Epigenetics, HOXA10.

MON-137

Analysis of epigenetic pathways induced by enhancer and insulator in genetic constructs transfected into Drosophila S2 cells

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Enhancer and insulator are ones of the key regulators of gene expression. We developed a system designed for the study of these elements in transfected genetic constructs. The system prevents the effects coming from other regulatory elements in cis on epigenetic states in different regions of the constructs. Previously we detected in Drosophila cultured S2 cells a promoter-independent bidirectional synthesis of non-coding RNAs in the intergenic region of the constructs induced by enhancer (enhancer RNAs, eRNAs). The synthesis was repressed by a single insulator or a pair of gypsy insulators (Tchurikov et al., 2009).

In this study we want to explore this eRNAs, and investigate epigenetic pathways launched by enhancer and insulator in genetic constructs transfected into S2 cells. With 5' RACE, primer extension and high-throughput sequencing approaches we detected that the most part of TSS of eRNAs are located at about 250 bp distance from the enhancer. We revealed, that enhancer induces the H3K4me3 and H3K18ac in the regions of eRNA synthesis. We observed that insulator acts as antagonist of enhancer, as far as it inhibits both the levels of eRNAs synthesis and H3K4me3 and H3K18ac marks produced by enhancers, and, at the same time, induces H3K4me1 and H3K27ac marks in the same region of the construct. Furthermore, study of insulator proteins binding shows that these proteins bind in different regions of genetic constructs. E.g., dCTCF, mod(mdg4)2.2 and Su(Hw) bind with enhancer and promoter regions in the absence of insulator. Interestingly, the introduction of one copy of gypsy insulator in the genetic construct suppresses this binding, while two copies of gypsy insulators are not as efficient, suggesting the interaction between them. Beside this, using ChIP we observed, that the genomic copies of gypsy and six different insulators from Bithorax Complex interact with nuclear lamina.

These data strongly suggest that enhancers and insulators mainly act indirectly, epigenetically, modulating histone modifications, synthesis of eRNAs, and regulating 3D chromosomal structures.

Keywords: Non-coding RNA, cis-regulatory elements, histone modification.

MON-138

Analysis of the epigenetic response induced by environmental conditions during seasonal adaptation of *C. carpio* fish

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For the *Cyprinus carpio* (carp) fish, seasonal acclimatization requires implementation of complex molecular and cellular mechanism that coordinates phenotypic plasticity. This process involves a reprogramming of gene expression, which in turn integrates a global homeostatic response. Previously, we have demonstrated that ribosomal genes (rDNA) activity is directly correlated with a change of activity and structure of the nucleolus. Moreover, this trait seems to be based in substantial changes in the number of active rDNA genes during carp acclimatization. Considerable evidence indicates that epigenetic mechanisms are important for regulation of this intricate process. In addition, cells must also have the ability to maintain their functions by constantly sensing and adapting to environmental variations (homeostasis). We are interested in the intersection of these two major cellular functions, namely in the issue of how transcriptional rRNA regulation is coordinated in response to oscillating environmental changes.

In the present work, we present some epigenetic mechanisms contributing to govern the reprogramming rDNA transcription in response to the seasonal adaptation of carp fish. Initially, we have determined the progression of the ratio of active/inactive ribosomal genes during acclimatization. Subsequently, we focused our attention in the rDNA regulation mediated by a molecular axis that involves TTF-I and NoRC chromatin-remodeling complex. Additionally, we have investigated the role of the eNoSC chromatin-remodeling complex during caloric restriction in carp cells. In the first case, we have established that NoRC contributes to silencing of rDNA during cold adaptation season. On the other hand, eNosC displays a compensatory effect that contributes to prevent a drastic cellular energy imbalance during carp acclimatization. Finally, we have studied the role of histones variants mH2A and H2A.Z in the transcriptional regulation of rDNA genes during carp seasonal adaptation. In both cases, we have observed that the activity of rDNA can be seasonally modulated through a dynamic exchange of canonical histones by their variants during the carp acclimatization.

In conclusion, our results demonstrate that epigenetic mechanisms play a central role in the reprogramming of gene expression of rDNA, which emerge as a molecular strategy to implement phenotypic plasticity during carp seasonal adaptation.

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Keywords: Epigenetics, Gene reprogramming, rRNA transcription.

MON-139

Analysis of the functional diversity of hINO80, a chromatin remodeling protein

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Chromatin remodeling complexes (CRM) are multiprotein complexes that contain a dedicated DNA dependent ATPase subunit of the SWI2/SNF2 family, that have been identified in organisms

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ranging from yeast to *Homo sapiens*. SNF2 like family members are further divided into several subfamilies according to the sequence features outside of their ATPase domain. The prominent subfamilies include ISWI, CHD1, SWI/SNF, and INO80 subfamilies.

The previous studies in our lab on human SWI2/SNF2 like protein hINO80 has demonstrated the catalytic and DNA binding activity (Bakshi *et al* 2006). The recent work in our lab has shown essentiality of dINO80 for Drosophila development, as *dIno80* knock-out is late embryonic lethal and these embryos show misexpression of homeotic genes (Bhatia *et al*, 2011). The DNA binding motif of INO80 has been identified by SELEX approach. In order to validate the regulatory role of hINO80 in human cell lines we cloned the INO80 binding motif both upstream and downstream the reporter gene. The strategy involved cloning the sequence having INO80 motif in a reporter vector pGL3 and monitor the expression of the reporter gene in the presence/ absence (knock down) of hINO80. By monitoring the expression of luciferase reporter gene under both conditions the regulatory role of hINO80 has been studied and we hypothesize INO80 to be functionally more diverse than what is known in literature so far.

We have bioinformatically analysed the distribution of INO80 and YY1 binding motif in the sequences upstream and downstream of human protein coding genes. While there are genes with both INO80 and YY1 binding motif, there are also several genes that have INO80 binding motif alone and YY1 motif alone. This raises the possibility of YY1 independent function of INO80. We have validated the binding of INO80 at selected target sites *in vivo* by Chromatin Immunoprecipitation (ChIP). We have also demonstrated the direct and specific DNA binding of hINO80 complex by EMSA. We have shown that INO80 can regulate reporter gene expression through its binding site.

Our results suggest an additional function of INO80 in regulating gene expression perhaps by direct binding to the DNA and acting as a recruiter, through its binding motif. The nature of the INO80 complex that mediates this function remains to be deciphered.

Keywords: Chromatin Remodelling, Epigenetics, microRNA.

MON-140
ASXL2 links ERα to histone methylation and determines antiestrogen response in ERα-positive breast cancer

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Despite the importance of correlations between transcriptional activity and epigenetic regulation of Estrogen receptor α (ERα) in breast cancer, their mechanisms and physiological roles are not clearly understood. Here we demonstrated that Additional sex comb-like 2 (ASXL2) plays pivotal role in ERα-positive (ERα+) breast carcinogenesis through recruiting co-activator complex containing LSD1 and UTX and removing repressive histone codes on ERα-target promoter upon E2 stimulation, supporting cooperation of ASXL2 complex in genome-wide level. ASXL2 expression is strongly correlated with ERa level, showed close association with overall good survival rate of breast cancer patients. Our data suggest that the necessity and majority of ASXL2 on ERα activity and the possibility of ASXL2 as new diagnostic marker and therapeutic target of breast cancer.

Keywords: ASXL2, breast cancer, epigenetics.

MON-141
Characterizing the zinc finger binding interactions of PRDM9 with the DNA of recombination hotspots

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Meiotic recombination events cluster in 1–2 kb regions of the genome termed recombination hotspots. Hotspots have been characterized for a long time, but little is known on how and why a region is targeted for recombination. The histone methyltransferase PRDM9 has been identified as a key trans-regulator of hotspot activity in mammals. It is believed that PRDM9 recognizes specific DNA motifs via its tandem array of zinc fingers and then epigenetically marks the local chromatin by its histone methyltransferase activity. Specific DNA motifs recognized by PRDM9 are a key factor in determining hotspots, yet there are many cases in which motifs are neither necessary nor sufficient to determine a hotspot, and in many instances the motifs are found more often outside than within hotspots. Thus, we still do not fully understand the binding determinants of PRDM9 to DNA. We are therefore analyzing PRDM9 binding affinities to diverse DNA substrates *in-vitro* using Electrophoretic Mobility Shift Assays (EMSAs), as well as more quantitative biophysical techniques. We have confirmed that binding to a specific hotspot sequence is much stronger than to a random DNA sequence or even to the predicted binding sequence (derived by the Persikov algorithms). Moreover, the addition of high amounts of specific or unspecific DNA leads to an increased PRDM9 affinity, suggesting either a cooperativity effect or a change in accessibility to the DNA-binding region. Furthermore, epigenetic modifications such as DNA methylation as well as the nature of the regions flanking the motif have a strong effect on the binding. Our results show that DNA binding motifs recognized by the zinc finger array do not capture all the aspects of the binding site information.

Keywords: Meiotic recombination, PRDM9, Protein-DNA binding assays.

MON-142
Comparative analysis of methylation/deletion patterns of chromosome 3 in different cancer types using NotI-microarrays

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Cancer is characterized by numerous genetic and epigenetic alterations. NotI-microarrays earlier developed by Dr. Eugene R. Zabarovsky are unique tool enabling simultaneous identification of DNA hypermethylation and deletions – two major reasons of tumor suppressor gene (TSG) inactivation. In the present work, methylation and/or deletion (M/D) frequencies were evaluated in lung (40 samples), ovarian (18), cervical (48), renal (23), breast (47) and colon (24) primary tumors using specific to chromosome 3 NotI-microarrays containing 180 NotI-clones associated with 188 genes. The important role of chromosome 3 in cancer is well known, but large-scale simultaneous analysis of genetic and epigenetic aberrations was not still performed. In this study in each cancer type we identified at least 25 genes with 15% or higher

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M/D frequency. These genes include TSGs or TSG-candidates (for example, *VHL*, *CTDSPL*, *ITGA9*, *EPHB1*, and *ALDH1L1*) and genes that were not previously considered as cancer-associated (e.g., *LRRN1*, *GORASP1*, *FGD5*, *TOPAZ1*, and *PLCL2*). In general, patterns of M/D were distinct in different cancer types. Nevertheless, the same genes were altered often. Proteins encoded by identified genes are involved in signaling pathways and biological processes frequently affected during carcinogenesis. However, for a part of the genes, functions of their proteins are still unknown: *LRRN1*, *LRRC3B*, and *TOPAZ1*, for example. In several examined samples at least 20 out of 180 NotI-sites were affected simultaneously. Probably, these samples represent CIMP+ tumor phenotype. Results of the bisulfite sequencing analysis were in concordance with NotI-microarray data and confirmed methylation as a frequent event in examined cancer types. Frequent (>40%) and significant (>4-fold) down-regulation was shown for several genes with high M/D frequency (for example, *CTDSPL*, *ITGA9*, *ALDH1L1*, *LRRN1*, and *FGD5*) using quantitative PCR. Finally, obtained data allowed to suggest potential sets of methylation markers for diagnostics of each studied cancer type and identification of pathomorphological characteristics of tumor.

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Keywords: chromosome 3, DNA methylation, NotI-microarrays.

MON-143
Comparative chromatin evaluation of human embryonic stem cells and induced pluripotent cells

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Pluripotent cells including embryonic (ESCs) and induced pluripotent stem cells (iPSCs) have extraordinary characteristics originated from their chromatin structure and regulation. One of the regulating levels of chromatin is post-translational modifications (PTM) on histones. PTMs are very important molecular determinants in epigenetic regulatory mechanisms. Also the pattern of PTMs which termed epigenome, has a critical role in regulation of pluripotency, differentiation and the fate of ESCs and iPSCs. Some PTMs such as H3K9ac and H3K9me2 are well known as epigenetic marks which correlate with active and repressive chromatin, other ones like bivalent marks (H3K4me3 and H3K27me3) are characteristic features of pluripotent cells. So, examination of nucleosomes as target of PTMs can be an adequate and rapid way to compare total levels of epigenetic marks on chromatin of ESCs and iPSCs. In this study the chromatin of human ES cells before and after differentiation was analyzed and compared with chromatin of iPS cells originated from differentiated ESCs and also fibroblasts as committed cell line. For this respect, an enzyme-linked immunosorbent assay (ELISA) based method was used for the quantitative evaluation of H3K9ac/me and bivalent marks, as PTMs on intact nucleosomes to compare their patterns on ESCs and iPSCs. Results showed significant changes of all examined epigenetic marks through differentiation of ES cells as well as the pluripotency induction of differentiated cells. Although on the other side, remarkable similarities were seen in PTM patterns of the two pluripotent ESCs and iPS cell. This experiment indicates the association of epiproteomic signature of cells with cellular identity, pluripotency, differentiation and reprogramming state of cells.

Keywords: epiproteome, histone modifications,, pluripotent cells.

MON-144
Comparison of 5hmC enichment techniques

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Epigenetic regulation is the process by which gene expression can be regulated without affecting the underlying DNA sequence. Direct modification of DNA bases and histone tails are 2 ways in which this regulation can occur. One well studied epigenetic mark is that of direct chemical modification to cytosine bases in the dinucleotide "CpG". The most abundant forms of this modification are that of 5-methylcytosine (5mC) as well as the more recently rediscovered 5-hydroxymethycytosine (5hmC) mark. The latter of these marks is of particular interest due to its apparent relationship with transcriptional activity when found in genic regions. The focus of my PhD is on the changes in the epigenetic profiles of cells during both reprogramming and the loss of the DNA methyltransferase responsible for maintaining the methylation pattern of the genome, DNMT1. Analysis has shown that certain epigenetic profiles, such as that of 5hmC, can be used as a good identifier of tissue type and state. As such current 5hmC research is focussed on the distribution of this modification genome wide.

Although several 5hmC enrichment techniques have been described, the majority of work focuses on antibody based enrichment protocols (HmeDIP). However, antibody based techniques may have some limitations and sequence bias. Thus part of the focus of my work has been to compare this widely used and well accepted 5hmC enrichment technique to newer alternative approaches, namely glycosylation based chemical capture techniques and JBP based affinity purification. From this analysis we compared the efficiencies of all three techniques, using genome wide arrays along with qPCR to give a side by side comparison of the results of the protocols. Data from this research has shown that the JBP affinity based approach yields very low levels of enrichment post purification, whilst chemical capture protocols result in comparable 5hmC patterns to those generated using HmeDIP. Nevertheless we do find a number of regions which show a striking yet reproducible difference between the two techniques which could be the result of either antibody bias or a failure in the chemical capture to bind to these regions. Further work has given us a better understanding of the basis of these differences allowing us to determine which protocol or combination of techniques gives the most accurate picture of the epigenetic landscape.

Keywords: None.

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MON-145
CpG islands promoter methylation the main cause for GNMT gene decreased expression in pancreatic adenocarcinoma

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Pancreatic cancer remains a major challenge for therapy and biomarkers identification. Many epidemiological and clinical studies had proved the importance of early detection to decrease the mortality caused by pancreatic cancer.

Epigenetic silencing of tumor suppressor genes is a major contributor to neoplastic transformation and is an area of intense research. Identification of genes which undergo cancer-specific CpG island hypermethylation and correlation of these data with tumor stage, progression, and long-term prognosis are becoming increasingly common.

The aim of this study was to identify new factors involved in pancreas oncogenesis.

The results of bioinformatic analysis of microarray data found a down regulation of GNMT gene (glycine N-methyl transferase) in pancreatic adenocarcinoma. GNMT posses CpG islands in the promoter and is an important gene involved in methyl group metabolism and in maintaining a normal methylation status of the genome.

To test the hypothesis that GNMT is epigenetic regulated in PDAC, we evaluated the *GNMT* gene expression and promoter methylation status in 30 paired samples of PDAC and normal pancreatic tissue. We found significantly higher methylation frequencies ($p < 0.001$) in PDACs (2.82–100%, median=36.05%) than in controls (0.28–14.02%, median=4.39%). The *GNMT* gene expression was decreased in PDACs compared to normal pancreatic tissue in 26/30 cases (86.67%). Fold change expression of investigated gene was between −0.02 and −4.55, median = −2.42, suggesting an important role for the downregulation of this gene in PDAC. Furthermore, we show that the treatment with 5-aza-dC increased *GNMT* mRNA expression and decreased viabiliy in PDAC cells. Collectively, these data indicate that *GNMT* is aberantly methylated in PDAC, and may be a major mechanism for gene silencing. Methylation of *GNMT* gene is directly correlated with disease stage and with tumor grade, indicating that these epigenetic effects may be important regulators of PDAC progression, and may serve as prognostic biomarker for PDAC.

The study was supported by POSDRU 89/1.5/S/60746 and PN-II-PT-PCCA90/2012 research grants.
Keywords: epigenetic alteration, promoter methylation, pancreatic cancer.

MON-146
CTCF target sequences within the chicken alpha-globin gene locus

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CTCF is a highly conserved multifunctional transcription factor of vertebrates. This protein is implicated in diverse regulatory functions, including transcriptional activation/repression, insulation, imprinting, X chromosome inactivation. CTCF role in the global organization of chromatin architecture was supported in a lot of works. CTCF protein binds DNA sequences named insulators. Insulators block influence of regulatory genome elements on genes located on the other side of insulator. Chicken alpha-globin gene domain is an open chromatin domain both in erythroid and nonerythroid cell types. However expression of the most nonglobin genes in domain doesn't change in consequence of the erythroid cells differentiation. We supposed that CTCF binding has a crucial role in alpha-globin genes insulation and domain structure reorganization linked with erythroid cell differentiation. We selected CTCF-binding sequences from fragments of the chicken alpha-globin locus DNA by two dimentional EMSA (Electrophoretic Mobility Shift Assay) in vitro. CTCF binding for some of this sequences was shown in vivo by chromatin immunoprecipitation and ChIP-seq (chromatin immunoprecipitation with massively parallel DNA sequencing) analysis on erythroid and lymphoid chicken cell lines. Some differences in CTCF-binding of this fragments in this cell lines may suggest a role of CTCF protein in alpha-globine gene expression. We compared data get by two dimention EMSA, ChIP-seq analysis and CTCF binding site motifs location. This comparison allow us to suggest, that vertebrate genomes contain a lot of sequences that can effectively bind CTCF in vitro, but don't bind it in vivo. As well DNA fragments that bind CTCF in vivo can have relative weak CTCF affinity in vitro and weak CTCF binding site consensus similarity.

Keywords: alpha-globin, CTCF.

MON-147
Detection of active promoters in human genome libraries using reporter assay

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Promoters are indispensable functional elements in all expression systems used in a wide range of medical and biological research. For many applications strong and cell-type specific expression of genes of interest is needed. Therefore massive functional approach for detection and isolation promoters active in specific cell types may be a very useful technique. For detection of active promoters in genome libraries we obtained sonicated PCR-amplified human library and cloned it between two reporter genes of green and red fluorescent proteins located in "head-to-head" orientations in plasmid vector. Over 400000 DNA fragments with size of 300–1500 bp were cloned and pool of plasmids obtained. After transient transfections of two human cell lines we analyzed green fluorescence using flow cytometer and find that 1% and 7% of true GFP-positive cells in A-431 and 293T cells, respectively. We also inserted in plasmid vector CMV enhancer and cloned library fragments into this construct between two reporter fluorescent protein genes. CMV enhancer increased the number of true GFP-positive cells up to 6% and 18% in A-431 and 293T cells, respectively, and therefore can be used for detection of weak or inactive cell-specific promoters in genome libraries.

Keywords: active promoters, genome libraries, massive functional assay.

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MON-148
DNA methylation alterations in ovarian cancer: impact on cancer initiation, progression and response to therapy

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We used methylated DNA immunoprecipitation (MeDIP) in combination with CpG island tiling arrays to characterize at high resolution the DNA methylation changes that occur in the genome of serous epithelial ovarian cancer (EOC) during disease progression. The DNA methylation profiles of five serous borderline, five serous grade 1/stage III/IV, five serous grade 3/ stage I and five serous grade 3/stage III/IV EOC tumors were compared with those of five normal human ovarian tissue samples.

We found widespread DNA hypermethylation at CpG islands in serous tumors that occurs even in less invasive/early stages of ovarian tumorigenesis. This hypermethylation preferentially included key developmental/homeobox genes and is possibly associated with repressive chromatin marks such as Polycomb group proteins-mediated gene silencing. Contrary to DNA hypermethylation, significant DNA hypomethylation was observed only in high-grade (G3) serous tumors. The later observation was further confirmed when comparing the DNA methylation profiles of primary cell cultures derived from matched tumor samples obtained prior to, and following chemotherapy treatment from two serous EOC patients with advanced disease. To our knowledge this is the first report that has shown the presence of massive DNA hypomethylation in advanced serous EOC, associated with the possible induction of a number of oncogenes, implicated in cancer progression, invasion/metastasis and probably chemoresistance. Our data raise the concern that demethylating drugs that are currently being used in advanced EOC disease (representing the majority of serous EOC cases) might have adverse effects due to activation of oncogenes and prometastatic genes. Understanding the relative roles of hypomethylation and hypermethylation in cancer could have clear implications on the therapeutic use of agents targeting the DNA methylation machinery.

Our epigenomic approach has also led to the identification of novel aberrantly methylated gene targets in serous EOC, including hypermethylated genes with potential tumor-suppressor gene function, and hypomethylated genes, involved in disease progression. These genes could represent new therapeutic targets and/or novel biomarkers indicative of EOC etiology.

Keywords: DNA methylation, epigenomics, ovarian cancer.

MON-149
DNA methylation analysis of selected genes comprised in the epidermal differentiation complex (EDC) during epidermal differentiation

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The epidermal differentiation complex (EDC) incorporates a syntenic and linear cluster of 4 gene families encoding the S100 proteins, the small proline rich proteins (SPRRs), the late cornified envelope proteins (LCE) and the S100-fused type proteins (SFTPs), all involved in keratinocyte differentiation process. We presupposed that epigenetic factors may play a considerable role in the regulation of EDC gene expression. As a research model we employed human epidermal keratinocytes (primary HEKa cells) in undifferentiated and differentiated form (the latter maintained in higher calcium concentration). Methods of real time PCR, genomic DNA isolation, bisulfite conversion and conventional sequencing were applied. First, we examined the expression pattern of several S100 genes during epidermal differentiation. Subsequently, we verified if the observed changes in mRNA level were reflected by changes in DNA methylation pattern. We performed the analysis for S100A6, S100A7, S100A8, S100A13 genes, for the NICE1 gene located within the LCE subcluster, and also for genes encoding involucrin and loricrin - the established markers of keratinocyte differentiation. Additionally, we examined DNA methylation within a recently identified conservative non-coding element endowed with enhancer properties. Gene areas harboring extremely CpG rich regions (defined as CpG islands) along with relatively CpG poor regions were selected for analysis. The analyzed DNA fragments were localized in the immediate proximity of transcription start sites or in both exons and introns. We did not observe distinct changes in CpG methylation in the analyzed gene regions between the initial and final stage of keratinocyte differentiation. Furthermore, the actual methylation state did not preclude the gene expression level. Other EDC fragments are to be investigated to localize differentially methylated regions (DMRs) of potential regulatory significance.

Keywords: Bisulfite sequencing, DNA methylation, Epidermal Differentiation Complex (EDC).

MON-150
Effect of insertion of anticancer drug 6-thioguanine into DNA on the functioning of mammalian de novo DNA methyltransferase Dnmt3a

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6-Thioguanine (G^s) is a cytotoxic drug which is widely used for the treatment of acute leukemia. However, the mechanism of G^s cytotoxicity is still unknown. *In vivo* G^s forms 2'-deoxy-6-thioguanosine triphosphate which incorporates into DNA. DNA methylation at CpG sites is an epigenetic modification of the genomes that plays an important role in regulation of many biologic processes including gene regulation. DNA methyltransferase Dnmt3a is involved in establishment of new methylation patterns in genome. The incorporation of G^s into CpG sites may affect the activity of Dnmt3a. Here, we examine the impact of G^s on cytosine methylation mediated by catalytic domain of Dnmt3a (Dnmt3a-CD). 30-mer DNA duplexes containing G^s either in the CpG site or adjacent to it (G^s-DNA) were obtained. Also we designed two-site substrates with G^s replacements. Using two-site substrates we took into account that the active form of Dnmt3a is a tetramer with two catalytic centers.

An introduction of the G^s into the CpG site near the target cytosine led to decrease of methylation as compared to the unmodified substrate. This effect may be due to the perturbation of the contact of Dnmt3a-CD with the 6-oxo group of the guanine which is important for the formation of a catalytically competent complex Dnmt3a-CD/DNA. Occurrence of the G^s opposite the target cytosine or in the CpG flanking sequences virtually has no effect on the methylation efficiency. Importantly, that in the case of two-site substrates incorporation of one or two G^s residues caused approximately equal decrease of the V_0 values. Probably, methylation of both sites by two catalytic centers of

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tetramer Dnmt3a-CD occurs interdependently and modification of one of the CpG-sites affects methylation of non-modified neighboring CpG site.

Binding of Dnmt3a-CD to G^s-DNA shows positive cooperativity like in the case of unmodified substrates. The K_d values for G^s-DNA/ Dnmt3a-CD complexes slightly increased regardless of the position of the G^s residue in DNA.

Absorption band of 6-thioguanine is above 300 nm where unmodified DNA and the enzyme are transparent. This allowed us to use G^s residue as spectroscopic probe and to study structural changes of G^s-DNA in complex with Dnmt3a-CD by circular dichroism in the region of 300–400 nm. The local disturbance of stacking interactions of bases in the CpG-site within G^s-DNA/ Dnmt3a-CD complexes were observed probably due to the flipping of the target cytosine out of the double helix.

Taken together, these results showed epigenetic contribution of 6-thioguanine incorporation into DNA via impact on the functioning of Dnmt3a.

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Keywords: DNA methylation 6-thioguanine.

MON-151
Protein-DNA binding in the absence of consensus motif

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Genome-wide identification of protein-DNA binding preferences of ~100 of *Caenorhabditis elegans* transcription factors (TFs) has revealed that a significant fraction of binding events occur in highly occupied target (HOT) regions where few specific transcription factor binding sites (TFBSs) have been detected. These measurements also revealed that a significant fraction of consensus TFBSs do not bind TFs. Furthermore, *in vitro* measurements of *C. elegans* TFs binding preferences indicate that TFs systematically bind DNA sequences that lack known consensus motifs. These observations strongly suggest that alongside with conventional specific binding, additional recognition mechanisms affect protein-DNA binding preferences.

Here we demonstrate that DNA sequence repeats characterized by certain symmetries, statistically affect protein-DNA binding. Using a simple statistical mechanics model, we compute the nonconsensus protein-DNA binding free energy originating from TFs binding to such sequence repeats in the *C. elegans* genome. We use the term nonconsensus protein-DNA binding in order to emphasize the point that specific, consensus TFBSs do not contribute to this effect. We show that the nonconsensus protein-DNA binding free energy is negatively correlated with the overall TF occupancy genome-wide. In addition, we show that genomic background surrounding specific TFBSs influences specific protein-DNA binding via the modulation of nonconsensus protein-DNA binding.

Finally we will present our recent measurements in which we used high-throughput protein-DNA binding assays to measure the binding levels and free energies of binding for several human TFs to tens of thousands of short DNA sequences with varying repeat symmetries. Based on statistical mechanics modeling, we identify a new protein-DNA binding mechanism induced by DNA sequence symmetry in the absence of specific base-pair recognition, and experimentally demonstrate that this mechanism indeed governs protein-DNA binding preferences.

Keywords: Pre-initiation complex, protein – DNA interactions, Transcription.

MON-152
Epigenetic regulation of piwil2 transcription in testicular tumors

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DNA methylation is a major epigenetic modification with a very important role in regulation of gene expression. During tumor development methylation pattern changes, this process is especially significant for gene regulatory regions and leads to changes in transcription level of appropriate genes. There are four PIWI-like proteins (PIWIL) in humans which are involved in embryonic stem cells development and promote precancerous stem cells proliferation. Many researchers report *piwil* genes transcription level changes in neoplasias, in particular, in testicular tumors.

We studied the influence of *piwil2* CpG island (CGI) methylation on its promoter activity in transiently transfected Tera-1 (testicular embryonic carcinoma) and A549 (lung adenocarcinoma) cell lines. The luciferase assay has showed a notable reduction of methylated promoter activity in both cell lines. Demethylation of CGI by 5-aza-2'-deoxycytidine and trichostatin A restored *piwil2* transcription inTera-1 other then A549 cell line.

Additionally, *piwil2* CGI methylation level was measured by methylation-sensitive high resolution melting (MS-HRM) analysis in testicular tumors (11 samples) and adjacent to tumor normal tissues. Also, *piwil2* transcription levels were determined in the same samples. There was no correlation between these two parameters in tumor and normal tissues. Absence of pronounced epigenetic regulation of *piwil2* transcription in testicular tumors could be due to expression of mRNA isoforms from alternative promoters, distant from CGI region.

In vivo and *in vitro* data obtained points to high tissue-specific manner of *piwil2* transcription: promoter demethylation appears to be a necessary but not sufficient condition for transcription. Probably, there is another expression regulation mechanism for *piwil2*, which is not connected with methylation level of CpG-rich promoter region.

The work was supported by RFBR grant 14-04-32314 and "Molecular and Cellular Biology" Programme of the Presidium of the Russian Academy of Sciences.
Keywords: epigenetics, methylation, CGI.

MON-153
Epigenetic role of the nuclear factor NF-Y on inhibitor of DNA binding (ID) helix-loop-helix family genes in human embryonic carcinoma cells

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Epigenetic mechanisms such as incorporation of histone variants/ substitutes have been shown to play key roles in regulation of gene transcription. Among them, NF-Y (Nuclear Factor Y) is a helix-loop-helix histone substitute protein which specifically binds to the CCAAT box of the target genes and causes their regulation. The CCAAT box is one of the cis-regulatory elements present in eukaryotic promoter regions of numerous genes such as basic helix-loop-helix ID (Inhibitor of DNA binding) factors. The ID regulatory proteins have important roles in development, by inhibiting differentiation and stimulating proliferation. Regarding the critical roles of *ID* genes in development, the potential role of NF-Y transcription factor on these NF-Y-regulated genes was aimed in this study. For this respect, we looked

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into expression levels of *ID* genes in a human embryonic carcinoma cell line, named NT2/NTera2, before and after 7 days-induction of differentiation. Afterwards, NF-Y incorporation on the regulatory regions of *ID* genes was evaluated quantitatively using chromatin immunoprecipitation (ChIP) coupled with real-time PCR on chromatin extract of NTera2 cells before and after differentiation. The results showed a significant incorporation of NF-Y on the CCAAT regulatory regions of *ID* genes before induction of differentiation, whereas a reduction was observed in the midst of induction term. In addition, the coordination in gene expression profile of *ID* genes with NF-Y incorporation during proliferation and differentiation implies for the first time that the NF-Y transcription factor might play a central role in transcriptional regulation of *ID* genes in embryonal development.

Keywords: ID, Nuclear Factor Y.

MON-154
Functional analysis of H2A ubiquitination in Polycomb repression

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To directly investigate the function of histone post-translational modifications in a metazoan, we use a system that permits the conditional replacement of the endogenous canonical histone proteins with mutated histone proteins in *Drosophila*. We investigated the role of histone modifications in Polycomb repression and recently showed that cells containing a histone H3-K27 point mutation fail to repress transcription of genes that are normally repressed by PRC2, the methyltransferase that modifies H3-K27. Here, we present our analysis on the role of histone H2A monoubiquitination in Polycomb repression. Unexpectedly, we found that repression of Polycomb target genes is maintained in cells containing mutant versions of H2A or H2Av that can no longer be ubiquitinated by PRC1-type complexes. This suggests that H3-K27 methylation but not H2A monoubiquitination is critical for maintaining Polycomb repression in Drosophila. We are currently investigating why H2A monoubiquitination is nevertheless essential for completion of embryogenesis and viability.

Keywords: development, histone modifications,, polycomb silencing.

MON-155
Global DNA methylation evaluation and DNMT1 gene expression in thyroid neoplasia

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Introduction: Thyroid carcinoma is the most common endocrine malignancy worldwide. Thyroid cancer initiation and progression occurs through gradual accumulation of various genetic and epigenetic alterations. DNA methylation is an epigenetic modification which is catalyzed by DNA cytosine-5-methyltransferases (DNMTs) and occurs at the 5-position (C5) of the cytosine ring, within CpG dinucleotides. DNMT1 is responsible for the maintenance of DNA methylation patterns in mammals and plays a crucial role in the epigenetic control of gene expression. Recently many studies have reported the implications of global DNA hypomethylation in several cancer types but little is known about this epigenetic event in thyroid neoplasia. Study aims to investigate the relationship between global DNA methylation status and DNMT1 expression levels in several types of thyroid tumors.

Methods: For this purpose 26 patients who underwent thyroidectomy were enrolled and divided into the following three groups acording to histopathologically exam: 1–10 subjects with papillary thyroid carcinoma (PTC); 2–8 patients with follicular adenoma (FA) and 3–8 patients with nodular goiters (NG). DNA and total RNA were extracted from tissue samples (26 matched tumoral and their adjacent normal tissue as controls) using commercial kits. DNA global methylation levels were assesed in patients samples by measuring 5-methylcytosine (5-mC) levels using ELISA. DNMT1 mRNA levels were quantified by qRT-PCR (ABI). Statistical analysis was performed with GraphPad Prism 5.0.

Results: The obtained data showed that for all 3 groups the global DNA methylation levels were lower than that of controls. Moreover a significant lower level of 5-mC was found in PTC patients compared with adjacent normal tissue. ($P < 0.007$) Results did not reveal significant differences between groups regarding DNMT1 gene expression levels. For PTC samples DNMT1 mRNA levels were found decreesed compared with controls, a positive correlation between DNMT1 expressions and global DNA methylation was noted for this group of patients.($P < 0.001$) Global DNA hypomethylation may play a important role for thyroid neoplasia but more studies are needed in order to elucidate the involvement of this epigenetic modification in thyroid tumorigenesis.

Keywords: DNMT1, global DNA methylation, thyroid cancer.

MON-156
HDAC inhibitors are potential regulators of tumor growth and tumor microenvironment

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Glioblastoma (GB) is one of the most difficult human malignancies to treat due to frequent dysfunctions of tumor suppressors and oncogenes, epigenetic aberrations, diffusive growth and changes in the tumor microenvironment. GB are abundantly infiltrated by microglia and peripheral macrophages that instead of developing anti-tumor immune responses, polarize into an anti-inflammatory phenotype and support invasion, angiogenesis and suppress the adaptive immunity. Recent evidence points to deregulation of histone modifying enzymes and aberrant epigenetic histone modifications in GB. The altered expression of histone deacetylases (HDACs) was detected in glioblastoma, but there was no systematic studies of neither expression or impact of selected HDAC inhibitors (HDACi) on glioma cells and microglia exposed to glioma.

Using immunofluorescence and Western blotting we found that VPA (valproic acid) and TSA (trichostatin A) increase histone H4 acetylation both in glioma and microglial cells. Treatment with TSA, but not VPA, affected viability of glioma cells. HDACi at non-cytoxic concentrations inhibited glioma-induced morphological changes of microglia, however only TSA inhibited upregulation of gene expression in activated microglia. The analysis of various HDAC gene expression revealed changes in the level of HDAC class II mRNAin microglia stimulated by glioma. These results suggest the role of HDAC class II in glioma pathology. We demonstrate the inhibitory effect of HDAC inhibitor on glioma growth and polarization of microglia into the proinvasive phenotype that points to a new prospect of therapeutic strategy based on direct modulation of tumor cells and its microenvironment.

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Keywords: glioma, histone deacetylase, tumor microenvironment.

MON-157
Highly conserved ENY2/Sus1 protein binds to *Drosophila* CTCF and is required for barrier activity

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ENY2/Sus1 protein is highly conserved multifunctional protein that is component of the deubiquitinylation module of SAGA histone-acetyltransferase complex involved in transcription regulation and of TREX-2 complex involved in the mRNA export. Structures of ENY2/Sus1 within these complexes suggest that this protein serves as adaptor molecule, binding to either Sgf11 (in SAGA) or Sac3 (in TREX-2) using the same surface for these interactions and so cannot bind both partners simultaneously. Also the Drosophila homologue of ENY2/Sus1 protein interacts selectively with the zinc-finger domains of Su(Hw), this interaction is required for barrier activity of Su(Hw)-dependent genomic insulators (that prevents spreading of heterochromatin in the genome). Other *Drosophila* insulator protein dCTCF marks active promoters and boundaries of many histone H3K27 trimethylation domains associated with repressed chromatin. In particular, dCTCF binds to such boundaries between the parasegment-specific regulatory domains of the *bithorax* complex. In this study we demonstrate that the Drosophila ENY2 protein is recruited to the zinc-finger domain of dCTCF and is required for the barrier activity of dCTCF-dependent promoters and insulators. Inactivation of ENY2 by RNAi in BG3 cells leads to the spreading of H3K27 trimethylation and Pc protein at several dCTCF boundaries. Homologous model of Drosophila ENY2 protein structure has been created based on known structures of human and yeast homologues. Using molecular docking we found several possible conformations of interaction between ENY2 and dCTCF zinc-finger domain and confirmed this using GST-pulldown assay in vitro. According to protein docking results this interaction utilizes the same interface of ENY2 protein as its known partners Sgf11 and Sac3. We tested this proposition using coexpression of tagged ENY2 and Sgf11 together with dCTCF zinc-finger domain. Copurification assays revealed that these interactions are mutually exclusive, suggesting that ENY2 protein might not be involved in recruitment of the SAGA-complex, which is known to promote formation of the transcriptionally-active chromatin. The results suggest that evolutionary conserved ENY2 is responsible for the recruitment of an as yet unidentified complex that is essential for establishing a barrier between active and silenced chromatin domains.

Keywords: chromatin boundaries, chromatin insulator, polycomb silencing.

MON-158
Histone deacetylase inhibitors as differentiation agents in breast cancer cells

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Approximately two thirds of breast tumours overexpress estrogen receptor alpha (ERα) at the time of diagnosis. Antiestrogen therapy has been effective in blocking the proliferative effects of ERα, but unfortunately a significant proportion of patients will relapse due to resistance. Histone deacetylase inhibitors (HDACis) are a new class of anticancer agents with anti-proliferative activity. *In vitro*, HDACis have been shown to suppress ERα expression and are currently being tested in clinical trials in combination with antiestrogens for treatment of ERα-positive breast tumours. Here, we report that the HDACis Trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA) abrogate expression of several luminal lineage transcription factors including ERα, FOXA1 and GATA3, all of which are known to regulate estrogen signaling and cell proliferation in MCF-7 breast cancer cells. As knockdown of FOXA1 reduces the expression of both ERα and GATA3, its suppression by TSA might contribute to loss of these transcription factors. Using gene expression microarrays, we observe that TSA treatment does not lead to increased expression of basal-like markers or of transcription factors controlling epithelial to mesenchymal transition. On the other hand, several markers of lactogenic differentiation, including the SREBF1-driven cholesterol biosynthesis pathway, are induced by TSA treatment *in vitro*. Consistently, we show reduced protein expression of ER, FOXA1 and GATA3 during lactation in the mouse mammary gland. In addition, markers of neuronal differentiation were also increased. We further show that chemical inhibition of the histone acetyltransferase P300 prevents the induction of SREBF1 expression and reverses the effects of TSA on ERα, GATA3 and FOXA1. This indicates that the effects of TSA are mediated via increased acetylation of protein substrates. Our study suggests that treatment of ERα-positive mammary cells by HDACis used in the clinic induces features of several differentiation pathways and contributes to its antiproliferative activity.

Keywords: breast cancer, Differentiation, Histone deacetylase inhibitors.

MON-159
Histones acetylation modulates differentiation and neovascularization potential of fetal stem cells

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The process of neovascularization is a complex process involving a series of interconnected steps leading to the formation of new blood vessels when vascular lesions occur in adult bodies. The major steps involved proliferation, chemotaxy, migration, adherence and differentiation of endothelial progenitor cells (EPCs) to the target situs, and their organization in vascular networks. Recent analysis of epigenetic changes showes that histone acetylation regulates pluripotency of EPCs and their differentiation capacity by controlling the expression of genes and transcription factors that are required for differentiation and neovascularization. To investigate the role of histone acetylation in regulation of neovascularization we used VPA (Valproic acid), a histone deacetylases (HDACs) inhibitor that mentained histone in an acetylated state. DNA intercalation with acridine orange and western blot analysis revealed that in the presence of VPA, histone H3 is acetylated at lysine 9, suggesting an acetylated state of chromatine. qRT-PCR data indicated that the expression of molecules involved in EPCs differentiation VE-cadherin, eNOS, VEGFR2, vWF and CD31 were decreased significantly after treatment with VPA. Furthermore flow cytometry assay revealed that at protein level the expression of surface markers CD31,

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CD105, VE-cadherin and VEGFR2 were inhibited whereas the expression of CD34 and CD45 remained unchanged, demonstrated that HDACs are involved in endothelial differentiation of progenitor cells. The proliferation of EPCs assesed by mesurements of telomerase activity and western blot analysis of PCNA showed a decreased proliferative potential, as well migration capacity assesed by wound-healing assay. Adherence capacity was also influenced by VPA, starting 12 hours after stimulation VPA has led to a decreased in adherence of EPCs. EPCs chemotaxis to anigogenic factors such as angiopoietin, VEGF, and SDF1α mesured in real time with xCELLigence system was stimulated by VPA. *In vitro* angiogenesis assay using Matrigel showed that in the presence of acetylated histones the number of capillary-like networks was reduced by up to 75%. Scanning electon microscopy photomicrographs showed that in the presence of VPA, EPCs lose the ability to form capilary networks on colagen scafolds, suggesting that histone acetylation inhibits the neovascularization process *in vitro*. Discovering the involvment of epigenetic mechanisms that regulate human vasculature development and differentiation will provide us greater insights into a variety of disorder, where the need of vasculatization is essential.

Keywords: acetylation, Epigenetics, stem cells.

MON-160
Identification of *Arabidopsis* KUMONOSU gene involved in DNA methylation and heterochromatin-associated silencing

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In plants, multiple layers of epigenetic regulators are required for heterochromatin-associated silencing, including many repetitive sequences and transposable elements (Regal and Mathieu, Biochim Biophys Acta., 2011). However, the control mechanism of tissue specific heterochromatic silencing is still unknown.

Here, we report the new *Arabidopsis* gene affecting heterochromatin-associated silencing, *KUMONOSU* (*KUN*). We isolated *kun* mutant from the screening affecting transgene silencing. In *kun* mutant, not only transgene, many endogenous repetitive sequence and transposable elements were activated. Moreover, the expression of specific genic regions was also affected and DNA methylation level of many target genes was slightly decreased in the mutant. Interestingly, release of silencing of the reporter transgene was observed specifically in vascular tissues of *kun* mutant, which may indicate possible tissue-specificity in the regulation of heterochromatin silencing.

Keywords: Epigenetics, heterochromatin, Silencing.

MON-161
Impact of glucose and O-GlcNAc transferase on expression of Polycomb genes in breast cancer cells

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Cancer is not only genetic disease but it can arise also from epigenetic abnormalities. The results of epidemiological studies indicate that obesity or hyperglycemia may increase the risk of some cancers, including breast cancers. Moreover, high blood glucose seems to be an important factor of cancer progression. Recent studies suggest that O-GlcNAcylation which consists in addition of N-acetylglucosamine on serine or threonine residues of proteins may play a key role in the regulation of the epigenom in response to cell metabolic status. Two enzymes are responsible for cyclic O-GlcNAcylation: O-GlcNAc transferase (OGT), which catalyzes the addition of the GlcNAc moiety to target proteins and O-GlcNAcase (OGA) which, removes the sugar moiety from proteins. The results of recent studies suggest that OGT may link cell metabolic status with transcriptional repression caused by Polycomb proteins. Abnormal OGT expression and O-GlcNAcylation are features of breast cancer cells but their role in Polycomb-dependent gene regulation remains to be elucidated. In this study we investigated the effect of hipo- normo- and hiperglycemia conditions on expression of O-GlcNAc cycling enzymes, expression of Polycomb genes, i.e. EZH2, SUZ12, RING1B and BMI-1 as well as histone modification levels in breast cancer cells (MCF7, MDA-MB-231 and Hs578t) and non-tumorigenic epithelial mammary cell line (MCF10A). Moreover, the impact of glucose and OGT down-regulation by RNAi on expression of several Polycomb target genes involved in cell differentiation and epithelial mesenchymal transition was analyzed. The results showed significant glucose-dependent changes in mRNA and protein level of OGT, OGA, EZH2 and BMI-1 in breast cell lines. Significant glucose-dependent increase in EZH2 protein expression was especially evident in poorly differentiated breast cancer cells MDA-MB-231 and Hs578t, which show high invasive phenotype. Cells grown in high glucose showed decreased BMI-1 protein and H2A ubiquitinylation levels compared to cells grown in low glucose. OGT down-regulation caused decreased O-GlcNAcylation in cells and was correlated with reduced EZH2 protein level but not BMI-1 level. OGT interference influence expression of some Polycomb target genes. There was increased expression level of FOXC1 and CDH1 and decreased level of HOX10A in cells with OGT depletion. The results of our preliminary studies suggest direct link between aberrant O-GlcNAcylation which is a nutrient-responsive modification and EZH2-dependent repression in breast cancer cells.

Keywords: O-GlcNAc transferase, Polycomb, breast cancer.

MON-162
Impact of surfactants on soft wheat meiosis

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Surfactants are broadly used in the national economy as active components of detergents, and in release of the products, based on synthetic and natural fibers. Oil and chemical industries, manufacture of construction materials also serve as important consumers.

A number of works is available on various biological effects and violations of structure and functions of organisms under synthetic surfactants, most of which depict biochemical changes. However, there is absolutely no data available on the possible genetic consequences.

The objective of our study is the comparative analysis of genetic toxicity of surfactants varying in their chemical nature at the level of chromosome violations in soft wheat microsporogenesis.

Kazakhstanskaya 3 and Shagala soft spring wheat varieties were used as the study material. The following non ionogenic surfactants: Triton X-100, Triton X-305, Twin 85, Twin 65 and Twin 20, frequently used in biological research, were chosen in a 1% concentration (with five hours treatment prior to sowing in the field). Seeds, processed by the distilled water, served as control. Pollen mother cells were temporally fixed in Carnoy solution, washed three times with 70% ethanol solution. Acetocarmine staining was used for estimation of chromosome aberra-

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tions in wheat microsporocytes. Chromosome violations were considered in 13,280.00 cells under the light microscope with appropriate statistical processing.

The maximum number of chromosome violations in Kazakhstanskaya 3 is caused by Twin 85, Triton X-305, Twin 65, and Triton X-100 (29.2 ± 0.9%, 25.7 ± 0.9%, 25.6 ± 0.8% and 32.5 ± 0.7% respectively), rather than Twin 20 (19.54 ± 0.7%), with the difference reliable at 99% probability level and similar for Shagala; with up to 16 times higher general frequency in compare with the spontaneous level.

Mutations at metaphase I are represented by multi-, univalents, clumping of chromosomes (pycnosis), shift of the metaphase plate division spindle and overflowing of nuclear matter, anaphase I – lagging chromosomes, bridges and fragments, anaphase II – bridges with fragments, asynchronous fission, and nucleus free cells. At tetrad stage violations of both nuclear (dyads, triads, pentads and hexades) and cytoplasmic (changes of tetrad walls and absence of cytokinesis) nature are found.

It is possible that the increasing frequency of the aberrant cells in wheat gametes is associated with the surfactants collision of physiological processes occurring in a cell, resulting in disturbed enzymatic system, which in turn leads to the accumulation of chromosomal defects. Spindle violation might be caused by uneven growth of its fibers, due to which fast-growing threads form bends, resulting with the chromosomes delay in the equatorial part of the cell or impaired formation of daughter cells.

Keywords: meiosis, surfactants, wheat.

MON-163
In silico study of drought and salt tolerance in wheat

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The in silico study of genes is an important step because it helps guide the interpretation of experimental results and suggest new experiments. Salt-tolerant candidate genes in wheat and other cereals include HKT, NHX, SOS, and HAK genes. Drought-tolerant candidate genes are DRG, DHN, DRF, DREB, etc. These genes were searched, identified and downloaded from the NCBI database. Comparative studies and analyses such as search for candidate genes' functions, search for protein domains, multiple sequence alignments, and phylogenetic tree construction as well as primers design were carried outusing bioinformatics tools. The RT-PCR primers associated with the candidate genes for the studied traits were designed as molecular markers to assist theprogram for improvement in cereals precisely in wheatvia marker-assisted selection.

Keywords: Bioinformatics tools, Drought & Salt-tolerant Candidate Genes, Marker-assisted selection.

MON-164
Inhibition of DNA methylation alters chromatin organization, nuclear positioning and activity of 45S rDNA loci in cycling cells of *Q. robur*

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In *Quercus robur* there are two rDNA loci, the major (NOR-1) and the minor (NOR-2) locus. We aimed to deduce the transcriptional activity of the two rDNA loci in root tip cycling cells by

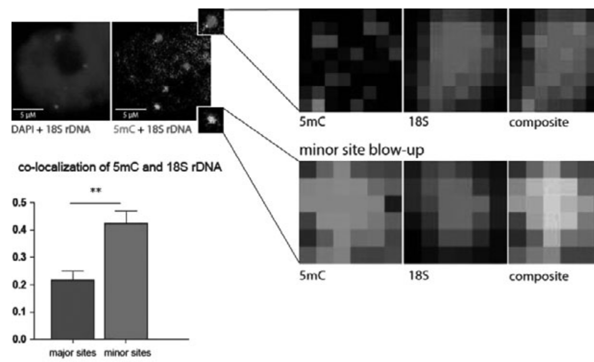

Fig. 1.

exhamining the position of rDNA loci in respect to the nucleolus, organizational patterns (topology) of rDNA chromatin in metaphases and interphases using light and electron microscopy, silver staining of NORs, epigenetic signatures of rDNA chromatin and the expression levels of 18S rRNA genes before and after treatment of root tips with the DNA methylation inhibitor 5-aza-2'deoxycytidine (5-aza-2'-dC). Our data indicates that in normal physiological conditions of *Q. robur* cells, a situation resembling nucleolar dominance is present where only rRNA genes within the NOR-1 locus are transcriptionally active and participate in the formation of the nucleolus. To investigate the role of the other, transcriptionally inactive NOR-2 locus, we induced its reactivation by treatment with 5-aza-2'-dC. We observed that a decrease in levels of DNA methylation at NOR-2 locus and an increase in total level of rRNA transcripts do not affect the contribution of this locus in nucleolar formation, suggesting its loss of function. In addition, we propose a correlation between rDNA chromatin topology, location of different rDNA fractions related to the nucleolus, epigenetic signature and the activity of rRNA genes within the active NOR-1 locus.

Keywords: DNA methylation, rDNA locus, transcriptional activity.

MON-165
Integrative genome-wide analysis reveals novel pathways and transcription factors involved in non-small cell lung cancer drug resistance

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Background: The mechanism of acquired drug resistance in non-small cell lung cancers (NSCLC) is poorly understood. The transcriptome and epigenetic signature associated with drug resistance, in particular, are not well-defined. We performed an integrative genome-wide analysis with the aim of identifying unique gene expression patterns, pathways, transcription factors (TFs), DNase I hypersensitive sites (DHSs), and enhancers involved in drug resistance.

Methods: We conducted RNA-sequencing (RNA-Seq) to examine differentially expressed genes and pathways, DNase I sequencing (DNase-Seq) to identify DHSs and infer TFs binding sites, and chromatin immunoprecipitation sequencing (ChIP-Seq) to locate H3K4me2 histone modification across enhancers in the gefitinib-resistant NSCLC cell line PC9R and its gefitinib-sensitive parental cell line, PC9.

Results: We discovered several genes and pathways, many of which are related to the epithelial-mesenchymal transition (EMT). We also identified differential distributions of both DHSs and enhancers and inferred TFs, which are closely associated

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with gene up-regulation in NSCLC gefitinib-resistant cells. Finally, we showed that drug-resistant cells exhibit an increased invasion ability and an up-regulation of EMT markers found in gefitinib-resistant lung cancer patients.

Conclusion: This integrative genome-wide analysis of genes, pathways, DHSs, enhancers, and TFs that are associated with acquired drug resistance may deepen our understanding of the mechanism underlying NSCLC drug resistance.

Keywords: drug resistance, Non-small lung cancer, transcription factor.

MON-166
MATH-BTB domain protein AtBPM1 directly interact with DMS3, important component of RNA-directed DNA methylation in plants

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In *Arabidopsis thaliana*, the MATH-BTB (BPM) proteins comprise a small family of six members. BPMs act as substrate-binding adaptors for the Cullin3-based ubiquitin E3 ligase, and interect with a broad range of Ethylene response factor/apetala2 transcription factors and with homeodomain-leucine zipper transcription factor ATHB6 affecting fatty acid metabolism and abscisic acid signaling. We showed previously that AtBPM1, localizes predominantly in nucleolus of plant cells indicating a Cullin3 independent function. To further elucidate the molecular function of AtBPM1, we identified AtBPM1 binding and functional partners using tandem affinity purification and mass spectrometry. Different stress-related proteins were identified in AtBPM1 protein complexes such as Catalases CAT2 and CAT3, Nucleoside diphosphate kinase III, Glycine-rich RNA-binding proteins GRP7 and GRP8, MLP-like protein 423, Polyketide cyclase/dehydrase and lipid transport superfamily protein. Moreover, we have identified a set of DNA repair and chromatin remodeling proteins, such as Nucleosome assembly protein NAP1, Tudor-SN proteins, DNA-damage-repair/toleration protein DRT102, WD-40 repeat family protein/beige-related, Chromatin remodeling 34, DNA mismatch repair protein Msh6-1 as well as a known components of RNA-directed DNA methylation, defective in meristem silencing 3 DMS3 and RNA-directed DNA methylation 1 RDM1. Furthermore, direct interaction of AtBPM1 and DMS3 was confirmed by yeast two hybrid and pull down assays and DNA methylation in plants overexpressing AtBPM1 was reduced. These results, for the first time, links MATH-BTB proteins with DNA methylation and related mechanisms.

Keywords: chromatin remodeling, protein-protein interactions, RNA-directed DNA methylation.

MON-167
Methylation analysis of selected genes associated with carcinogenesis: downregulation of APC, AR, GPX3, PTGS2, TIMP2, RASSF1 and DKK3 genes expression is regulated by hypermethylation

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PNC (perinucleolar compartment), is a subnuclear structure forming in cancer cells. PNC prevalence (a percentage of cells containing at least one PNC) is correlated with higher grade and stage of breast cancer - is potential cancer biomarker. Previous studies have shown that increasing PNC prevalence positively correlates with PTB expression, the main component of the PNC structure. Therefore we hypothesized that the expression of other genes whose proteins are part of the PNC structure, and general tumor suppress genes may also vary in the cell lines PNC prevalence high vs. low cells.

Aim: Our first aim was to analyze the expression of several tumor suppressor genes including: APC, AR, DKK3, GPX3, PTGS2, RASSF1, TIMP2 Secondly, we evaluated whether DNA methylation is involved in regulating their expression.

Methods: We have evaluated gene expression using 2 fluorescence detection systems: SYBRGreen dye-based assay (reference gene MRPL19) and hydrolysis probe assays (reference genes PBGD and GAPDH). Methylation analysis for 16 tumor suppressor genes in prostate cancer cell lines with low (PC3), and high (PC3-M, PC3-M LN4) PNC prevalence and fibroblasts was performed using EpiTectMethylII PCR system based on detection of remaining input DNA after cleavage with a methylation-sensitive and /or methylation dependent restriction enzyme.

Results: We evaluated gene expression and methylation of 7 tumor suppressor genes. Interestingly, DKK3 expression was 20-fold lower respectively in PC3M, PC3MPro4 and PC3MLN4 compared to PC3 line and suitably more than 100-fold compared to fibroblasts. These lines are also characterized by increased level of methylation of the gene DKK3 (over 90%) as compared to PC3 and fibroblasts (below 1%). In addition, PTGS2 and RASSF1 expression was minimum 7.5 fold higher and 10-fold higher in VH10 versus all 4 prostate cancer cell lines and correlated with methylation below 1.5% in VH10 and 99% in PC3M for both gene. APC and AR gene expressions were both 2-fold lower in prostate cancer cell lines versus VH10. Methylation results are consistent with theirs expression ones: APC and AR were methylated in average 0.3% and 8.4%, respectively in VH10, whereas in PC3 LN4 and PC3M cells lines methylation for both genes was above 65% .

Keywords: None.

MON-168
Methylation of glycosylation genes in cancer

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Aberrant protein glycosylation is a well-documented and universal feature of malignant transformation as well as tumor progression. The intricate network of enzymes that carry out protein glycosylation is controlled by epigenetic mechanisms, which under physiological conditions integrate information from the environment and fine tune cellular metabolism. Glycosylation affects the function of extracellular and membrane proteins by incorporating sugars into their structure, making the carbohydrate moiety an integral part of the glycoprotein, which affects its function. Since glycoproteins are positioned at the interface between the cell and its surroundings, their involvement in all steps of tumor progression is unsurprising. We focused on the changes of epigenetic control in glycosylation-related genes, both the enzymes which directly modify protein molecules and regulatory proteins such as HNF1A, the master regulator of plasma protein antennary fucosylation. Publicly available methylation data was used to compare the epigenetic regulation of glycosylation-associated genes in cancer cells, matching healthy tissue and, when applicable, matching cell lines. Remarkably complete datasets involving hepatocellular carcinoma and lung cancer enabled us to identify the key differences between normal and malignant cells in the pattern of regulation of glycosylation, while the data on melanoma progression gave insight into the role of glycosyla-

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tion in metastasis. This study will guide our further experimental work involving direct glycan analysis in liver tissue, which will be aimed to confirm the effect of epigenetic regulation of glycosylation genes on observed glycan structures.
Keywords: cancer epigenetics, glycosylation, methylation.

MON-169
Molecular evaluation of chromatin remodeling and ALT in pediatric brain tumors

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Pediatric high-grade gliomas (pHGG) are histologically similar to high-grade gliomas of adults (aHGG), however they have different characteristics and molecular mechanisms involved in the progression and gliomagenesis.

Chromatin remodeling is of primary importance in regulating gene expression, apoptosis, DNA replication and repair. Dysfunctions in chromatin-remodeling mechanisms have been associated with disease development, and particularly, alterations in chromatin structure can lead to deregulated expression of tumor suppressor genes or oncogenes and cancer initiation.

Our analysis was focused on 3 molecular factors involved in chromatin remodeling pathway: H3.3, ATRX and DAXX. These three proteins colocalize in pericentric eterochromatin and the ATRX-DAXX complex is involved in the deposition of the H3.3 variant at heterochromatic regions of the genome and cooperate providing stability to the telomeres. Loss of ATRX or DAXX gene promote tumorigenesis through altered gene expression and genomic instability; in particular, their absence can lead to telomere instability and elongation of chromosomal endings, even in the absence of telomerase, and then through an alternative mechanism of telomeres' elongation (ALT).

The study was conducted on 83 pHGG cohort, with age range of 0–16, including: Glioblastomas (GBM), Peripheral Neuroectodermic Tumors (PNET), Pylocit Astrocytomas (AP) and Anaplastic Astrocytomas (AA).

ATRX, DAXX and H3.3 expression has been investigated at protein level by Immunohistochemistry (IHC), at genomic level through the analysis of messenger RNA by reverse transcription polymerase chain reaction (RT-PCR) followed by sequencing and mutational analysis for H3F3A and H3F3B. Furthermore, presence of ALT has been investigated by the Quantitative Fluorescence in situ Hybridization (Q-FISH) analysis.

In this work we evaluated the components of chromatin remodeling in high grade pediatric glial neoplasms to define a possible correlation between the presence of alterations of ATRX dAXX and H3.3 genes and histology, grading (WHO) and age at onset.
Keywords: Alternative lengthening Telomere (ALT), Chromatin remodeling, pHGG.

MON-170
Novel regulatory elements of the 9p21 region: the role in breast cancer development

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The 9p21 region, harboring the ARF/CDKN2A/B tumor suppressor locus, is known to be frequently deleted in breast cancer.

Integrated analysis of copy number alteration-expression revealed that 9p21 loss-of-heterozygosity (LOH) does not affect expression levels of the 9p21 tumor suppressor genes. This could be explained by the fact that all three genes are epigenetically silenced in breast cancer patients. On the other hand, the analysis of The Cancer Genome Atlas (TCGA) breast cancer dataset revealed that loss of the 9p21 region is associated with poor survival. Further interrogation of ENCODE data has revealed a disproportionate number of regulatory elements within the 9p21 region, suggesting their potential role in tumor suppression.

By applying circular chromosome conformation capture (4C), we examined the network of long range chromosomal interactions in primary human mammary epithelial cells and two breast cancer cell lines, MCF7 and MDA-MB134-VI. We identified chromosomal regions that physically interact with 9p21 regulatory elements. This allowed us to create a catalogue of genes, in which expression may be modulated by regulatory elements located in the 9p21 region. Strikingly, gene set enrichment analysis (GSEA) revealed that the identified set of genes significantly overlap with genes that are differentially expressed in breast cancer patients with 9p21 LOH. Using genome editing techniques we are generating isogenic cells targeted with a 9p21 deletion. These models will allow us to confirm the contribution of 9p21 regulatory elements to the regulation of the identified genes. Among the genes that become abnormally up-regulated due to loss of the 9p21 regulatory regions, we will also search for genes involved in known cancer-related pathways.

This study will provide an opportunity to unravel the link between genomic architecture, gene regulation and function to determine the role of regulatory elements in cancer development.
Keywords: 9p21, breast cancer, chromatin organization.

MON-171
Nucleosome rearrangement as a feedback mechanism between DNA methylation and transcription factor binding

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During differentiation of embryonic stem cells, chromatin reorganizes to establish cell type specific expression programs. Here, we have dissected the linkages between DNA methylation (5mC), hydroxymethylation (5hmC), nucleosome repositioning and binding of the transcription factor CTCF during this process. By integrating MNase-seq and ChIP-seq experiments in mouse embryonic stem cells (ESC) and their differentiated counterparts with biophysical modeling, we found that the interplay between these factors depends on their genomic context. The mostly unmethylated CpG islands have a reduced nucleosome occupancy and are enriched in cell type-independent binding sites for CTCF. The few remaining methylated CpG dinucleotides are preferentially associated with nucleosomes. In contrast, outside of CpG islands most CpGs are methylated and the average methylation density oscillates so that it is highest in the linker region between nucleosomes. Outside CpG islands binding of TET1, an enzyme that converts 5mC to 5hmC, is associated with labile, MNase-sensitive nucleosomes. Such nucleosomes are poised for eviction in ESCs and become stably bound in differentiated cells where the TET1 and 5hmC levels go down. This process regulates a class of CTCF binding sites outside CpG islands that are occupied by CTCF in ESCs but loose the protein during differentiation. We rationalize this cell type dependent targeting of CTCF with a quantitative biophysical model of competitive binding with the histone octamer in dependence of the TET1, 5hmC and 5mC state.
Keywords: chromatin, DNA methylation, nucleosome.

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MON-173
Protective effect of lithium chloride on cytotoxicity of lead nitrate in non-adherent bone marrow cells

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The harmful effects of lead nitrate on the body have been well documented. It has been show that Lithium chloride, as an element that is used for clinical purposes, has significant protective effect on the cells treated with lead. Lead can damage all type of tissues especially blood and hematopoietic stem cells. Bone marrow is the most important tissue that hematopoiesis occurs in it. Therefore any toxic substance that disturbs this process can lead to various diseases such as anemia and cancers. In this study the protective effect of lithium against lead-induced cytotoxicity in mouse non-adherent bone marrow cells are investigated.

The cells were prepared from mouse femurs and cultured in the absence and presence of various concentration of lithium chloride or lead nitrate. Cell viability was quantified using trypan blue and MTT assays. Also Histones and high mobility group B (HMG B) proteins were extracted and analysed on SDS-PAGE and immunobloted. When the cells were first treated with 160 μM of lithium chloride for 3 h and then exposure to different concentrations of lead nitrate, lead-induced cytotoxicity was overcome. With exposure of non- adherent bone marrow cells to lead nitrate the content of HMG B and histone H1 on the gel was decreased as metal concentration increased. However, pretreatment of the cells with lithium chloride prevented proteins reduction. Western blot analysis against HMGB and histone H1 antibodies confirmed the results. This study suggests that we could take advantage from lithium chloride potential for medical purpose in reduction of lead undesirable effects.

Keywords: chromatin proteins, Cytoprotective effect, lithium chloride.

MON-174
Protein-DNA binding in the absence of specific base-pair recognition

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Until now, it has been reasonably assumed that specific base-pair recognition is the only mechanism controlling the specificity of transcription factor (TF)-DNA binding. Contrary to this assumption, here we show that nonspecific DNA sequences possessing certain repeat symmetries, when present outside of specific TF binding sites (TFBSs), statistically control TF-DNA binding preferences. We used high-throughput protein-DNA binding assays to measure the binding levels and free energies of binding for several human TFs to tens of thousands of short DNA sequences with varying repeat symmetries. Based on statistical mechanics modeling, we identify a new protein-DNA binding mechanism induced by DNA sequence symmetry in the absence of specific base-pair recognition, and experimentally demonstrate that this mechanism indeed governs protein-DNA binding preferences.

Keywords: Protein-DNA interactions, transcription regulation.

MON-176
RARβ gene methylation in primary glioblastomas

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Background: In our study, patients who have received a diagnosis of GBM response to treatment and survival the relationship between of the tumor suppressor gene methylation pattern RARβ is aimed to reveal. Other is the objective of our study, yet you find new areas of application MS-HRM method for use in the determination of the GBM samples. According to our information rarbeta for GBM patients in the literature that there is only one paper. So our group of patients RARβ methylation frequency in GBM world literature will form the basis.
Results: In our study, GBM was diagnosed 40 cases related to the tumor samples were studied Eskisehir Osmangazi University Faculty of Medicine, Neurosurgery Department by the operated and the Department of Pathology by histopathological examining.
Conclusions: GBM in 24 cases of 40 patients (60%) proportions of different quantitative methylation was determined of RARβ gene. Examples of 24 cases of the methylation rate according to our assessment of samples 3 of which 25% methylated, 5 were 50% methyl, 4-one 75% methylated and 12 cases 100% RARβ gene hypermethylation demonstrated. The mean survival time of the patients with methylation RARβ period of 19 months, and 15 months of nonmethylated is calculated as cases. Our case study 28 patients received treatment, the remaining 12 cases were not specified in the patient files of any treatment protocol. When statistically evaluated patients who received treatment with chemotherapy and radiotherapy of patients with a 25 month survival was determined. Patients who received radiotherapy alone or no treatment protocol was applied in cases between 15 and 20 months, a significant difference in survival has been observed. On the other hand, in response to retinoic acid mediated marker for chemotherapy to be RARβ. Chemotherapy, which may be important for resistance to retinoids.

Keywords: glioblastoma, methylation, RARβ.

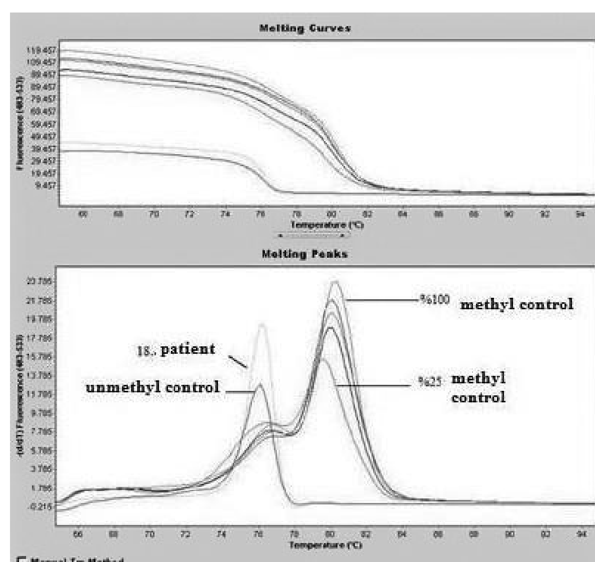

Fig. 1.

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MON-177
Recognition of 5-methylcytosine and 5-hydroxymethylcytosine by methyl-directed restriction endonucleases

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DNA methylation is a covalent DNA modification that is abundant in most forms of life. 5-methylcytosine (5mC) and the recently discovered 5-hydroxymethylcytosine (5hmC) are involved in the epigenetic regulation of gene expression in eukaryotes. Bacterial methyl-directed restriction endonucleases (MD-REases) are promising molecular tools for the studies of 5mC and 5hmC distribution in eukaryotic genomes. Understanding the mechanism of modified DNA recognition by these enzymes might open new avenues for the design and improvement of methylation profiling tools.

We have focused on elucidating of molecular mechanisms of modified DNA recognition by two groups of MD-REases:

1 MspJI family enzymes that are specific for 5mC and 5hmC in various sequence contexts;
2 PvuRts1I family enzymes that recognize two 5hmC or glucosylated 5hmC nucleotides separated by ˜20 base pairs.

Structural comparison of DNA-binding domains of MspJI, AspBHI (both belong to the MspJI family), and PvuRts1I with known protein structures revealed similarities to the eukaryotic SRA (set and ring associated) domains that interact with 5mC and 5hmC-containing DNA. In the crystal structures the SRA domains flip out the modified cytosine base from the DNA duplex and position it within a protein pocket. MspJI, AspBHI and PvuRts1I endonucleases also have similar protein pockets, but direct evidence for base flipping by these enzymes is still missing.

We have employed chemical modification-based assays to detect base flipping by MD-REases and have performed mutational analysis of protein loops that may be involved in recognition of DNA nucleotides adjacent to the modified base. Our results indicate that DNA recognition mechanism employed by eukaryotic SRA domains is conserved among MspJI and PvuRts1I-like restriction enzymes: all these proteins employ similar structural elements for DNA binding and use base-flipping for the recognition of modified cytosine.

Acknowledgements: This work was supported by the Research Council of Lithuania (grant MIP-027/2012 to G.S.).
Keywords: 5-methylcytosine, protein-DNA interactions, restriction endonuclease.

MON-178
RNA mediated trans-activation: its therapeutic potential in anaplastic thyroid cancer

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RNA mediated Trans-Activation is a proposed mechanism involved in the setting of the epigenetic state of chromatin.

It has been studied first in *Drosophila melanogaster* where it seems that at least some transcribed loci are marked as transcriptionally active by their own transcripts. This effect acts *in trans* and in some cases could give rise to a transgenerational paramutational-like effect.

This work studies the RNA mediated trans-activation in the light of one of its possible applications, specifically its use as a therapeutic strategy for the incurable and highly aggressive ATC (Anaplastic Thyroid Cancer). We explore the possibility to reactivate the Thyroid specific NIS (Natrium-Iodide Symport) via the expression of ncRNAs with sequence homology with transcripts from the NIS coding locus itself. The reactivation of NIS in ATC would make them susceptible to treatment with radioiodine. Preliminary data strongly suggest that we reached our goal.

This work suggests that RNA trans-activation is a conserved mechanism and it may be used in human to manipulate the gene expression.

Keywords: Chromatin Remodelling, Epigenetics, regulation of gene expression.

MON-179
Role of histone methylation in transcriptional repression of Ras-association domain family tumor suppressor, RASSF8

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Ras proteins are well-known for its critical role in cell proliferation. "Ras-association domain containing family" (RASSF) proteins are non-enzymatic Ras effectors which comprises of ten members. RASSF members are predominantly identified as tumor suppressors and downregulated by promoter hypermethylation in cancers. One such member, RASSF8 is involved in maintenance of adherent junctions. It is interesting to note that the downregulation of RASSF8 was observed in many cancers despite insignificant promoter hypermethylation suggesting a novel mechanism regulating RASSF8 function. Hence, we aimed to understand the mechanism by which RASSF8 is downregulated in cancers. Treatment of MCF-7 cells with 5-aza deoxycytidine, a demethylating agent results in unaltered the RASSF8 expression which indicated the possibility of other epigenetic marks regulating RASSF8 expression. Interestingly, BIX-01294, an inhibitor of G9a, a histone methyltransferase increased the RASSF8 expression in MCF-7 cells. G9a is a histone methyltransferase (HMT) which catalyse mono- and di-methylation of Histone H3 at Lysine9 residue. Subsequently, a well-known member of HMT, SUV39H1 catalyse trimethylation of H3K9 leading to chromatin condensation resulting in transcriptional repression. Over-expression of G9a and SUV39H1 downregulates RASSF8 promoter activity. Experiments with RASSF8 promoter deletion constructs using reporter assays identified the region between −350 and −266 to harbour the repressor element. Further Chromatin immunoprecipitation confirmed the occupancy of Histone H3, H3K9me2, and H3K9me3 in the same region in RASSF8 promoter. Together, our data implicates histone methylation independent of DNA methylation in transcriptional regulation of RASSF8.

Keywords: Histone methylation, transcription regulation, Tumor suppressor.

MON-180
Role of Sp1 in butyric acid-induced HIV-1 gene expression

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The ability of human immunodeficiency virus-1(HIV-1) to establish latent infection and its re-activation is considered critical for the progression of HIV-1-associated diseases. Accumulating evidence has indicated that histone acetylation and deacetylation regulates HIV-1 gene expression. The bacterial metabolite butyric acid has been shown to be a potent inhibitor of histone deacetylases (HDACs), leading to transcription of HIV-1

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proviruses. We previously demonstrated that butyric acid-producing bacteria in the vaginal and oral cavities and gut can strongly induce histone acetylation and HIV-1 replication from latently infected cells by inhibiting HDAC; thus, [Editor1] co-infection with anaerobic bacteria is an important risk factor for progression of AIDS. However, the molecular mechanism by which butyric acid activates HIV-1 gene expression is not well understood. In this study, we found that Sp1 binding sites are considerably involved in butyric acid-mediated activation of HIV-1 gene expression as indicated by the results of a luciferase assay using HIV-1 long terminal repeats (LTRs) of various mutants. Sp1 knockdown by small interfering (si) RNA and the Sp1 inhibitor mithramycin A inhibited the effects of butyric acid. Furthermore, we observed that cAMP response element-binding (CREB)-binding protein (CBP) was involved in butyric acid-induced Sp1-dependent HIV-1 gene expression. A chromatin immunoprecipitation assay analysis revealed that Sp1 and HDAC1 are present in the HIV-1 LTR promoter in TZM-bl cells and dissociate from the promoter concomitantly with the association of acetylated histone H3 and CBP upon butyric acid stimulation. Furthermore, siRNA knockdown of Sp1 resulted in decreased recruitment of CBP to the promoter. These results suggest that butyric acid stimulates HIV-1 promoter activity through the inhibition of Sp1-associated HDAC activity and recruitment of CBP to the Sp1 sites on HIV-1 LTR. Our findings suggest that Sp1 should be considered as a therapeutic target in new anti-viral therapies against HIV-1 infection aggravated by butyric acid-producing bacteria.

Keywords: HIV/AIDS, butyric acid, HDAC.

MON-181

Sequences proximal to the HCF-1 cleavage site are novel binding sites for O-GlcNAc transferase to facilitate HCF-1 glycosylation and proteolysis

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O-GlcNAc transferase (OGT) is a cellular glycosyltransferase that modifies a large number of proteins by the addition of a sugar molecule, N-Acetylglucosamine, to serine or threonine residues in the form of O-linked-beta-N-acetylglucosamine (O-GlcNAc). One of the most extensively glycosylated proteins is the human Host cell factor-1 (HCF-1), a transcriptional co-regulator of cell cycle progression.

We have recently shown that OGT not only glycosylates, but also proteolytically cleaves HCF-1 at six sites during protein maturation. OGT's unusual dual role for HCF-1 is believed to link cellular nutrient levels to cell cycle progression. However, how these two post-translational modifications –glycosylation and proteolysis– are regulated and coordinated with each other, is currently unknown.

We have performed mutational analyses of sequences proximal to the HCF-1 cleavage site and combined mutants of OGT in our cellular or in vitro assays. We have identified an HCF-1 sequence that contains a cluster of novel glycosylation sites. This sequence has a high affinity for OGT and augments substrate glycosylation and proteolysis. A dissection of the two OGT activities further shows that glycosylation of the OGT–binding sequence is not required for proteolysis. Thus, we provide evidence for a novel OGT–binding sequence that efficiently recruits OGT to facilitate glycosylation and proteolysis of HCF-1.

Keywords: Epigenetics, Glycosylation, O-GlcNAc transferase.

MON-182

Significance of DNA methylation in regulation of hypoxia-inducible factor -3α expression in colorectal cancer

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Introduction: Colorectal cancer (CRC) is the most frequent gastrointestinal malignancy. The development of solid tumors, including CRC, is associated with hypoxic conditions as a result of immense proliferation of tumor cells. Main adaptive response that allow cells to adjust to changed environment is hypoxia-inducible factor -α (HIF-α), which is an oxygen-sensitive component of the HIF-1 transcription factor. There are three isoforms of HIF- α subunit: HIF- 1α, HIF- 2α and HIF- 3α. HIF- 1α and HIF- 2α share similar protein structure and may bind together with HIF-β subunit to activate HIF-dependent gene transcription. HIF3- α, on the other hand, act as a weak transcription factor and is reported to suppress HIF-1α or HIF-2α-mediated gene expression. Relatively little is known about regulation of *HIF-3α* gene expression in CRC. Presence of CpG island within promoter region of *HIF-3α* prompted us to investigate it role in colorectal tumorigenesis. DNA hyper or hypomethylation of gene regulatory sequences alter expression of cancer related genes in CRC.

Materials and Methods: Using real time PCR and western blotting we determined *HIF3-α* expression in primary colorectal cancer and histopathologically unchanged colorectal tissue from the same one hundred patients with respect to patient survival. DNA methylation level within promoter region of analyzed gene was evaluated using bisulfite sequencing and high-resolution melting analysis (HRMA). We also assessed the effect of DNA methyltransferase inhibitor, 5-Aza-2'-deoxycytidine (5-dAzaC), on *HIF-3α* in HCT116 and DLD-1 colon cancer cells under hypoxic and normoxic conditions.

Results: We found significantly lower HIF3-α mRNA and protein level in primary colorectal tissue than in histopathologically unchanged colorectal tissue. Moreover, Kaplan-Meier analysis revealed a benefit of high HIF-3α protein level in cancerous tissue in patient survival compare to low HIF-3α protein level. The reduced *HIF3-α* expression was also preliminary correlated with increased DNA methylation in the CpG island of the *HIF3-α*. Additionally we observed that 5-dAzaC significantly increased *HIF3-α* expression level in HCT116 cancer cells under hypoxic conditions, whereas under normoxic neither DNA methylation nor changes in expression level were observed.

Conclusions: Our findings present that *HIF-3α* is decreased in CRC and high HIF-3α protein level in cancerous tissue might be prognostic factor for CRC. Additionally, initially observed methylation-induced epigenetic silencing of *HIF3-α* may facilitate better understanding the mechanism of hypoxia response.

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Keywords: colorectal cancer, DNA methylation, Hypoxia.

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MON-183
SMRT sequencing defines the sequence requirements for the positioning of base J into DNA

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Some T's in nuclear DNA of trypanosomes and *Leishmania* are hydroxylated and glucosylated to yield base J (β-D-glucosyl-hydroxymethyluracil)(Gommers-Ampt et al., Cell, 1993). Base J replaces 1% of T in the Leishmania genome and is only found in telomeric repeats (99%) and in regions where transcription starts and stops (1%) (Van Luenen et al., Cell 2012). This highly restricted distribution must be co-determined by J-Binding Proteins (JBPs) 1 and 2 that catalyze the initial step in J synthesis, the hydroxylation of selected T-residues in DNA. To determine the DNA sequences recognized by JBP1/2, we used SMRT sequencing of DNA segments inserted into plasmids grown in *Leishmania tarentolae*. We show that SMRT sequencing recognizes base J in DNA and that the signal generated is characteristic and DNA context dependent. *Leishmania* DNA segments that normally contain J also pick up J when present in the plasmid, whereas control sequences do not. Even a segment of only 10 telomeric (GGGTTA) repeats is modified in the plasmid. This modification requires JBP2, as it does not occur in JBP2 null cells. We show that J spreads from the plasmid insert into adjacent plasmid sequences. We find a consensus sequence for J insertion of T-(N)12-A and infer that JBP1 is able to bind to J and then hydroxylate a T at position +13 (but not −13) on the complementary strand, explaining the unusual modification of the (GGGTTA)n repeats in which only the second T is modified and the unusual kinetics of JBP1 binding to J-DNA (Heidebrecht et al., JACS, 2012). We present a speculative model for the *de novo* J insertion and spreading involving G-quadruplets.

Keywords: DNA modification, Telomeres, TET/JBP1.

MON-184
Solubilization of huntingtin aggregates: success lies with a combinatorial effort, rather than a singular target

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Protein misfolding is a common motif recurring in many neurodegenerative disorders. Proteins containing long, homopolymeric stretches of glutamine are especially prone to misfolding which results in their aggregation, causing diseases like Huntington's disease (HD) and various ataxias. Increased oxidative stress, cellular toxicity and impairment of many cellular pathways have been well documented in HD. Strategies like the use of osmolytes, ubiquitination and activation of autophagy have shown some success in inhibiting aggregation of mutant huntingtin (mhtt). A better understanding of the various players will help in devising a more rational and comprehensive solution. Here, we show that glycerol3-phosphate dehydrogenase (Gpd1), a key enzyme in the glycerol synthesis pathway, plays a unique and pivotal role in regulating the expression of silent information regulator-2 (Sir2), a major histone deacetylase, in a yeast model of HD. Absence of Gpd1 showed significant increase in Sir2 expression, which could not be reversed even on supplementing these cells with Gpd1. Increased expression of Sir2 correlated with solubilization of mhtt. Gene expression analysis showed marginal upregulation of chaperones and High-osmolarity glycerol (HOG) pathway genes in Δ*Gpd1* cells. Interestingly, even when Sir2 activity was inhibited by nicotinamide, solubilization of mhtt remained high in Δ*Gpd1* cells. This led us to discover the third component of this multifactorial response, viz. tailless complex polypeptide (Tcp1), one of the subunits of cytosolic chaperonin Tcp1-ring complex (TRiC). The expression of Tcp1 was found to be significantly higher in Δ*Gpd1* cells, where Sir2 expression and solubility of mhtt were high. It has been reported that prior acetylation and methylglyoxal (MGO) mediated glycation of αA-crystallin enhances its chaperone activity {*Biochim Biophys Acta* 1832; 195–203 (2013)}. Coimmunoprecipitation of Tcp1 with acetylated lysine and MGO antibodies showed Tcp1 to be hyperacetylated as well as glycated in Δ*Gpd1* cells. This increased its chaperonin activity, leading to higher solubilization of mhtt. Thus, in addition to Sir2 activity, enzymatic and non-enzymatic modifications of Tcp1 are responsible for increased solubilization of mhtt. As expected, oxidative stress level and toxicity were reduced in these cells. Overall, we propose a novel role for Gpd1 in regulating Sir2 expression and its downstream effectors like Tcp1, by facilitating the enzymatic and non-enzymatic modifications of the chaperone.

Keywords: chaperonin, glycation, protein aggregation.

MON-185
Spatial interaction of CpG islands in the interphase nucleus

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Using 4C-seq, we characterized the genome-wide patterns of spatial contacts of several CpG islands (CGIs) located on chromosome 14 in cultured chicken lymphoid and erythroid cells. We observed a clear tendency for the spatial clustering of CGIs present on the same and different chromosomes regardless of the presence or absence of promoters within these CGIs. Accordingly, we observed preferential spatial contacts between the Sp1 binding motifs and other GC-rich genomic elements, including the DNA sequence motifs capable of forming G-quadruplexes. On the contrary, an anchor placed in a gene/CGI-poor area formed spatial contacts with other gene/CGI-poor areas on chromosome 14 and other chromosomes but not with GC-rich elements. These results corroborate the two-compartment model of the spatial organization of interphase chromosomes and suggest that the clustering of CGIs constitutes an important determinant of the 3D organization of the eukaryotic genome. Using ChIP-seq, we mapped genome-wide the CTCF deposition sites in the chicken lymphoid and erythroid cells that were used for the 4C analysis. We observed a good correlation between the density of CTCF deposition sites and the level of 4C signals for the anchors located in CGIs but not for an anchor located in a gene desert. It is thus possible that CTCF contributes to the clustering of CGIs observed in our experiments.

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Keywords: 4C, CpG island, genome 3D organization.

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MON-186
Specific epigenetic code in crested newt spermatogenesis

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An exciting challenge in developmental biology field is to decipher the molecular mechanisms involved in cellular differentiation and to understand the processes that control and regulate genes expression. The study of nuclear molecular architecture during gametogenesis represents one approach toward deciphering the molecular organization and function of the eukaryotic chromatin. As spermatogenesis progresses, there is a widespread reorganization of the haploid genome followed by extensive DNA compaction. It is becoming increasingly evident that the dynamic composition of chromatin plays an important role in the activities of enzymes and in the processes that act upon it.

In order to gain an insight into mechanisms controlling histone hyperacetylation/histone replacement during spermiogenesis, we have investigated in vivo effect of TSA on *Triturus cristatus* spermatogenesis. We focused our investigations on the dynamics of chromatin structure after treatment with trichostatin A (TSA), a histone deacetylase inhibitor. Ultrastructural and molecular analysis of the newt testicular tissue after TSA treatment indicated a major chromatin remodelling in sperm nuclei, evidenced by chromatin decompactation and the absence of sperm-specific proteins. Thus, histone deacetylation could be interpreted as an early signal of histone replacement by highly basic sperm-specific proteins a process tightly linked to nuclear condensation. The analysis of the electrophoretic profiles of DNA restricted with MspI/HpaII isoschisomere enzymes showed a global DNA demethylation in the TSA-incubated testicular tissue. ChIP assay and Immunocytochemistry (ICC) with specific antibodies to histones modifications (H4 hyperacetylation, H2A ubiquitination, H3K9 methylation) evinced a modulation of the chromatin structure during spermatogenesis that requires changed patterns of histone proteins and histone modifications which contribute to restructuring of chromatin. Some of these epigenetic modifications are involved in genomic imprinting.

Keywords: epigenetic modifications, spermatogenesis, Trichostatin A.

MON-187
Stearoyl-CoA desaturase regulates sirtuin 1 activity in skeletal muscle

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Recent studies indicate that epigenetic modifications such as histone methylation and acetylation are essential for the regulation of lipid-mediated metabolic pathways and energy metabolism. The introduction of such modifications and the balance between acetylation and methylation are crucial for gene expression. NAD-dependent deacetylase sirtuin-1 (SIRT1) affects histone H3 lysine 9 acetylation status and therefore contributes to epigenetic gene silencing. The aim of our study was to elucidate whether stearoyl-CoA desaturase 1 (SCD1), an important control point in lipid and insulin signaling, affects the activity of SIRT-1 in skeletal muscle. Our study showed that the level of histone H3 K9 acetylation is significantly reduced in skeletal muscle with diminished Scd1 expression and increased in muscle with Scd1 overexpression. Opposite results were obtained when methylation profile of H3K9 was analyzed. The level of Sirt1 gene expression was decreased in skeletal muscle of mice with muscle-specific overexpression of Scd-1 (mSCD-1 Tg). Also, SIRT1 protein content was affected in SCD-1 knockout mice. Importantly, skeletal muscles of SCD-1 KO mice are extremely insulin sensitive and have high metabolic rate. Muscle-specific overexpression of SCD-1 severely impairs glucose tolerance in mice fed high-fat diet compared to wild-type mice. Our results show that SCD1 influences histone H3 acetylation/methylation ratio via SIRT1 activity and suggest that modifications in H3K9 may contribute to insulin sensitivity regulation in skeletal muscle and susceptibility to insulin resistance development.

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Keywords: histone modifications,, lipid metabolism, skeletal muscle.

MON-188
Terminin protein DTL/MOI and trimethylguanosine synthase TGS1 are required during *Drosophila* oogenesis

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The Drosophila CG31241 is a bicistronic gene, which encodes a telomere protein DTL/MOI and the RNA trimethylguanosine synthase TGS1, required for sn/snoRNA biogenesis. Telomere protection and sn/snoRNA 5' cap hypermethylation are essential processes in Drosophila. In the absence of DTL chromosome ends become the targets of DNA break repair enzymes and form fusions which will lead eventually pupa lethality. The absence of the TGS1 protein causes failures in TMG cap modification of the sn/snoRNAs and results in small disc phenotype and late L3 larval lethality.

Despite the sever disturbance of these basic functions, the DTL/TGS1 double mutants are able to survive until the late L3 larval stage, which raises the possibility that the maternal product could rescue the animals so far. Using transgenic animals, carrying somatic line specific DTL or TGS1 expression system, we examined the role of DTL and TGS1 in the germline. Loss of TGS1 resulted in decreased fecundity and fertility, dysfunctional production and maturation rate of the eggs. Additionally, the dorsal appendage and the length of the eggs were decreased. In the ovary, higher occurrence of apoptosis at the 8th developmental stage, and aberrant nurse cell nuclear morphology was detected.

In germline specific DTL mutant ovaries, the number of the nurse cells was altered, ring channels showed aberrant morphology and the developing cyst could not leave the germarium. These morphological observations showed unstable penetrance as expected from the stochastic nature of the uncapped telomere behavior.

Our observations suggest that both DTL and TGS1 are necessary for proper oogenesis. Our data suggest that similarly to somatic cells in the germline DTL is involved in chromosome end protection. On the other hand the phenotype of TGS1 mutants suggests that its function is required for proper chromatin formation.

Keywords: None.

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MON-189
The developmental dynamics and disease potential of random monoallelic gene expression

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The majority of autosomal genes in mammals are believed to be expressed from both alleles. However a number of imprinted genes exist and an increasing number of loci may be expressed monoallelically in a random fashion, similarly to the X chromosome in females where one of the two Xs is randomly chosen for inactivation. Classical examples of random monoallelic expression (RME) include members of large gene families, such as immunoglobulins and odorant receptors. Single copy genes have also been reported to show RME but the *in vivo* relevance and underlying mechanism of this pattern of expression remain poorly understood. We have identified a series of RME genes in clonal neural progenitor cell lines derived from highly polymorphic male and female mouse embryonic stem cells. We show that RME occurs during differentiation and, once established, the monoallelic state can be highly stable and DNA methylation does not always account for this extremely stable epigenetic state. We also demonstrate that RME of single copy genes can occur even in the absence of DNA sequence polymorphism *in vivo*. Several of the genes we identify play important roles in development and have been implicated in human autosomal dominant disorders, including the *Eya* genes and the BOR syndrome. We propose that monoallelic expression of such genes might contribute to the fine-tuning of the regulatory pathways they control during development, but in the context of a mutation on one allele, this may predispose such genes to loss of function in a proportion of cells and thus contribute to disease.

Keywords: Epigenetics, Monoallelic expression, Mouse development.

MON-190
The histone composition in neutrophils of patients with community-acquired pneumonia and chronic obstructive pulmonary disease

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The aim of the work was to study the histone composition in neutrophils of patients with community-acquired pneumonia and chronic obstructive pulmonary disease (COPD). Patients were divided into 3 groups. 29 patients with COPD, moderate severity, mixed form (emphysematous and bronchial), exacerbation, respiratory insufficiency of grade 2 were included in first group. 20 patients with community-acquired pneumonia and COPD were included in second group. 16 patients with community-acquired pneumonia were included in 3-d group. The control group consisted of 32 healthy persons. All patients and healthy subjects had received the full information on probable inconveniences at the blood sampling before giving their written informed consent.

For neutrophils separation we used the standard procedure. Purity and viability were assessed by trypan blue dye exclusion. The samples of >85% neutrophilic leukocytes with >90% viability were obtained. In neutrophils of all subjects the composition of histone H1, H2A, H2B, H3, H4 were detected following the protocol of L.Markusheva et al (2000).

The results obtained have demonstrated the significant increasing of H1 histone (by 88%, $p < 0.05$), total fraction of H2A, H3,H4 histones (by 61%, $p < 0.05$) and H2B histone (by 56%, $p < 0.05$) in neutrophils of COPD patients in comparison with healthy persons.

The increasing of H1 histone (by 36%, $p < 0.05$), total fraction of H2A, H3, H4 histones (by 2 times, $p < 0.001$) and H2B histone (by 5.5%, $p < 0.001$) was determined in neutrophils of patients with community-acquired pneumonia in comparison with healthy persons.

The decreasing of total fraction of H2A, H3,H4 histones (by 82%, $p < 0.05$) in neutrophils was established in patients with community-acquired pneumonia and COPD in comparison with healthy persons.

Our results have demonstrated opposite changes of histone composition in neutrophils of patients with community-acquired pneumonia and COPD in comparison with results of first and second groups of patients. Such kind of histone decomposition could be connected with different epigenetic mechanisms.

Keywords: Histones, neutrophils, pneumonia.

MON-191
The RSC remodeling complex as essential component of the remodeler network for chromatin remodeling at the yeast PHO5 promoter

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The yeast *PHO5* promoter was the first and still is one of the best characterized examples of a massive chromatin transition that is an absolute prerequisite for transcription activation. The comprehensive search for involved cofactor(s) revealed a complex network of five remodelers from all four major subfamilies in yeast. We showed recently that RSC, the only remodeling complex essential for viability in yeast, is a major component of this network. In continuation we wished to fully clarify the role of RSC, especially if it is essential for *PHO5* promoter opening. We applied new strategies for more complete RSC ablation than the previous inactivation of its catalytic subunit Sth1 by the temperature sensitive degron allele $Sth1^{td}$. First, we combined the deletion of *RSC2*, encoding a subunit of a major RSC complex isoform, with inactivation of $Sth1^{td}$ during *PHO5* induction at the nonpermissive temperature (37 °C). Second, we constructed a double mutant containing a Tet-Off-promoter driven *STH1* gene and the *rsc2* deletion allele and examined chromatin remodeling upon physiological induction at 30 °C. In contrast to the $sth1^{td}$ single mutant, both double mutants achieved no appreciable *PHO5* promoter opening even after prolonged induction suggesting an essential role of RSC complex. The same was true for a *PHO5* promoter variant activated by the non-physiological activator Gal4, i.e. the RSC effect is not specific for induction through PHO signaling nor for promoter activation by the native activator Pho4. We also demonstrated that RSC activity is not only essential for opening but also for the maintenance of open chromatin at the *PHO5* promoter.

Keywords: chromatin remodeling, gene transcription, yeast PHO5.

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MON-192
The SUMO-specific isopeptidase SENP3 regulates the SET1/MLL methyl transferase complex and controls fate of human mesenchymal stem cells

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Cellular differentiation processes critically rely on the control of gene expression programs. The ubiquitin-like SUMO system regulates gene expression, but the molecular insights in this process are incomplete. Here we show that the SUMO-specific isopeptidase SENP3 controls H3K4 methylation by regulating histone-modifying SET1/MLL complexes. SET1/MLL complexes are composed of a histone methyltransferase and the regulatory components WDR5, RbBP5, Ash2L and DPY-30. MLL1/2 complexes are particularly important for the activation of *HOX* genes and contain menin as an additional component. We demonstrate that SENP3 is associated with MLL1/2 complexes and catalyzes deSUMOylation of RbBP5. This is required for activation of a subset of *HOX* genes, including the developmental regulator *DLX3*. In the absence of SENP3, the association of menin and Ash2L with the *DLX3* gene is impaired leading to decreased H3K4 methylation and renders chromatin less permissible for transcription as was evident by reduced recruitment of active RNA polymerase II. Importantly, the SENP3-DLX3 pathway dictates osteogenic differentiation of human mesenchymal stem cells thus delineating the importance of balanced SUMOylation for the epigenetic control of gene expression programs. This study also esbalished novel molecular mechanism that governs the associataion of of histone methyl transferase complex to its target gene.

Keywords: SET1/MLL histone methyl transferase, Stem cell fate deterrnimation, SUMO, chromatin structure.

MON-193
The transcriptional co-repressor Ski localizes in the pericentromeric region of mitotic chromosomes, regulating the expression of targeted genes at the early G1 phase of the cell cycle

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Ski is a nuclear protein that is part of transcriptional co-repressor complexes containing histone deacetylase activity. Ski is expressed throughout the cell cycle, reaching its highest levels in mitosis. In order to establish the role of Ski during mitosis, a detailed characterization of Ski localization during this stage of the cell cycle in mouse embryonic fibroblasts (MEFs) was performed. It was observed that Ski was forming pairs of well defined dots in the pericentromeric region of some mitotic chromosomes. Giving that Ski is a transcriptional co-repressor, we hypothesized that during mitosis, Ski was targeting pericentromeric genes for silencing after mitosis is completed, i.e., early G_1. Thus, we compared overall gene expression in MEFs knock out for Ski (*Ski-/-*) and MEFs *Ski-/-* expressing an ectopic protein (Ski-/-_hSKI), synchronized at early G_1. Total RNA was extracted and Cy3/Cy5 labeled cDNA was hybridized to a mouse exonic oligonucleotide array (MEEBO). The analysis revealed that 265 genes were differentially expressed in the presence of Ski at G_1. Among these, 161 were repressed and 104, overexpressed. Although there was a homogeneous coverage of the probes on the different chromosomes and along each chromosome, 75 (47%) out of 161 repressed genes were located in the first 50 Mb of chromosomes and 65% of them on chromosomes 9, 13 and 17. The most repressed genes corresponded to three pericentromeric genes (*mmp-3, mmp-10, mmp-13*) located in a cluster at chromosome 9. Microarray results were validated by real-time RT-PCR assays and the occupancy of Ski on the promoter of *MMP3* gene was confirmed by a chromatin immunoprecipitation (ChIP) assay. The presence of Ski correlated with a decrease in H3Lys9 acetylation and an increase in H3Lys9-3me levels in mitotic and G_1 cells, and at chromosome level, acetylation of histone H3 at Lys9 (H3Lys9) was decreased in regions that co-localized with Ski. These results suggest that Ski direct the mitotic bookmarking of pericentromeric genes, repressing the expression of those genes at the beginning of the next cell cycle (early G_1). Supported by National Counseling of Science and Technology (CONICYT) Grant FONDECYT 1120222 and Chilean Ministry of Education Grant STIPAS MECESUP 0717.
Keywords: mitotic bookmarking, ski.

MON-194
The yeast HMG proteins Hmo1 and Nhp6 exert a differential stimulatory effect on ATP-dependent nucleosome remodeling activity

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ATP-dependent chromatin remodeling activity plays a relevant role in several aspects of DNA metabolism, including transcriptional regulation. Several protein complexes harboring this activity have been described, with the yeast SWI/SNF (switching defective/sucrose non-fermenting) as the founding member. These complexes can facilitate the access of DNA-interacting proteins to their cognate sequences by transiently exposing stretches of nucleolsomal DNA, by mobilizing histone octamers in *cis* (sliding) or evicting them towards naked DNA stretches or histone chaperones. A number of elements have been described as factors stimulating or affecting ATP-dependent nucleosome remodeling, such as certain histone modifications, DNA sequences and also High Mobility Group (HMG) proteins. Little is known about the influence of HMG proteins on the activity of these complexes and no studies have addressed the influence of different types of yeast HMG proteins on the activity of complexes such as ySWI/SNF. In order to gain insight into these aspects we performed different types of *in vitro* nucleosome remodeling assays, observing that the yeast HMG proteins Nhp6A, Nhp6B and Hmo1 are able to stimulate SWI/SNF sliding activity. However, only Hmo1 stimulates other biochemical outcomes of ySWI/SNF activity and binding of this complex to the nucleosome. Different experimental approaches were used to explore the mechanism by which Hmo1 stimulates ySWI/SNF acitivity. These stimulatory effects exerted by Hmo1 appear to be dependent on the C-terminal tail of this protein, as a deletion mutant of this protein, lacking the last 35 residues, is not able to stimulate SWI/SNF activity nor binding to the nucleosome. Stimulation of SWI/SNF action by Hmo1 was also observed *in vivo*. In a *hmo1* deletion mutant of *Saccharomyces cerevisiae*, changes in mRNA levels of SWI/SNF target genes were observed. In the absence of Hmo1, nucleosome positioning on promoter regions of these genes and physical association of ySWI/SNF to these regions were also affected. Supported by grant CONICYT, FONDECYT/Regular 1130818.
Keywords: chromatin remodeling, HMG proteins, SWI/SNF.

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MON-195
Theoretical study of DNA methylation in nucleosome

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Cytosine methylation in DNA is a major epigenetic signal, and plays a central role in propagating chromatin status during cell division, regulation of gene expression, transcriptional regulation, maintenance of genomic stability and in the development of cancer (1). However the mechanistic links between DNA methylation and histone methylation are poorly understood.

We have performed biased molecular dynamics (MD) simulations and free energy and mesoscopic calculations of the base flipping on histone-bound DNA, free DNA (linker DNA) and curved DNA of the same sequence. By using umbrella sampling techniques we were able to calculate the energy of flipping cytosine base on different positions on histone-bound DNA and compare it with experimentally observed values (2). The simulation results give us an atomistic view on the methylation favourable spots, energetics involved and the influence of DNA binding to the histone protein.

Free energy calculations indicate that methylation disfavours cytosines, which are placed in the region of the minor groove that is pointing away from histones, while it favours CpG step placed in the region with the major groove pointing away from the protein. This confirms the proposed mechanism of methylation of a CpG step with base flipping done in the major groove (1). These findings also correspond with recent study done in our group on nucleosome binding affinity and cytosine methylation (3).

The study of nucleosome-like curved DNA suggested that the curvature of DNA is responsible for energetically more favourable base flipping at a CpG step in the nucleosome rather than in the linker DNA of the same sequence. Our results suggest that physical properties of DNA might play a significant role in epigenetic regulatory mechanisms and might influence nucleosome formation.

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Keywords: free energy, methylation, Nucleosome.

MON-196
VHH-based universal histone binding domains: novel tools for a different look on and manipulation of chromatin

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The engineering of histone binding domains, non-endogenously encoded by the genome, therefore without intrinsic cellular functions, constitutes a major issue in the epigenome editing and visualization. Using an immune library of VHHs (or nanobodies®), the monomeric antigen-binding domain of camelidae heavy chain antibodies, we made several rounds of phage display affinity selection against histone H2AX extracted from chromatin. Among the selected clones, we identified several VHHs, referred to as chromatibodies, presenting the unique ability to specifically recognize chromatin, from human to yeast, in cell immunostaining. In vitro, chromatibodies bind core histones, exhibiting a marked preference to the H2A-H2B dimers compared to the histone monomers and the H3-H4 tetramer. Expressed as a GFP fusion in human cells or in drosophila, chromatibodies retain their ability to specifically bind chromatin, and similar to conventional core histone-GFP probes, allow non-invasive live chromatin imaging. However, unlike H2B-GFP, fluorescent chromatibodies exhibit a high exchange mobility on chromatin, as revealed by FRAP experiments. By varying the nature and the number of chromatibodies modules linked to GFP, we have been able to modulate the mobility and specificity of the probe to chromatin. We therefore have developed a family of genetically encoded affinity reagents to chromatin that potentially have the ability to carry any modifying activities to the nucleosomes, a capability which could be used to manipulate epigenetic marks at the scale of the whole genome.

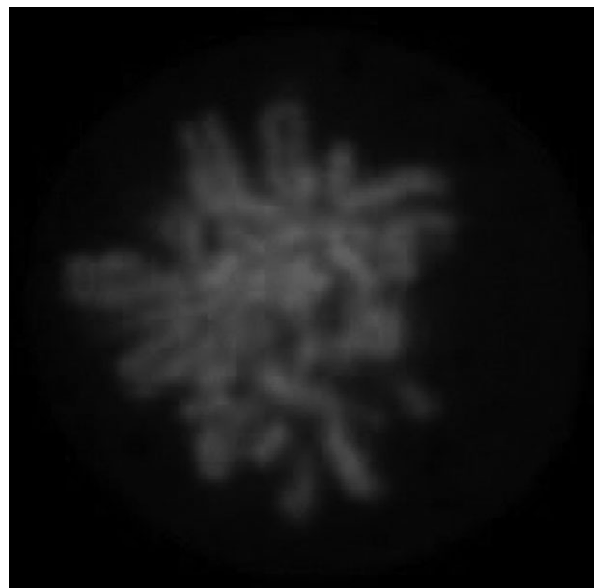

Fig. 1.

Keywords: Chromatin imaging and targeting, Histone-binding module, VHH single domain antibodies.

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CSIII-02 – Development & Evolution

MON-198
A new pathway for embryo implantation: the uterine LEFTY2/AKT/SGK1 axis

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Background: Pregnancy requires attachment of the embryo to a receptive endometrium followed by implantation. Endometrial SGK1 is rapidly induced in response to the post-ovulatory rise in progesterone but expression is transiently down-regulated during the window of implantation. Previous analysis of mid-secretory endometrial samples revealed that SGK1 is deregulated in unexplained infertility, although the underlying mechanisms are not understood.
Methods: AKT inhibitor screening & functional analysis *in vitro* and *in vivo*, protein analysis, qRT-PCR, embryo transfer and histological assessment uterine sections.
Findings: In endometrial cells, an increase in SGK1 activity is reciprocated by a decrease in AKT phosphorylation, pointing towards a homeostatic mechanism that balances the activities of these related kinases. To test this hypothesis, we screened 9 AKT inhibitors in Ishikawa cells, an endometrial epithelial cell line, and identified 2 AKT inhibitors (INH-III and V) that strongly enhanced endometrial SGK1 phosphorylation. Transient flushing of the non-pregnant uterus in mice with AKT inhibitors INH-III or V strongly upregulated SGK1 phosphorylation. Moreover, AKT inhibition *in vivo* down-regulated the E3 ubiquitin-protein ligase Nedd4-2, thereby enhancing epithelial sodium channel (ENaC) expression on the luminal epithelium. To assess the impact of AKT inhibition on implantation, uteri of pseudopregnant female mice were flushed with INH-III or vehicle prior to embryo transfer. Ten cultured blastocysts were transferred to a single uterine horn and the implantation sites counted after 3 days. We show that transient exposure of the uterus lumen to INH-III prior to embryo transfer significantly reduced the number of implantation sites compared to vehicle control. Finally, we demonstrate that recombinant LEFTY2, an important inhibitor of the NODAL signalling pathway produced by decidualizing stromal cells, induces a reciprocal increase and decrease SGK1 and AKT phosphorylation, respectively; and is sufficient to block implantation in mice.
Conclusion: We provide evidence that the activities of SGK1 and AKT are homeostatically balanced in the endometrium of human and mice. LEFTY2-dependent AKT inhibition enhances SGK1 phosphorylation and abolishes embryo implantation by perturbing uterine fluid handling. Our results indicate that the LEFTY2/AKT/SGK1 axis is a promising target for fertility control as well as for the prevention of implantation failure, especially in the context of assisted reproductive technologies.
Keywords: Endometrium, Implantation, Kinases.

MON-199
A set of LRR-RLK genes quantitatively regulate root growth under iron-limited conditions in Arabidopsis

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Plant development is highly plastic and is profoundly influenced by the environment. The sessile life style of plants has led to the development of diverse mechanisms required for the perception and response to fluctuating environments. Drought, changing temperatures, lack of sufficient nutrients, exposure to toxic minerals, and soil compaction are just a few examples of the environmental constraints that roots are exposed to during plant growth and to which they respond by altering their developmental program. We used a geographically diverse set of 450 natural accessions of *Arabidopsis thaliana* to identify genes that quantitatively regulate root growth responses to Iron (Fe) deficiency using genome wide association mapping. We identified more than 20 genomic loci that were statistically highly significantly associated with changes of root growth rate upon iron deficiency. Among genes in proximity of these associations, a cluster of 3 *Leucine-rich repeat receptor-like protein kinases* (*LRR-RLK*) genes and a kinase gene showed strong signatures of epistatic interactions. Each of the single mutant lines of these signaling genes displayed a significant root growth rate reduction on iron deficient media but not on full media, showing that this gene cluster is involved in growth regulation under iron limited conditions. Due to their tissue specific expression pattern, we hypothesize that these 4 genes are coordinating growth responses in different tissues of the root. We are currently testing this hypothesis and exploring the epistatic interactions of these genes and their alleles.
Keywords: GWAS, Iron deficiency, Plant root development.

MON-200
A two loci genetic incompatibility leads to offspring respiratory deficiency within the Saccharomyces cerevisiae species

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Understand the molecular basis of how reproductive isolation evolves within a same species offers great insight into the patterns of genetic differentiation as well as the onset of speciation. Previously, we examined the landscape of post-zygotic reproductive isolation within the *Saccharomyces cerevisiae* species by analyzing a large number of crosses among genetically diverse strains, and identified chromosomal rearrangements as the major cause leading to the observed cases of reduced offspring viability. By contrast, genetic incompatibility results from epistatic interactions between diverged genes remained undetected, indicating that this type of mechanism is probably rare and might have a modest effect to the offspring viability under permissive laboratory con-

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ditions. To further our understanding of the prevalence of genetic incompatibility relative to the onset of reproductive isolation in this species, we selected 30 crosses that are compatible (offspring viability >90%) on standard laboratory media (YPD), and scored their offspring viability in the presence of different conditions (e.g. carbon sources, temperature, chemicals). One cross between a clinical isolate and the laboratory reference strain S288c was found to be incompatible, showing reduced offspring viability of 75% on media containing non-fermentable carbon sources (e.g. glycerol and ethanol). Using bulk segregant analysis strategy, we mapped the regions and genes that are involved in the incompatibility. We performed allele replacements and confirmed that interactions between incompatible alleles result in respiratory deficiency. This study demonstrates the first example of genetic incompatibility related to specific ecological environment in the *S. cerevisiae* species.

Keywords: evolution, genetic incompatibility, yeast.

MON-201
Additional sex comb-like 1 and Nmyc controls alveolar epithelial cell formation during mouse embryonic development by Retinoic acid signaling

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Retinoic acid (RA) plays a pivotal role during lung development at late gestation. RA plays important roles in cell-to-cell communications. Asxl1 has been identified as an interacting protein of RA receptor (RAR) and functions as either co-activator or repressor in a cell type-specific manner. To investigate physiological functions of Asxl1, we generated Asxl1-null mice. Homozygous Asxl$^{-/-}$ embryos died shortly after birth, respiratory failure and remarkably decreased the expression levels of several epithelium markers. To identify how Asxl1 might regulate lung epithelial cell, we compared mRNA expression profiles in lung from E18.5 Asxl1$^{-/-}$ mice and wild-type littermates. One of the altered up regulation genes is Nmyc, which encodes a factor that down regulate by RA. Furthermore, there is an overlap in gene expression in the Asxl1$^{-/-}$ mcie and Nmyc overexpression TG mice lung. To identify as these data, Nmyc and target gene expression validate at E18.5 lung samples. To demonstrate that the Nmyc induction upon Asxl1 knock down, we performed the Asxl1 knock down in human lung carcinoma cell lines A549 in normal medium condition. In this cancer cell line, knock down of Asxl1 resulted that increased Nmyc expression. Additionally, we found that RARa repressed the transcription activity of Nmyc promoter. Overall, our data suggest that RA combined with Asxl1 is essential for lung epithelial cell development, which may be critical for air breathing at birth.

Keywords: Asxl1 knock out mouse, Nmyc, Retinoic acid.

MON-202
Adverse glucocorticoid effect on angiogenesis is associated with Akt/mTOR pathway in vitro

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Glucocorticoids have been used during pregnancy for several reasons but overexposure to glucocorticoids may have adverse effects. Despite the widespread use of dexamethasone and other steroids in clinical practice, it's still not known how proangiogenic factors and their receptors are affected from glucocorticoid exposure. Therefore, we aimed to investigate the effect of glucocorticoid exposure on VEGF, PIGF, VEGFR1 and VEGFR2. We also investigated whether glucocorticoid effect on angiogenesis mechanisms is Akt/mTOR pathway dependent or not. HUVECs were incubated with synthetic glucocorticoid, Triamnicinolone Acetonide (TA). TA administration led to a decrease in VEGF and VEGFR1 expression at mRNA and protein levels and an increase in VEGFR2 protein expression. PIGF protein expression was unaffected by TA treatment but mRNA expression levels were decreased dose dependently at 48 and 72 hours incubation. Phospho-Akt expression was unaffected, but phospho-p70S6K and phospho-4EBP1 expression was decreased time and dose dependently. According to Matrigel analysis, vascular network forming capacity was decreased time and dose dependence of TA. In summary, we observed that glucocorticoids have negative effects on angiogenesis mechanisms, which was dependent on the alterations of cellular and soluble angiogenic protein and mRNA levels and their respective vascular network forming capacities in an Akt/mTOR pathway dependent manner.

Keywords: Akt/mTOR, angiogenesis, glucocorticoids.

MON-203
An unique N-glycosylation in Drosophila BMP-type ligand Screw contributes to a robust BMP morphogen gradient in embryogenesis

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Nature exhibits an amazing variety of species. However, despite this immense diversity of animals, their development is controlled by common genetic mechanisms, which are widely conserved among metazoan phyla. One very well studied example for such highly conserved molecules are Bone Morphogenetic Proteins (BMPs), which belong to the TGF-β family. Three BMP-type ligands have been identified in *Drosophila* – Screw (Scw) and Glass bottom boat (Gbb), which are BMP5-8 type ligands, and Decapentaplegic (Dpp), a BMP2/4-type ligand. It has been considered that the *scw* gene evolved by gene duplication from *gbb*, and that it is exclusively found in higher Diptera [1]. Both, Scw as well as Gbb form a functional unit with Dpp [2, 3]. While Scw:Dpp heterodimer is the primary ligand for dorsal-ventral patterning in the early embryo [2], Gbb:Dpp heterodimer is crucial for crossvein development of the pupal wing [3]. Additionally, in both contexts a conserved kind of transport mechanism is acting, which is crucial for proper signalling outcome [2–4]. A further indication for the conserved mechanism during embryogenesis and crossvein formation is that Scw can rescue *gbb* mutant phenotypes in the wing. Intriguingly, *vice versa*, Gbb is not able to recover *scw* mutant phenotype in the early embryo [1]. Taken together, these facts raise the question of what is the fundamental difference that causes the differing signalling outcome of Scw and Gbb?

In the course of this study we are focusing on the differential N-glycosylation of highly conserved signalling molecules and how these modifications might be utilized for species- and context specific signalling.

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Keywords: BMP signalling, Drosophila embryogenesis, Post-translational modifications.

MON-204
Analysis of human and rabbit metallothioneins by Brdicka reaction and mass spectrometry

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Metallothioneins (MTs) form a large family of evolutionarily conserved low-molecular-weight proteins (~6 kDa), found in practically all life forms, in vertebrates, invertebrates, fungi and even in plants. MT plays a special role in maintaining the homeostasis of metals essential for the proper functioning of the human body, including Zn, Cu. MT is also responsible for detoxification of heavy metals such as Cd and Hg and removal of free radicals. Mammalian MTs were separated into two charge-separable isoforms, designated as MT fractions 1 and 2. The six amino acids of rabbit MT sequence show marked differences compared with the sequences of other mammalian MTs. The biological significance for this difference remains unclear.

The present study demonstrates an analytical approach of employing two detection techniques: Brdicka reaction and matrix-assisted laser desorption/ionization - mass spectrometry analysis (MALDI-MS) to characterize MTs from human and rabbit liver.

By MALDI-MS, rabbit and human MT were identified and additionally to monomers of MTs (~6 kDa), which are the major peaks in mass spectrum, small signals from the dimers of MTs were also observed, which are present both in human and rabbit MT.

During MT analysis by Brdicka reaction, changes in signals - RS_2Co (−1.25V which represents current response of MT complex with components of Brdicka electrolyte), Cat1 (−1.35V) and Cat2 (−1.48V) correspond to hydrogen evolution from the supporting electrolyte catalyzed by the MT - were observed. Concentrations of MT – 0.025, 0.5 and 0.1 mg/ml were analyzed. With decreasing MT concentration, RS2Co and Cat signals decreased and shifted to more positive potential. Signals Cat1 and Cat2 are more exposed with decreasing MT concentration. Character of the mentioned MT signals changed with different MT concentrations. The best signal for human MT was observed in concentration 0.025 mg/ml and for rabbit MT in concentration 0.05 mg/ml.

Methods used in this report allow MTs identification, permit to quantify the purity and content of its isoforms, and allow studying its quantification and polymerization.

Acknowledgement: Support from CEITEC CZ.1.05/1.1.00/02.0068 is highly acknowledged.
Keywords: Brdicka reaction, mass spectrometry, metallothionein.

MON-205
Brk regulates wing disc growth in part via repression of Myc expression

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The biological and molecular mechanisms, which regulate tissue size, represent an essential but unresolved problem in developmental biology. The main question is how developing growing tissues know when they have reached their correct final size and therefore should stop growing. The developing wing of Drosophila melanogaster is one model system that has been extensively used to address this question. One signaling pathway that strongly influences wing tissue size is the TGFß pathway, which is activated by Dpp – a gradient morphogen member of the TGFβ family growth factors. Flies lacking Dpp in the wing have extremely small wings, whereas overactivation of the Dpp pathway leads to excessive tissue overgrowth, clearly indicating that Dpp signaling is required to support tissue growth and also that the size of the wing correlates with the activity of the Dpp pathway. Dpp promotes growth via repression of the transcription factor Brinker. Although the transcriptional targets of Brinker that control the patterning of the wing, like vg, salm and omb, are known, the transcriptional targets and processes for cell growth and proliferation are not yet fully elucidated. In this project, a genome-wide approach was used to identify Brinker transcriptional targets, performing a ChIP-Seq of endogenous Brinker from wing imaginal discs. I identified the growth regulator Myc as a target of Brinker and showed that myc together with the microRNA bantam explain a significant fraction of the growth inhibition caused by Brinker. This work sheds light on the mechanisms by which Dpp signaling controls tissue growth.

Keywords: Dpp, Drososphila, Wing growth.

MON-206
Cell cycle analysis of neural progenitors in the developing ferret neocortex

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The evolutionary expansion of the neocortex reflects an increase in neurogenesis, which in turn is due to increased numbers and rounds of cell division of cortical progenitors. The length of the cell cycle is known to be an important parameter determining the proliferative versus neurogenic potential of cortical progenitors. Here, we have studied the length of the individual cell cycle phases of the various progenitor populations in the developing neocortex of a gyrencephalic mammal, the ferret (*Mustela putorius furo*), in order to gain further insight into possible causes underlying neocortical expansion.

Progenitor types were identified by immunofluorescence for Pax6 and Tbr2. These two transcription factors are sequentially expressed by cortical progenitors, in correlation with their progressive restriction towards a neurogenic fate. We identified an abundant progenitor population positive for both of these markers, found in all germinal zones during neurogenesis. We propose these progenitors to be capable of transit-amplification and to include proliferative intermediate progenitors. These Pax6- plus Tbr2-positive progenitors would thus have the potential to expand the progenitor pool and the total number of neurons of gyrencephalic brains.

The length of each cell cycle phase of the four different cortical progenitor populations (Pax6+Tbr2−, Pax6+Tbr2+, Pax6−Tbr2+, Pax6−Tbr2−) was determined by cumulative EdU labeling of postnatal day 1 (P1) ferrets. The greatest difference between these

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progenitor types was in the length of their S phase, suggestive of differences in their proliferative versus neurogenic potential. Unexpectedly, and in contrast to previous observations in other animal models, we found little variation in G1 phase length.

In addition, to complement the EdU labeling data, a live imaging method in ferret organotypic brain slice culture was established. Cells in the developing neocortex were labeled with a GFP-expressing adenovirus in order to follow their cell cycle and cell division and to study different progenitor types and their lineages.

Keywords: Cell cycle, Cortical development, Neurogenesis.

MON-207
Characterization of second heart field cardiac progenitor cells in the developing heart

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Cardiac progenitor cells of the second heart field (SHF) contribute to the poles of the elongating embryonic heart. Failure or perturbation of second heart field development leads to congenital heart defects. Recent studies have provided initial evidence of how distinct regions of the definitive heart are pre-patterned in the progenitor cell population. In particular, the myocardium at the base of the aorta and pulmonary trunk were shown to be prefigured in the outflow tract (OFT), where *Tbx1* has been demonstrated to be crucial for the population of progenitor cells giving rise to subpulmonary myocardium. On the basis of this observation, we investigated the molecular and genetic mechanisms underlying heterogeneity in cardiac progenitor cell population, in particular the *Tbx1*-independent subpopulation that give rise to the future subaortic myocardium. Using the *A17-Myf5-nlacZ-T55* (*T55*) enhancer trap transgene which is expressed in the superior wall of the OFT and the future subaortic myocardium, we investigated the differences in expression pattern, signaling inputs and spatio-temporal pre-patterning of the future subaortic and subpulmonary myocardial progenitors. Our results indicated that the *T55* transgene defines a distinct sub-domain of progenitors within the SHF, specifically in the anterior part of the SHF. We demonstrated that this subpopulation is mainly not derived from the *Hoxb1*-expressing progenitors that contribute to the inferior wall of the OFT and subsequently to the myocardium at the base of pulmonary trunk. We also showed that part of the *T55* sub-domain, located in the most cranial region of the anterior SHF, does not express TBX1, thus most likely being the subaortic myocardial progenitors. Moreover, our preliminary results revealed that *Tbx1*-dependent and independent subpopulations of progenitor cells are characterized by different transcriptional signature and temporal specification; where cells from subaortic myocardial progenitors differentiate earlier than the subpulmonary myocardial progenitors. The molecular mechanisms underlying such differences are currently under investigation. Altogether, this study will provide insights into how cardiac progenitor cells are pre-patterned to prefigure clinically important regions of the definitive heart.

Keywords: Outflow tract, Second heart field, Sub-aortic myocardium.

MON-208
Colour pattern formation in zebrafish

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Colour patterns are a striking feature of animals; they evolve rapidly and play an important role in natural as well as sexual selection. It has been proposed that colour pattern formation in

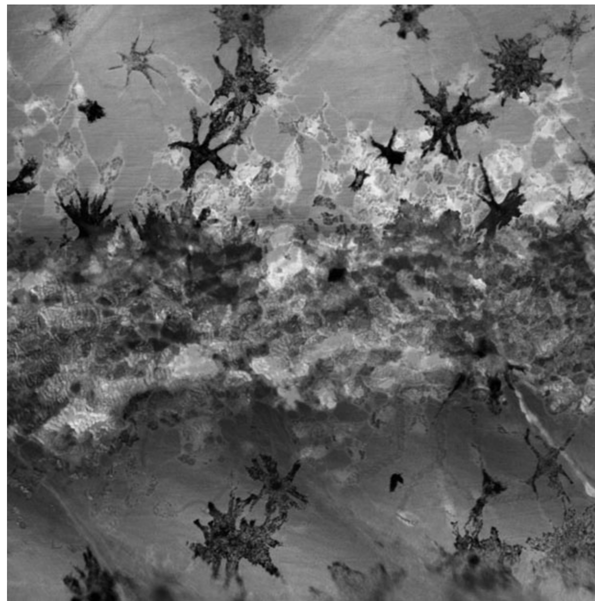

Fig. 1.

adult vertebrates depends on Turing-type interactions between pigment cells, however little is known about the actual developmental mechanisms underlying the complex and prolonged ontogeny of this important adult feature. Zebrafish (*Danio rerio*) owe their name to a repetitive pattern of dark stripes and light interstripes parallel to the anteroposterior body axis which develop during juvenile stages. By inducible Cre/loxP-mediated recombination in neural crest-derived progenitors, we created labelled clones of skin pigment cells that were imaged over several weeks in juvenile and adult fish. Metamorphic iridophores arise from postembryonic stem cells located at the dorsal root ganglia (DRGs) of the peripheral nervous system. They emerge in the skin at the horizontal myoseptum to form the first interstripe and proliferate while spreading bidirectionally along the dorsoventral axis. Patterned aggregation of iridophores during their dispersal generates a series of interstripes that define the stripe regions. Melanophore progenitors appear *in situ* in the presumptive stripe region where they melanise and expand in size to form compact stripes. Thus, although depending on mutual interactions between different pigment cells, stripes and interstripes are formed by a completely different cellular route.

Keywords: Colouration, Pattern formation, zebrafish.

MON-209
Comparative analysis of the adult and embryo major yolk proteins in the sea urchin

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The major yolk protein, MYP, is localized to the egg and coelomic fluid of the adult sea urchin. While the egg-localized protein has been extensively studies, relatively little is known about the coelomic fluid-localized form of the MYP. We have conducted a comparative biochemical analysis of these proteins. Sucrose density gradient fractionation revealed unique elution profiles for these proteins. V8 peptide mapping revealed that both species had very similar primary structures while circular dichroic and endogenous tryptophan fluorescence analyses revealed unique

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secondary and tertiary structural features. Both proteins exhibit unique responses to calcium suggesting differing requirements for calcium-dependent binding to membranes and protein-dependent, membrane-membrane interactions. We discuss the functional implications arising from these structural differences.
Keywords: Embryo, Adult, Comparative.

MON-210
Comparison of several molecular processing approaches of ancient DNA from medieval human remains in Latvia

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In the work with ancient DNA (aDNA) within human evolution studies a complete recovery of genetic material preserved in the sample together with avoiding of contamination with modern DNA are the most important issues. The aDNA usually is present in small amounts and is in various states of degradation. In addition, the archeologic material may contains PCR inhibitors which can be co-purified with aDNA sample. Therefore numerous aDNA extraction methods have deen developed.

In this study, two different aDNA extraction approaches, i.e. commercial-kit based and the protocol of Rohland & Hofreiter (Nature protocols, 2007), were compared. aDNA was isolated from samples of medieval human skeletal remains dated by 15th – 17th centuries, Latvia, followed by PCR amplification of two genetic markers for sex determination. Simultaneously, in order to control contamination, blank samples were included in each experiment of aDNA isolation. The results show that PCR amplification was successful for a set of samples regardless of the extraction method used. In contrast, two samples failed for the PCR amplification in the case of both approaches. These results indicate that different aDNA methods can be used in archeological research and rather the initial DNA degradation level or conditions that may influence the sample preservation should be considered.
Acknowledgements: The research was supported by a grant from the European Social Fund (ESF) no.1DP/1.1.1.2.0/13/APIA/VIAA/038.
Keywords: ancient DNA.

MON-211
Computational and experimental sequence analysis of Y-nups

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Nuclear pore complexes are composed of 30 evolutionarily conserved proteins or nucleoporins (nups) assembled into sub-complexes. The Y-shaped Nup84/Nup107-160 sub-complex (Y-complex) forms the outer ring scaffold of the pores and is composed of 9 key subunits (Y-Nups) in vertebrates (7 in yeast) with common structural features yet elusive sequence similarities. We deployed computational and experimental sequence analysis involving extensive sequence comparisons, and protein domain detection using the *Drosophila melanogaster* Y-Nups, as queries and augmented the limited set of known protein associations for Y-Nups across eukaryotes. Using established protocols for low-complexity masking, sensitive iterative sequence profile searches, automated sequence clustering and visualization of sequence similarity, we assigned the detected homologies into nine Y-Nup families. Our analysis increased the compendium of known Y-Nups by more than 63% with the resulting multiple sequence alignments sharing as low as <10% identity between certain members and their homologs. In the above alignments using an adaptive length threshold we were able to detect 27 novel multi-domain architectures for Y-Nups that were further validated by genomic sequence comparisons, as well as, their presence in multiple species. RNA-seq expression data revealed that Y-Nups associated with a subset of the discovered domains are found to be under tight coordinated regulation across diverse human and mouse cell types and tissues, implying that they function in conjunction with the nuclear pore. Six (out of the 27) detected cases were strongly supported by the three set criteria (by genome, by expression, by frequency) and point to moonlighting roles of Y-Nups, outside of the pores related to DNA-RNA metabolism and mitotic control and warrant further experimental analysis.
Keywords: nucleoporins, sequence analysis.

MON-212
Conformational changes of intrinsically disordered otolith matrix macromolecule-64

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Otoliths in fishes and their homologues otoconia in higher vertebrates are part of gravity and linear acceleration detection system. The structures are biominerals composed of calcium carbonate and a small fraction of organic component including proteoglycans, glycoproteins, collagens and noncollagenic proteins. Despite of the low content of organic molecules in the otolith, they play a crucial role in biomineralization by controlling the shape, size and polymorph of crystals.

Otolith matrix macromolecule – 64 (OMM-64) is one of the protein involved in biomineralization control. It was identified by screening a cDNA library of rainbow trout inner ear. OMM-64 is a constituent of high-molecular-weight aggregates (HMWAs) along with otolin-1, heparan sulfate glycosaminoglycans and other unidentified component. It has been demonstrated that the HMWAs control the morphology and polymorph of calcium carbonate. In the presence of HMWAs aragonite crystals are formed, whereas OMM-64 and otolin-1 itself induce vaterite and calcite respectively. Till now it is not known whether the presence of these two proteins is sufficient to form aragonite or another HMWA constituent is necessary. To understand biomineralization process separation and structural and functional analyses of each HMWAs component is absolutely imperative. That is why the first stem of our study is investigation of structural properties of OMM-64.

It has been previously shown that OMM-64, like many others proteins involved in biomineralization, belongs to intrinsically disordered proteins (IDPs). IDPs are very flexible, can interact with multiple partners, gain transient structures and even undergo disorder-order transition. The aim of our work was to investigate whether OMM-64 is able to adopt ordered structure.

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In this study conformational behavior and unique response of OMM-64 to environment conditions are presented. Increasing the content of ordered secondary structure in the presence of TFE detected by far UV CD spectra indicated that OMM-64 has tendency to gain ordered structures. Decrease of Stokes radius in the presence of counter ions: Na^{+}, K^{+}, Mg^{2+}, Ca^{2+} was observed implying existence of squeezed partially collapsed conformation. The strongest effect was observed in the presence of calcium ions, which are ligand for the protein. The effect of temperature, pH and chemical denaturant was also investigated. 'Turned out' response to heat and changes in pH was demonstrated. Cooperativity of denaturant induced unfolding was not observed. In low concentration of guanidium chloride (GdmCl) OMM-64 was more compact than without or in high concentration of denaturant.

Acknowledgment: This work was supported by a statutory activity subsidy from the Polish Ministry of Science and Higher Education for the Faculty of Chemistry of Wroclaw University of Technology.
Keywords: biomineralization, IDP, OMM-64.

MON-213
Conservativeness and features of pre-nervous serotonergic signaling system in early embryonic development

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Neurotransmitters, such as serotonin, catecholamines and acetylcholine have numerous non-neuronal functions in addition to classic one. These signaling molecules exist in eggs and early embryos of a wide variety of animal groups and are functionally active during early embryonic development, long before the appearance of the nervous system. It is suggested that the primary function of these substances was humoral regulation of the functional state of the cell, and neurotransmitter function arose secondarily in nerve cells.

Non-neuronal functions of serotonin were most intensively studied since the beginning of the researchers in this field. This biogenic amine is commonly occurring in embryos at early stages of development. Pharmacological experiments on embryos of sea urchins have shown that serotonin is functionally active during the period of cleavage and is required for cell cycle regulation and blastomere interactions. Using molecular genetic techniques, we investigated the composition of the serotonergic system of early embryos three model objects belonging to different phylogenetic groups - sea urchin *Paracentrotus lividus*, clawed frog *Xenopus* and mouse.

Enzymes of serotonin synthesis are expressed at early stages of development, and it is the neural form of tryptophan hydroxylase that is presented in early embryos. Early embryos of all three species have a membrane transporter SERT performing the uptake of serotonin from the extracellular environment to the cytoplasm. Vesicular monoamine transporter VMAT is also expressed during early development of mice and frogs that is required for the accumulation of serotonin in the excretory vesicles and further intercellular signaling. It is interesting that in the early stages of development *Vmat2* gene is expressed, which is typical for the nervous system.

Receptors are the key components of the serotonergic signaling system. In all species investigated several serotonin receptors were expressed simultaneously at early developmental stages. This may be associated with multifunctionality of serotonin at this stage of development. In the case of mice and frogs, receptors that are expressed on the early stages of development influence the same second messenger system (adenylate cyclase) in the opposite way. This fact may indicate a sensitive concentration-dependent serotonergic regulation of early development or its complex spatio-temporal organization.

Our results suggest that the mechanisms of serotonergic signaling in early embryogenesis are generally similar to those in the nerve cells. However, the multiplicity of possible mechanisms of action is one of the characteristics of pre-nervous embryonic serotonergic system.

Keywords: development, neurotransmitter, serotonin.

MON-214
Defining cell type-specific transcriptomes in the early plant embryo

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Multicellular land plants (Embryophytes) develop as a result of the continuous formation of tissue layers and organs made possible by the establishment of diverse cell types and their specification, in space and time, during embryogenesis. A fundamental difference between plants and animals is the cellular and physiological constraints that prevent migration of plant cells. Plants and animals each developed independently from unicellular ancestors and, consequently, plants depend on their own cellular and molecular mechanisms to form their multicellular body. In seed plants, embryogenesis establishes the basic body organization of an apical-basal and radial pattern while the remainder of the plant body develops through the activity of the meristems – stem cell niches in which pluripotent stem cells continuously divide to produce differentiated cells for organ formation. These stem cells are prevented from differentiation by the so-called organizer cells and, while active throughout plant life, they are first specified during embryogenesis. A key unanswered question in plant biology is how these cell types are specified in the early embryo. The embryo of the plant *Arabidopsis thaliana* is a perfect model to study these processes, as most cell divisions are highly invariable and well characterized and therefore highly predictable. During embryogenesis a distinct developmental stage can be discerned (early-globular stage) in which precursor cell types of the future root apical meristem, including the first stem cells and the organizer cell, are being specified. Recently, important insights concerning cell fates have been gained through cell-specific transcriptomics during post-embryonic development in plants. In the present study our aim has been to determine the transcriptome of the first stem cells, the organizer cell and precursors of the root apical meristem to characterize the underlying genetic network responsible for their specification during embryogenesis. To accomplish this we have adapted a genome-wide approach that allows single cell transcriptomic studies in the embryo.

Keywords: Plants, Stem cells, Transcriptomics.

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MON-215
Discovery of a novel zebrafish calponin homology-domain containing transcript using systematic search of RNA sequencing data

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Early development is a complex process which may be governed in part by gene transcripts either specific for this time in development or by specific isoforms of transcripts that continue to be present during later time points. With the introduction of massive parallel RNA sequencing, whole transcriptome investigations became possible through assembling entire transcript from overlapping sequencing reads. Zebrafish (Danio rerio) is an established vertebrate model within developmental biology research. However, the current version of the zebrafish transcriptome annotation is not complete and there are still novel transcripts to be found. We developed a bioinformatics pipeline for systematically searching massive parallel RNA sequencing data for the presence of novel transcripts, aiming at discovering novel transcripts which could be involved in early development. By using data from massive parallel RNA sequencing of early zebrafish embryos we identified a number of putative novel transcripts. Among five fragments from two putative genes chosen for proof-of-principle validation we found a calponin homology (CH)-domain containing transcript. The identified transcript differs from the previous known zebrafish transcript originating from gene located on chromosome 20. In addition, this novel zebrafish gene displays similarity with the human CLMN gene. The CH-domain is found in cytoskeletal and signal transduction proteins, including actin-binding proteins. When transiently knocking down the transcript using morfolinos early development is affected. In summary we have developed a bioinformatics pipeline for systematically searching RNA-seq data after novel transcripts and by using this pipeline we have identified and characterized a novel CH-domain containing gene expressed during early development in zebrafish.

Keywords: Bioinformatics, development, zebrafish.

MON-216
Dynamics of proteomic spectrum of amniotic fluid during different periods of intrauterine ontogenesis

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Background: Prenatal development period, which is important for ontogenesis, is connected with deep biochemical transformations in the mother-placenta-fetus system, where amniotic fluid (AF) plays an important role. The ability of this biological environment to react quickly by the modification of its composition to all changes arising in the indicated system allows to use the study of its proteomic spectrum to receive objective information about the character of gestation course and condition of a fetus.

The objective of this study is the analysis of proteomic spectrum of AF during normal and complicated pregnancies.

Methods: The investigation was performed in women with physiological pregnancy (n = 24) and placental insufficiency (PI, n = 20). AF was obtained in the 2nd trimester (23–24 weeks) by means of transabdominal amniocentesis due to medical reasons and during amniotomy in the 1st stage of labor (39–40 weeks). The proteomic analysis of AF was carried out using the two-dimensional electrophoresis (1st dimension: IPG-strip, pH 3–10, 17 cm; 2nd dimension: 8–16% polyacrylamide gel) with the subsequent silver staining of protein and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of peptides extracted from gel. Proteins were identified using the Mascot program, Swiss-Prot and NCBI data bases.

Results: The dynamics of AF proteomic spectrum in physiological pregnancy are characterized by up-regulation of 3 proteins in the 3rd trimester: transthyretin, calgranulin A and calgranulin B, which were not revealed in the 2nd trimester. During the pregnnacy complicated by PI, regardless of its term, 6 proteins are down-regulated (epidermal fatty acid-binding protein, plasma retinol-binding protein, chorionic somatomammotropin hormone, haptoglobin, peroxiredoxin-2, insulin-like growth factor-binding protein-1 (IGFBP-1)) and 5 proteins are up-regulated (zinc-α-2-glycoprotein, NKG2D ligand 2, fragment IGFBP-1, fibrinogen β chain, putative CDC37-like protein). In the 2nd trimester in case of PI the proteins, which are specific for this period of gestation, are up-regulated: hippocalcin-like protein 1 and α-1-microglobulin/bikunin precursor.

Conclusion: The determined modification of protein production can have both compensatory character aimed at the prolongation of gestation and negative consequences. The results of the present study allow to expand our understanding of the formation of AF protein spectrum during intrauterine ontogenesis and molecular mechanisms of PI development. The revealed proteins of AF difference may be used as markers of this obstetric pathology.

Keywords: Proteins; proteomic spectrum; intrauterine ontogenesis.

MON-217
Effects of in utero Di-n-hexyl phthalate and Dicyclohexyl phthalate exposure on fetal testicular development in rats

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The aim of the study is to investigate the effects of di-n-hexyl phthalate (DHP) and dicyclohexyl phthalate (DCHP) on the male reproductive development *in utero*. Pregnant rats were exposed to DHP and DCHP at doses of 0 (vehicle), 20, 100 and 500 mg/kg/day, by gavage, on gestational days (GD) 6–19. A significant decrease in the anogenital distance (AGD) of male fetuses was observed at all doses of DHP and DCHP. The AGD/cube root of body weight ratio in male fetuses was also significantly reduced compared to control group. The litters with resorption, percentage of resorption and inhibin B levels increased in treatment groups. Despite this, testosterone and MIS/AMH levels in treatment groups decreased. Although FSH and inhibin B levels of male pups exposed to DHP and DCHP increased, FSH/inhibin B ratio decreased in treatment groups. Reduced testosterone production in response to DHP and DCHP exposure appeared to be related to changes in testosterone metabolism, as shown by decreased 3β-HSD immunoexpression. The increased percentages of large Leydig clusters were observed after exposure to DHP and DCHP *in utero*. Histopathological examination of the testis on GD20 revealed changes at all doses. Relative integrated immunodensities of 3β-HSD, MIS/AMH, PCNA and AR decreased after DHP and DCHP exposures. Altered fetal Sertoli cell development and function may caused by disrupted PMC function revealed by reduced AR production in these cells in treatment groups. In conclusion, DHP and DCHP may affect on the fetal testicular development as an anti-

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androgen. AGD, litters with resorption, resorption percentages and Leydig cell clusters may also reveal that embryotoxicity of DCHP is more potent than DHP.

Keywords: Di-n-hexyl phthalate, Dicyclohexyl phthalate, Endocrine disrupters, antiandrogens, iin utero testicular development.

MON-218
Endothelial Notch activity promotes angiogenesis and osteogenesis in bone

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Blood vessel growth in the skeletal system and osteogenesis seem to be coupled, suggesting the existence of molecular crosstalk between endothelial and osteoblastic cells. Understanding the nature of the mechanisms linking angiogenesis and bone formation should be of great relevance for improved fracture healing or prevention of bone mass loss. Here we show that vascular growth in bone involves a specialized, tissue-specific form of angiogenesis. Notch signalling promotes endothelial cell proliferation and vessel growth in postnatal long bone, which is the opposite of the well-established function of Notch and its ligand Dll4 in the endothelium of other organs and tumours. Endothelial-cell-specific and inducible genetic disruption of Notch signalling in mice not only impaired bone vessel morphology and growth, but also led to reduced osteogenesis, shortening of long bones, chondrocyte defects, loss of trabeculae and decreased bone mass. On the basis of a series of genetic experiments, we conclude that skeletal defects in these mutants involved defective angiocrine release of Noggin from endothelial cells, which is positively regulated by Notch. Administration of recombinant Noggin, a secreted antagonist of bone morphogenetic proteins, restored bone growth and mineralization, chondrocyte maturation, the formation of trabeculae and osteoprogenitor numbers in endothelial-cell-specific Notch pathway mutants. These findings establish a molecular framework coupling angiogenesis, angiocrine signals and osteogenesis, which may prove significant for the development of future therapeutic applications.

Keywords: angiogenesis, bone growth, osteoprogenitors.

MON-219
Epigenetic control of L1 retrotransposition contributes to skeletal muscle cell differentiation

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Regulation, evolution and impact of repetitive elements in human genome function is largely unknown. Recent reports indicate that LINE-1 (L1) occurs in brain cells, and stem cells, contributing to neuronal plasticity and somatic diversification. Defects in their mobilization are associated with neurological disorder, as Rett syndrome or Schizophrenia. However, whether somatic L1 retrotransposition regulation could positively contribute to cellular differentiation program is still unexplored. We investigated L1 epigenetic regulation and retrotransposition activity during differentiation of human primary muscle cells, finding that skeletal myogenesis supports a MyoD-dependent and Dystrophyn-nNOS dependent activity of this class of repetitive elements that results in the acquisition of additional L1 copies in differentiated cells.

Such mechanism is impaired during differentiation of muscle cells derived by patients affected by Duchene muscular Dystrophy, being HDAC2 aberrantly recruited at L1 promoter and their transcription repressed. Notably, functional restoration of dystrophin-nNOS-HDAC2 signaling and fiber functionality by HDAC inhibitors or dystrophin re-expression by exon-skipping could restore normal L1 expression levels and CNV either in the *mdx* mice and in DMD primary muscle cells.

We further prove that impairment of L1 mobilization by reverse transcriptase pharmacological inhibition reduces the differentiation ability of normal human primary muscle cells, specifically interfering with sarcolemma and muscle fibers correct formation. In this regard, L1-RC-seq (L1-retrotransposon–capturing and sequencing) revealed muscle site specific L1 insertions in the genome, recurrent in different individuals, with an enhanced CNV during control differentiation, and not in DMD patients where transcriptome program and muscle fiber formation is impaired.

We propose that L1 repetitive elements are positive players in cellular differentiation and cell identity, and their epigenetic deregulation a key trait in muscular dystrophy manifestation.

Keywords: Epigenetics, Retrotransposons, Cell Differentiation.

MON-220
Evaluation and analysis of different distance measure in context of stabilizing gene content method of phylogenomics as source of phylogenetic analysis

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With the advancement of technology and novel cost effective sequencing methods during the recent times, the number of organisms having whole genome sequences has increased exponentially. This huge chunk of high throughput data can be used in many different ways. Besides all the others use of the high throughput data, one of the very common question is to answer correctly the evolutionary history of the organism. There are various methods for the prediction of the evolutionary tree of organisms but the method based on whole genome content is believed to be a better solution to the problem due to consideration of all the genes of the organism. Moreover, consideration of orthologs as a measure of gene content is also quite reasonable due to the presence of better and efficient algorithms for orthologs prediction. Many recent works compare the different distance measure, some well known and some not so familiar ones, on the orthologs data set which can be used as a measure of similarity between the different datasets. In the present study we have compared the different similarity measures like Jaccard similarity index, Dollo evolutionary distance, Dice, Simpson, and Hamming similarity measure.

The study was done on the ortholog dataset of fungal species having whole genome sequence taken from KEGG database. The results show that Jaccard and Dollo distance measures show the most significant results which are also in compliance with the 16S rRNA phylogeny.

Keywords: Orthologs, Phylogenomics, Phylogeny.

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MON-221
Evolution of Prdm genes in animals: insights from comparative genomics and gene expression studies

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Prdm genes encode transcription factors with a PRDI-BF1 and RIZ homology (PR) domain and a variable number of zinc finger motifs. These genes show a wide variety of functions. In particular, several *Prdm* genes, such as *Prdm1* (also known as *Blimp1*) and *Prdm14*, have been shown to have important roles in somatic pluripotent stem cells and in primordial germ cells. Other genes, such as *Prdm8*, *Prdm12*, and *Prdm13*, are expressed in specific neural populations and are required for the proper development of these neural cells. Whereas the functions of *Prdm* genes have been carefully studied in some vertebrates, especially mouse, little is known about the evolution of this gene family. We have searched for *Prdm* genes in the fully-sequenced genomes of 91 different animal species representative of all the main animal lineages. We identified a total number of more than 900 *Prdm* genes in these species, the number of *Prdm* genes per species ranging from 2 to 19 depending on the species. To better understand how the *Prdm* gene family has evolved in metazoans, we performed phylogenetic analyses using the large set of *Prdm* genes we have identified. These analyses allowed to define 14 different subfamilies of *Prdm* genes and to establish that 11 of them are ancestral to bilaterian animals. Detailed analysis allowed to define the gene duplication and gene loss events that occurred in the different animal lineages. By studying a large number of non animal genomes, we also defined the most likely evolutionary origin of this gene family. To get insight into the evolution of the functions of these genes in bilaterian animals, we cloned the full set of *Prdm* genes from the emerging model species, the annelid *Platynereis dumerilii*, a slow-evolving species that is distantly related to both vertebrates and arthropods. Expression patterns of the cloned genes will be reported. Together, our data provide new insights in the evolution of this important family of transcription factors.

Keywords: annelids, neurogenesis, stem and germ cells.

MON-222
Evolution of regulatory properties of fructose-1,6-bisphosphatase

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Muscle and liver fructose-1,6-bisphosphatase (FBPase) isozymes are present in mammalian tissues. Both isozymes catalyze the hydrolysis of fructose-1,6-bisphosphate to fructose-6-phosphate and orthophosphate in the presence of divalent cations such as Mg^{2+}, Mn^{2+} or Zn^{2+}. Both are activated by NH_4^+ and K^+ but inhibited by fructose-2,6-bisphosphate, AMP and Ca^{2+}. However, in contrast to muscle FBPase, the liver isozyme is significantly less sensitive to inhibition by AMP and Ca^{2+}, which allows the liver isozyme to play a regulatory role in gluconeogenesis. The role of muscle FBPase is more complex: the muscle isozyme particpates in glycogen synthesis from carbohydrate precursors in striated muscles, but it is also engaged in the protection of mitochondria against stress stimuli and in the regulation of cell cycle progression.

Initial research was focused on the kinetic and structural properties of the liver isozyme and its role in glucostasis. However, comparison of all known primary structures of FBPase from microorganisms and invertebrates through lower vertebrates to birds and mammals – has revealed that the muscle isozyme is an ancestral form of vertebrate FBPase and that the low sensititivity of the liver isozyme to the inhibitors has evolved as an adaptation of vertebrates to hypo- and hyperglycemia. This raised the question of which changes in amino acid residues resulted in this low susceptibility of liver FBPase toward AMP and calcium.

Our studies demonstrated that E69 is essential for the high sensitivity of muscle FBPase to inhibition by calcium ions and that a point mutation, E69Q, desensitizes the muscle isozyme toward inhibition by Ca^{2+}.

The results of our studies also showed that the N-terminus of FBPase functions in the mechanism of allosteric inhibition of the enzyme by AMP. It turned out that the N-terminal region of muscle FBPase contributes to the hight susceptibility of the muscle isozyme to AMP and regulates its interaction with aldolase .

However, differences in the N-terminal sequence between the two FBPase isozymes only partially explained the large difference in their sensitivity to AMP. Thus, we hypothesized that these differences must be related to different conformation of the two isozymes.

As a follow-up of our previous study, we have crystallized human muscle FBPase to compare its structure with that of the human liver isozyme. The structural results demonstrate that while the inactive T conformation of both isozymes is practically the same, their active R-state differs essentially. Studying the crystal structures of FBPase in the T and R form we found a unique R-to-T conversion of human muscle FBPase, which explains its high sensitivity to AMP and the lower affinity of the liver isozyme to this inhibitor.

Keywords: None.

MON-223
Evolution of spider toxin diversity in *Cheiracanthium* and *Oxyopes*

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In the course of evolution living organisms acquired diverse adaptations enabling them to occupy a variety of ecological niches. Venoms of spiders promoted these animals in hunting insects. Most arachnid venoms are based on peptide toxins composing natural combinatorial libraries, i. e. complex mixtures of several groups of homologous toxins. The most probable mechanism underlying the generation of toxin diversity is multiple gene duplications followed by rapid diversifying evolution.

An analysis of two cDNA libraries from venom glands of *Cheiracanthium punctorium* and *Oxyopes takobius* spiders was performed. Translated *O. takobius* library contains precursors of toxins belonging to several structural classes: linear cytolytic peptides, neurotoxins with the inhibitory cystine knot fold (ICK, or knottin) maintained by 3, 4 or 5 disulfides, and chimeric two-domain toxins built from linear and knottin domains. One- and two-domain knottin toxins with 4 or 8 disulfide bonds are characteristic of *C. punctorium* cDNA library.

Nonsynonymous to synonymous nucleotide substitution ratio in the cDNA sequences encoding toxins reflects the tendency to positive/negative selection acting on the toxin genes. Many animal toxins undergo positive selection. In spiders two groups of knottin neurotoxins with 3 disulfide bonds were shown to undergo positive selection. We performed selection analysis in each structural

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group using the Nei-Gojobori and Goldman-Yang methods. Surprisingly, it turned out that evolution of most *O. takobius* and *C. punctorium* toxins is driven by negative selection. Diversifying selection affects only one-domain knottins containing 3 disulfide bonds. Thus, among the considered groups some undergo fast evolution, while others are more conserved. Probably the tendency to negative selection acting on most *O. takobius* and *C. punctorium* toxins is due to several factors: the ancient evolutionary age of spiders, non-specific mechanisms of toxin action, and the relatively high molecular mass of the toxins.

This work was supported by the Program of Molecular and Cell Biology of the Russian Academy of Sciences, and by the Scholarship of the President of Russian Federation.
Keywords: cDNA library, molecular evolution, spider toxin.

MON-224
Evolutionary origin of the Mycobacterial plasmid pAL5000 replication proteins RepA and RepB

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The Mycobacterial plasmid pAL5000 has been used extensively for the genetic engineering of Mycobacteria. The replication region of this plasmid comprises two open reading frames encoding the replication proteins RepA and RepB. Replication is initiated by the binding of RepB to the origin of replication located upstream of the *repA-repB* operon. Although the essential elements have been defined the precise mechanism of pAL5000 replication is not fully understood. The objective of this study is to investigate the function as well as evolutionary origin of the pAL5000 derived replication proteins RepA and B.

Plasmids similar to pAL5000 can be found in various Actinobacteria. In all plasmids of the pAL5000 family, RepA and RepB orthologs are found. However while the RepAs are well conserved, RepBs were found to be divergent. The phylogenetic affinities of these two replication proteins were also found to be different, indicating that the *repA-repB* operon was created by random breaking and joining events. However these two Rep proteins function together as indicated by the observation that their synthesis is tightly coupled. Moreover a RepA-RepB fusion was found to be fully active in supporting replication, which further confirms that they function as a pair. The ColE2 plasmids of Gram-negative bacteria are distantly related to pAL5000. These plasmids replicate using a single replication protein (Rep). However in the C-terminal region of ColE2 Rep, a RepB like domain can be found. It appears that the ColE2 Rep must have evolved through the fusion of pAL5000 like RepA and RepB domains.

RepB is a single domain Helix-turn-helix (HTH) family protein which shares considerable homology with region 4 of Extra Cytoplasmic Function (ECF) family of Sigma factors. The involvement of a domain, usually associated with transcription, in a replicative process indicates that early in evolution replication and transcription factors may have utilized same or similar DNA binding domain(s) for sequence specific recognition of DNA. While RepB, the origin binding factor, is a HTH family protein, RepA is related to primase-polymerases (prim-pol) class of ancient proteins which are known to function both as primases as well as polymerases. Previously RepA of ColE2 has been demonstrated to have primase activity. In our investigation it has been shown for the first time, that RepA of pAL5000 which is a ColE2 Rep homolog, can act as a DNA polymerase. Both RepA and RepB therefore represent ancient replication proteins and hence it is proposed that the pAL5000 replication system is of ancient origin.

Keywords: Plasmids, replication proteins, evolution.

MON-226
Extensive mouse regulatory DNA landscapes reveal global principles guiding cis-regulatory evolution

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To gain a detailed perspective on the evolutionary dynamics of regulatory DNA, we performed DNase I hypersensitivity mapping in 45 mouse cell and primary tissue types, and systematically compared these with human DHSs from orthologous cell and tissue types. While we identified a small core mammalian regulon comprising DHSs with preserved cell activity patterns that densely encode recognition sites for most known developmental and cell lineage regulators, the mouse and human genome have undergone extensive cis-regulatory rewiring via both innovation and functional repurposing (cell lineage switching) of DHSs driven by acquisition and interconversion of recognition sites for regulators of alternative cell fates. However, despite the weak conservation of individual recognition elements, the density of recognition sequences for cell-specifying master regulators is nearly identical in orthologous tissues. This finding suggests that the evolution of the cis-regulatory compartment is guided directly by a conserved program of spatiotemporal transcription factor activity patterns and indicates that evolutionary selection is preferentially occurring on the overall composition of of the cis-regulatory compartment versus individual cis-regulatory elements. Collectively, these data indicate that the limited regulatory DNA repertoire encompassed by mouse-human shared DHSs with preserved tissue selectivity may function as a scaffold around which diverse potential regulatory programs with convergent outcomes can be constructed with relative facility.

Keywords: chromatin organization, evolution, regulatory DNA.

MON-227
Genes involved in horizontal gene transfer across different environments

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Horizontal gene transfer (HGT; also named lateral gene transfer) enables the acquisition of new genes between closely or distantly related organisms and it occurs within and between all three domains of life (Eukarya, Bacteria and Archaea). This process can help microbes to adapt to different environments by allowing molecular innovations to be traded within ecological niches. The extent of HGT in natural populations from different environments remains largely unknown and existing data are mostly limited to particular habitats. We compared the extent of HGT from various natural populations using available metagenomic data. Since metagenomes represent total DNA from all microbes present in particular environment, they represent genetic profile of particular sample much better than traditional culture-dependent analyses (it is estimated that over 99% of bacteria living on Earth today cannot be grown in laboratory). We analyzed frequency and diversity of genes involved in HGT mechanisms in metagenomes from wide variety of environments: water (salt and fresh), soil, drainages, gut content, etc. MG-RAST database was used as a major source of

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different metagenomes and the abundance of total HGT as well as the abundance of six HGT subcategories: gene transfer agents (GTA), pathogenicity islands (PAIs), phages and prophages (PP), transposable elements (TE), plasmid related functions (PRF) and integrons (I), were determined. Finally, a detailed statistics analysis was used to present the obtained differences in HGT gene content in selected various environments.

Keywords: Horizontal gene transfer (HGT); metagenome, environment.

MON-228
Genetical analysis of human norovirus in underground water in South Korea

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Noroviruses (NoV) are important pathogenic viruses that cause foodborne and waterborne gastroenteritis in human. From January to September 2013, we obtained water samples from 550 geological locations in South Korea. Six out of five hundred fifty water samples showed positive result for human NoV by reverse transcription-polymerase chain reaction (RT-PCR). The amplified genomes of ORF 1-2 junction region of NoV from 6 positive samples were then sequenced, genotyped and analysed for sequence alignmnet with reference NoV strains. Approximately 1% (6/550) of the water samples was positive for NoV GI and GII. The genotypic distribution of the 6 NoV strains was as follow: GII-4, 50% (n = 3); GII-11, 16.7% (n = 1); GII-14, 16.7% (n = 1); GII-17, 16.7% (n = 1). The obtained genetic data can be used for prevention and control of foodborne NoV outbreak.

Keywords: foodborne, Norovirus, underground water.

MON-229
Glycans – the third revolution in evolution

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The development and maintenance of a complex organism composed of trillions of cells is an extremely complex task. At the molecular level every process requires a specific molecular structures to perform it, thus it is difficult to imagine how less than tenfold increase in the number of genes between simple bacteria and higher eukaryotes enabled this quantum leap in complexity. It is generally assumed that the appearance of self-replicating nucleic acids (the first revolution in evolution) provided the basis for the development of early life. Nucleic acids then recruited amino acids to create proteins, which are still the main effectors of life at the cellular level (the second revolution). However, the integration of different cells into a complex multicellular organism required an additional layer of complexity. The invention of protein glycosylation, through its inherent ability to create novel structures without the need to alter genetic information, enabled the entire new level of complexity. Contrary to proteins and nucleic acids, which are made from a direct DNA template, glycans are product of a complex biosynthetic pathway affected by hundreds of genetic and environmental factors. Therefore glycans enable adaptive response to environmental changes and, unlike other epiproteomic modifications, which act as off/on switches, significantly contribute to protein structure and functions. The importance of glycosylation is evident from the fact that nearly all proteins invented after the appearance of multicellular life are composed of both polypeptide and glycan parts.

Keywords: evolution, Glycosylation.

MON-230
HAND2 regulates the transcriptional circuits that compartmentalize the early mouse limb bud

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The genetic interactions and networks that govern limb bud patterning and outgrowth have been studied extensively, while much less is known about the relevant trans-acting factors, target genes and *cis*-regulatory modules. Using a mouse allele in which we have inserted a 3xFLAG epitope-tag into the endogenous HAND2 protein, we identify the genome-wide range of HAND2-binding regions in mouse embryos. By combining this approach with genetic analysis, we uncover a network of HAND2 target regulators that define a proximal limb compartment and are required for morphogenesis of proximal skeletal elements. Simultaneously, HAND2 orchestrates the transcriptional interactions that control the establishment of an anterior and posterior limb bud compartment. While HAND2 directly regulates *Gli3* transcription, is also needed to activate the TBX3 transcriptional repressor, which is essential to position the posterior *Gli3* boundary. This combination of cross-regulation with transcriptional relay provides HAND2-controlled circuits with robustness in defining limb bud compartments prior to activation of SHH signaling.

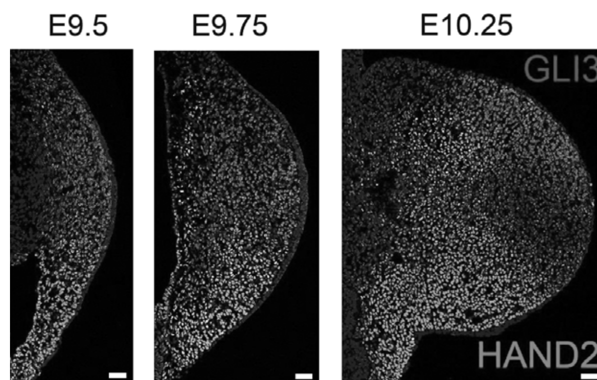

Fig. 1.

Co-immunolocalization of HAND2 (green) and GLI3 (red) in wild-type forelimb buds at E9.5 (25–26 somites), E9.75 (28–29 somites) and E10.25 (33–34 somites). Scale bars: 50 μm.

Keywords: ChIP-seq, Hand2, limb bud.

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MON-233
Identification and structure-function analyses of an Elk-1 antecedent in a primitive triploblastic deuterostome

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Gene expansion in early metazoans gave rise to multiple ETS paralogues with overlapping or indiscriminate biological functions. Elk-1, one of three mammalian ternary complex factors, is a well-conserved, ETS domain-containing transcriptional regulator of mitogen-responsive genes. Nonetheless, the *elk-1* gene could be deleted from the mouse genome apparently without adverse effect.

To gain insight into the biological role of Elk-1 through evolutionary conservation, we identified potential antecedent Elk-1 genes in extant early metazoans using database searches and sequence alignments. We then performed biochemical studies to determine whether sequences present in a putative Elk protein of the acorn worm *Saccoglossus kowalewskii*, a primitive hemichordate, were functionally orthologous to characteristic domains of human Elk-1. Our findings link the appearance of a structurally and functionally recognisable Elk-1 to the advent of triploblastic metazoans, implying an ancestral role for Elk-1 during the specification of mesoderm.

Keywords: ETS protein, mesoderm, transcription factor.

MON-234
In vitro reconstitution of a P granule-like phase

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It is well known that cells use membranes to spatially separate biological activities in specialized compartments e.g. nucleus, endoplasmic reticulum, Golgi bodies, mitochondria, lysosomes. On the other hand, compartmentalization is also possible in the absence of a membrane separating the compartment from its surroundings. Such membrane-less compartmentalization is observed in cellular structures containing both proteins and RNA, for instance nucleolus, Cajal bodies, P bodies, promyelocytic leukemia nuclear bodies, stress granules. One such membrane-less compartment in the germ-line cytoplasm of the nematode *Caenorhabditis elegans* is P granule. RNA and more than forty proteins concentrate in P granules. Deficiencies or mutations of P granule proteins result in problems with germ-line development and sterility. The molecular mechanisms of how P granules facilitate normal germ-line development remain remarkably unclear. Further, insight into how compartmentalization occurs and sustains in the absence of membrane is lacking. In order to address these issues, we are attempting to reconstitute *in vitro* a P granule-like compartment from purified components. We will present our progress in this direction.

Keywords: *C. elegans*, P granules, phase separation.

MON-235
Intrinsically disordered regions in calponin-like Chd64 from *Tribolium castaneum*

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20-hydroxyecdysone (20E) and juvenile hormone (JH) signalling pathways interact to mediate insect development but the mechanism of their interaction is poorly understood. Recently, two proteins, a calponin-like Chd64 and an immunophilin FKBP39 have been found to play a pivotal role in cross linking these two signalling pathways. GST pull–down and yeast two-hybrid assays revealed that Chd64 and FKBP39 can interact with each other and some nuclear proteins including proteins forming functional receptor for 20E: EcR and Usp and probable receptor for JH a Met protein. Importantly, Chd64 and FKBP39 were shown to bind JH response element (JHRE) in *Drosophila melanogaster*. Nevertheless, the molecular bases of the multiple interactions of the Chd64 and the FKBP39 with various partners remain unknown. We expect to elucidate the function of the Chd64by exploring its biochemical and biophysical properties. Researches on Chd64 from *D. melanogaster* are already carried out in our laboratory and we decided to extend them to a more generic arthropod *Tribolium castaneum. In silico* analyses predicted the existence of intrinsically disordered regions (IDRs) on both termini of the Chd64. Next, a protocol for overexpression and purification of Chd64 had been developed. Obtained recombinant was a subject for intensive biochemical and biophysical studies which aimed to reveal its structural organization. Hydrodynamic properties determined by means of analytical size-exclusion chromatography and analytical ultracentrifugation revealed the Chd64 has elongated, asymmetrical shape and it was further confirmed by small angle X-ray scattering (SAXS). The location of disordered regions was mapped by hydrogen deuterium exchange mass spectrometry (HDX MS). This technique reveled that Chd64 possesses two IDRs: one on the N- and one on the C- terminus, whereas the core of the protein is globular. Since disordered tails are often involved in diverse interactions, it is highly possible that in Chd64 they serve as platforms for multiple interactions with various partners and constitute the foundation for their regulatory function.

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Keywords: None.

MON-236
Investigating Prrx1 cis-regulation during limb development

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Paired related homeobox 1 gene (*Prrx1*) encodes for a transcriptional coactivator critical for skeletal development, particularly in the limbs, craniofacial region and the inner ear. An enhancer element located upstream of *Prrx1* was shown to drive the expression of the reporter gene in the limb mesenchyme. The replacement of the mouse enhancer by the orthologous region from bats caused the elongation of mouse forelimbs, suggesting that variations in *Prrx1* regulation may play a role in the evolu-

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tionary differences between the mouse and the bat limbs. However, this enhancer alone does not account for the entire *Prrx1* expression pattern and little more is known about its regulation. To decipher *Prrx1 cis*-regulation, we cloned individual enhancer candidates based on limb-specific p300 sites and histone modification marks upstream of a *lacZ* reporter gene driven by a minimal β-globin promoter. We plan to improve the existing transgenic reporter assay in the mouse by using *phiC* integrase-mediated site-specific integration at the *Hipp11* locus. Site-specific integration eliminates variations arising from positional effects and transgene copy number. In parallel, we will examine large genomic regions by testing *lacZ*-tagged mouse- and bat-derived bacterial artificial chromosomes (BACs) within the *Prrx1* topological domain. Additionally, we have access to DNA from multiple individuals of two related bat species, the Greater and the Lesser mouse-eared bats (*Myotis myotis* and *Myotis blythii oxygnathus*). Taking advantage of the available genomic data on mouse fore- and hindlimb-specific active chromatin marks and transcription factor binding profiles, we will perform deep sequencing of the homologous regions in bat to identify sequence differences between mouse and bat in putative limb regulators. We will carry out further transgenic reporter assays on limb enhancers showing major sequence dissimilarities between bats and mice, especially those near *Prrx1*, to determine if such mutations lead to expression variations. In summary, the results of transgenic experiments will shed light on the *cis*-regulation of *Prrx1*, while the data obtained from the deep sequencing may indicate how such differences could have evolved. This will in turn open a way to better understanding of how *cis*-regulation of genes is achieved and modulated during the evolution of various morphological forms.

Keywords: bats, limb development, Prrx1.

MON-237
Lauryl-poly-L-lysine: a new antimicrobial agent?

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The development of antibioresistance is a major worldwide health problem. The antimicrobial peptides are defined as cationic peptides, generally described by a substantial portion of hydrophobic residues. Here, we describe new tools, whose mechanisms of action differ from current antibiotics.

Thanks to Gemac's know-how in chemistry, our team has synthesized an active product with different satured and insatured fatty acid (FA) covalently linked to a polypeptide, Poly-L-lysine (PLL). These PLL compounds were studied by using specific analytical methods. The linkage of FA to a carrier is largely reported to increase the antibacterial effect of the linked FA, in comparison with free FA, free PLL or a mixture of both (FA free + PLL free). The compounds from C6 to C18 FA linked to PLL were assessed in vitro to evaluate their antibacterial activity. Some FA-PLLs showed a good ability to fight multiresistant bacteria. This bacterial evaluation was shown an optimum compound, Lauryl-PLL. The specificity of linkage lauric acid to the epsilon-NH2 lysyl of the PLL was studied in vitro with another carrier, such as poly-L-ornithine. A screening of Lauryl-PLL against a broad variety of bacteria was realized with Lauryl-PLL, in respect of clinical strains from patients. The determination of the minimum inhibitory concentration of Lauryl-PLL were 250 microM (concentration in small molecules linked) for Staphyloccocus aureus, 125 microM Pseudomonas aeruginosa, and 1000 microM for Escherichia coli. The kinetic activity of Lauryl-PLL was allowed highlight this bactericidal activity against S. aureus and P. aeruginosa strains. This product was found to be the most active product against Gram-positive bacteria, such as S. aureus and Streptoccocus pneumoniae, Gram-negative bacteria, E. coli and P. aeruginosa.

Individual linkage to PLL offers significant advantages, among other aspects, for stabilizing the linked fatty acids. Regarding the hydrophobic part of PLL compounds, the activities of PLL compounds are considered to be more efficacious against Gram-positive than against Gram-negative bacteria. The Lauryl-PLL compound, having an amphiphilic character, might show a better ability to destabilize the bacterial cell wall. Lauryl PLL, which is more hydrophobic than free PLL, could increase interactions with bacterial membranes. According to the literature, the antimicrobial cationic peptides interact with the negatively charged cell membranes of bacteria.

Thus, the Lauryl-PLL compound is a new and promising antimicrobial agent. The treatment of these strains with a very low quantity of product is devoid of undesirable side effects. This strategy opens new perspectives in anti-infective therapeutic strategies.

Keywords: biochemistry, fatty acids, polypeptide conjugated.

MON-238
Lineage tracing demonstrates multipotency of premigratory and migratory neural crest cells in vivo

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The neural crest (NC) belongs to the lineages with the broadest developmental potential, generating cell types as diverse as peripheral neurons, myelinating Schwann cells, and pigment cells. However, it is highly controversial whether *in vivo* this diversity is achieved by lineage segregation from multipotent mother cells or by differentiation of distinct lineage-restricted progenitors coexisting in the dorsal neural tube (dNT). Here, we performed *in vivo* fate mapping of single trunk NC cells both at pre-migratory and migratory stages using the *R26R-Confetti* mouse model. We combined quantitative analysis with mathematical evaluation to show that the NC population consists of only few fate-restricted cells, while the majority of NC cells are multipotent. Moreover, multipotency is maintained in migratory NC cells even after their emergence from the NT. Finally, our findings suggest the existence of multipotent neural crest stem cells (NCSCs) *in vivo*.

Keywords: Confetti mouse, Lineage tracing, Neural Crest Cells.

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MON-239
LRRC1 and SCRIB, two polarity genes from the LAP family, genetically interact during embryogenesis

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This work is focused on the functional study of LAP (LRR And PDZ) scaffold proteins which form an evolutionary conserved family involved in cell polarity. Among them, we are especially interested in Scrib functions. We have contributed to highlight Scrib role in the vesicular recycling pathway as well as in cell migration both involving interactions and regulation of the bPIX/GIT1 complex (Audebert *et al.* 2004; Nola *et al.* 2008). We cloned an atypical LAP member: *Lano* (LRR and no PDZ) / *LRRC1* (Saito *et al.* 2001). Phylogeny and characterization studies have demonstrated that *Lano* has an expression restricted to vertebrates and is a *Scrib* paralogue (Santoni *et al.* 2002).

During *Drosophila* development, *Scribble* (the invertebrate *Scrib* and *Lano* orthologue) inactivation leads to profound defects in the apico-basal (AB) cell polarity associated to uncontrolled cell proliferation (Bilder *et al.* 2000). Interestingly, this phenotype was partially rescued by the expression of a Scribble deletion mutant closely related to Lano (Zeitler *et al.* 2004), suggesting that Lano might be involved in the regulation of the AB cell polarity during the vertebrates development. In contrast to *Drosophila*, *Scrib* mouse invalidation does not affect AB cell polarity but gives rise to a massive defect in the neural tube closure linked to a disruption in convergence extension and planar cell polarity (Murdoch *et al.* 2001). This discrepancy leads us to investigate the function of its paralogue, *Lano*. For this purpose, we engineered mouse models invalidated either for *Lano* or for *Lano* and *Scrib* to evaluate a possible genetic interaction between them.

Keywords: Polarity, Embryogenesis genetic interaction.

MON-240
Mammalian cells, differentiated tissue cells and microorganisms renewal

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Our research interests encompass cell engineering, tissue engineering and metabolic engineering also Bio Banking (in vitro, in vivo, in situ, ex situ, in farm) and Biopreservation. The emphasis is on the application of systems analysis to biochemical and cellular systems and on the incorporation of physiological insight into the quantitative modeling of biological reactions. The systems employed include mammalian cells, differentiated tissue cells and microorganisms. Current research efforts emphasize employing genomic and proteomic tools in those research projects and exploring novel modeling approaches for quantitative description of cellular processes. A research project on mammalian cell culture focuses on the relationship between physiological regulation and global gene expression. Our research has allowed us to control Energy metabolism to reduce waste metabolite accumulation and to increase the productivity and Product quality. Currently, genomic and proteomic tools are used to unveil the control of cellular structure and physiological characteristics important to process. Metabolomics requires the unbiased identification and quantification of all of the metabolites present in a specific biological sample (from an organism or in vitro). Metabolites are generally labile species, by their nature are chemically very diverse, and often present in a wide dynamic range. For analysis of mRNA and proteins one 'only' needs to know the genome sequence of the organism and exploit thisinformation using nucleic acid hybridization or protein separation followed by MS. We tested and validated four different mathematical models of RNA interference by quantitatively fitting models'parameters to best capture the in vitro experimental data. We show that a simple model is the most efficient way to model RNA interference. Our experimental and modeling findings clearly show that the RNAi-mediated degradation of mRNA is subject to saturation effects. Work on tissue engineering encompasses stem cell renewal and the development of adult and embryonic stem cells to liver cells. Focuses are on the in vitro formation of liver tissue-like spheroids from stem cell derives liver cells and on the regulation of stem cell renewal and differentiation.

Keywords: None.

MON-241
Mitochondrial DNA lineage, genetic diversity and population structure of two pre-hispanic Maya populations

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The Mayan civilization began to settle in the Yucatan area of what is now Mexico. The maximum splendor of this civilization was during the Classic period and had the maximal expansion at the beginning of Postclassical period. The population from the archeological site El Rey Quintana Roo became a relevant city between 1300 to 1550 C.E. and Palenque is an ancient Maya city located at the base of the northern of Chiapas. Palenque was rich natural resources allowed this site a long occupation from the 200 to 900 CE. Because the importance of this civilization we study, maternal lineage, genetic diversity and population structure through the analysis of the sequences comprising the Hypervariable Region I of the mtDNA. The maternal lineage of Mayan pre-Hispanic populations showed the haplogroups C, D, and A with a frequency of 57.1%, 28.6% and 14.3% respectively. These results are different from those reported for populations of ancient Maya from Copan, Honduras that showed frequencies of 89% and 11% for haplogroups C and D respectively. Studies of genetic diversity index and neutrality test, suggested that the population from archeological site El Rey, Quintana Roo was a stable population due to the similarity of the values $\theta\pi$ and θs, and the Tajima D value of zero. In addition, we found significantly negative Fu's Fs values which can be interpreted as a signal of expansion. The pre-Hispanic population from Palenque Chiapas showed no signal of expansion. The phylogenetic analysis by network base on sequences from the ancient populations and contemporary sequences from the NCBI database showed the relationship among these populations and the contemporary Maya populations. In conclusion, we are suggesting that the Maya population pertain to the same culture but have a different genetic origin. We will enrich this study increasing the number of samples and comparing the sequences with those of contemporary populations.

Keywords: PRE-HISPANIC MAYA, Haplogroup, mitochondrial DNA.

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MON-242
Molecular analysis of beta amyrin 11-oxidase (Cytochrome P450 88D6) among *Glycyrrhiza* species grown in Turkey

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The genus *Glycyrrhiza* L. (Fabaceae) is represented with about 20 taxa worldwide and with six species in Turkey. *Glycyrrhiza* species have been used as medicinal plants and natural sweetener for many centuries. *Glycyrrhiza* extracts have been reported to have antimicrobial, antibacterial, antiviral, anticoagulant, hepatoprotective and antidiabetic activities. Glycyrrhizin which is a triterpene saponin is one of the major compounds of *Glycyrrhiza* roots and rhizomes. Glycyrrhizin is about 50 to 100 times sweeter than table sugar. Because of the economical value of glycyrrhizin, genes having key roles in glycyrrhizin biosynthesis are very important. The aim of this study was to conduct molecular characterization of Beta amyrin 11-oxydase which has a key function in glycyrrhizin production. With this respect, genomic structure along with intron and exon polymorphism of Beta amyrin 11-oxidase among *Glycyrrhiza* species grown in Turkey were determined. Studied specimens were collected from different localities in Turkey between 2009 and 2013. Beta amyrin oxidase fragments from genomic DNA and cDNA samples were amplified via nested primers designed for this purpose. The amplified fragments were sequenced using same primers used for amplification. Alignment of the cDNA and DNA sequences was achieved using BioEdit program. The phylogenetic trees were also constructed with the same software. According to molecular analysis, *Glycyrrhiza* taxa were divided into two groups. The first group included *Glycyrrhiza asymmetrica* and *G. flavescens*. The other group included *G. glabra*, *G. echinata* and *G. aspera*. In conclusion, the molecular analysis of beta amyrin 11-oxidase revealed that the gene has polymorphic intron along with polymorphic exons which displayed a correlation with the glycyrrhizin properties of *Glycyrrhiza* taxa. These results can be utilized to device beta amyrin 11-oxydase as a genetic marker to differentiate among *Glycyrrhiza* taxa for their glycyrrhizin production potentials.

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Keywords: Glycyrrhiza, Beta amyrin 11-oxidase, glycyrrhizin.

MON-243
Molecular composition of the nucleolus-associated bodies in mouse fully-grown oocytes: extended cytochemical, immunocytochemical and fish analysis of their proteins and RNAs

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It is well known that fully-grown mammalian oocytes, instead of typical nucleoli, contain prominent, but structurally homogenous bodies called 'nucleolus-like bodies' (NLBs). NLBs accumulate a vast amount of material, but their biochemical composition and thus functions still remain undefined. To shed light on these problems we have devised an assay to detect internal oocyte proteins with fluorescein-5-isothiocyanate (FITC) and applied two fluorescent RNA-binding dyes, acridine orange and pyronin Y, to examine whether NLBs accumulate RNAs. Our results unequivocally show that similar to typical nucleoli, proteins and RNAs are major constituents of functionally active (or non-surrounded) NLBs as well as of functionally silent (or surrounded) NLBs. We have also shown, by exposure of fixed oocytes to a mild proteinase K digestion, that the interior of NLBs of both types sequesters nucleolar proteins, which are involved in all major steps of ribosome biogenesis, including rDNA transcription (UBF), early pre-rRNA processing (fibrillarin), late rRNA processing (NPM1/nucleophosmin/B23, nucleolin/C23) and ribosome assembly (RPL26) but neither of the nuclear proteins tested, including SC35, NOBOX, topoisomerase II, HP1α, and H3. In order to learn whether NLBs accumulate ribosomal RNAs (rRNAs), we applied fluorescence *in situ* hybridization technique and antisense FAM-conjugated oligonucleotide probes to 45S pre-rRNA, 18S and 28S rRNAs. The results show that, in contrast to somatic-like nucleoli of preantral oocytes, mature and immature rRNAs are hardly detectable within NLBs of fully-grown oocytes. These observations can be explained either by migration of rRNAs from transforming nucleoli to other oocyte locations (e.g., to cytoplasmic lattices, Monti et al., 2013) or by additional compactization of rRNA within NLBs that makes it inaccessible to the hybridization probes. Overall, our study suggests several novel cytochemical and immunocytochemical approaches that allow researchers to examine composition of 'nucleoli' in fully-grown mammalian oocytes. It also provides the first evidence in favor of an NLB role in the storage of major nucleolar proteins that may explain the requirement of the NLB material for the initial stages of early embryo development in mammals.

Keywords: development, mammals, oocytes.

MON-244
Molecular phylogenetic and karyomorphological data about some Turkish *Teucrium* (Lamiaceae) taxa

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Teucrium L. genus belongs to Lamiaceae family (subfamily Ajugoideae) and is one of the largest genera at this family. *Teucrium* has about 260 species at all over the world and 250 species of them distributes around the Mediterranean basin.

Chromosome cytology is important when attempting to evaluate relationships and deduce phylogenetic sequences in angiosperms. The use of karyomorphological and karyosymmetrical parameters as characters for the reconstruction of phylogenetic relationship is valid. These parameters include chromosomal characters such as chromosome number, ploidy level and karyologic characters such as total haploid length of the chromosomes and satellite situation. Also karyotype symmetry indices such as A index, A1 and A2 indices which derive from karyomorphology of species are important characters for reconstruction of phylogenetic relationships too. In addition, DNA sequence comparison has been using to determine phylogenetic relationships of the organisms. However, using of DNA sequences is more controversial than using morphological characters. On the other hand, the accuracy of the molecular data can't be ignored. Using different gene regions (nrDNA, cpDNA, mtDNA) are facilitating the identification of the phylogenetic relationships.

In this study, some trees created using DNA data and karyomorphological data. The main purpose of this study is to ascertain molecular phylogeny (using DNA datas) and karyomorphological relationship within some *Teucrium* members using different trees. *Teucrium* genus has 47 taxa growing in Turkey and 12 of them were utilized for molecular phylogenetic and karyo-

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morphological trees. trnL-F gene region (cpDNA) were used for molecular data and sequences were processed using BioEdit and Clustal W. Phylogenetic tree was obtained utilizing MEGA 6.0. In addition, six important quantitative traits which are about karyomorphology were used for cluster analysis. All data were processed using NTSYS-pc Version 2.10. The standardized matrix was processed using the Interval Data of Similarity subroutine to obtain the EUCLID matrix. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering method of the Clustering subroutine SAHN was used to construct the dendrogram in operational taxonomic units. Consequently, molecular phylogenetic and karyomorphological trees basically support each other. But as trnL-F trees have about 820 characters (820 nucleotides) karyomorphological trees have six important characters. Improving to these characters is very important for the next studies.

Keywords: Karyomorphology, Teucrium, trnL-F.

MON-245
Network inference of serial gene regulation during *D. melanogaster* AP pattern formation

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In systems biology field, revealing the genetic control system during embryogenesis is one of important tasks, and various factors are considered to be regulators of serial events during embryonic development. To elucidate the mechanism of those developmental events, the regulatory relationships between the regulatory factors and specific expressed genes behind the phenomenon should be uncovered.

In this study, we applied one statistical method based on Structural Equation Modeling (SEM), combined with factor analysis, to expression profiles which were measured in embryogenesis. Since SEM can include not only the observed variables but the latent variables within the constructed network model, we can infer the causal relationships between genes and regulatory factors. Furthermore, we improved a new method by combination of cross-correlation and partial correlation to construct an initial model for SEM calculation. We applied our approach to estimate the regulatory network for Antero-Posterior (AP) pattern formation in *D.melanogaster* embryogenesis. In the inferred model, specific transcription factor genes were regulated by not only the other genes' expressions, but also the estimated factors. Since the each factor regulated the same type of genes, those factors were considered to be involved in maternal effects or spatial morphogen distributions. The interpretation of the inferred network model allowed us to reveal the regulatory mechanism for the patterning along the head to tail axis in *D.melanogaster*.

Keywords: gene expression regulation, network analysis, Structural Equation Modelling.

MON-246
Notch and retinoic acid signaling are required for the spatiotemporal distribution of prune2 mRNA in the zebrafish embryonic development

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PRUNE2 is identified as a susceptible gene for Alzheimer's disease. Isoforms of Prune2 regulate various cellular processes, such as neuronal cell differentiation and synaptogenesis. Although *prune2* is known to be expressed in the murine brain, its detailed expression pattern along embryonic development remains to be defined. Here we showed expression profiles of *prune2* transcripts in the zebrafish embryos. *prune2* exhibits low expression level in the early embryogenesis and in later stage embryos, larvae and adult zebrafish brain whereas it is predominantly expressed in the telencephalon, epiphysis cluster, nucleus of the tract of the post optic commissuare, spinal cord neurons, cerebellum, tegamentum, anterior lateral line ganglion, posterior lateral line ganglion and in the rhombomeres 2 to 5. In order to study details expression pattern, we performed two color whole-mount *in situ* hybridization (WISH) with a postmitotic neuron specific marker, *huC* to reveal that *prune2* is expressed in the post mitotic neurons. In addition, we demonstrated that *prune2* transcripts are significantly increased in the Notch signaling homozygous mutant, *mib* (mib^{ta52b}) suggesting that Notch signaling governs the transcription of *prune2* in the zebrafish embryos. Interestingly retinoic acid (RA) signaling modulates the *prune2* expression pattern in the zebrafish embryos. Currently we are addressing the functional roles of Prune2 in the zebrafish neurogenesis.

Keywords: prune2, CNS, post mitotic neurons, Notch, Retinoic acid.

MON-248
Optimal growth schedule of holometabolous insects

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In holometabolous insects, adult organs develop as imaginal discs within the growing larvae. Once larvae pass the critical size, juvenile hormone (JH) titers decline and imaginal discs begin to grow rapidly. The subsequent rise in ecdysteroid titers causes the larva to stop feeding and wander away from its food to find a place to metamorphose. The period between attainment of the critical size and ecdysteroid secretion is called the terminal growth period (TGP). Although all holometabolous insects are thought to have the critical size for metamorphosis, the mechanisms for nutritional regulation of the critical size and the TGP differ between species. For example, in Manduca sexta, low nutrition reduces the critical size and has no effect on the duration of the TGP, whereas in Drosophila melanogaster, low nutrition lengthens the duration of the TGP and has no effect on the critical size.

To study the ecological causes of the diversity in the mechanisms for nutritional regulation, we develop a resource allocation model of a holometabolous insect and analyze it with the Pontryagin's maximum principle with free-final-time. A typical optimal growth schedule is biphasic (the so-called bang-bang control): all nutrition is devoted to growth of a larval body until some switching time, after which point all nutrition is devoted to growth of an imaginal disc until the end of development. Regarding the larval body size at the switching time and the second phase as 'the critical size' and 'the TGP', we investigate how these values depend on nutrition for different fitness functions.

Considering that the fitness is proportional to the size of an imaginal disc at the end of development, the combination of a constant TGP and a nutrition-dependent critical size is optimal. On the other hand, the combination of a nutrition-dependent TGP and a constant critical size is optimal if the time to obtain a mature imaginal disc (fixed size) is minimized. These optimal strategies are in accord with the pattern observed for Manduca sexta and Drosophila melanogaster, respectively.

Keywords: Growth regulation, Holometabolous insects, Mathematical modeling.

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MON-249
Origin of 35delG mutation in the Belarus population: Where did it come from?

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Deletion *35delG* in GJB2 gene is the main cause of congenital hearing loss(HL) in many populations worldwide. It's account up to 70–90% of all GJB2 mutation among the European population. *35delG* is recessive leading to HL in homozygous state. There are regional differences in *35delG* mutation carrier rate even within Europe, where the prevalence is higher in southern (Greece 3.54%, France 3.35%, Italy 4%) and lower in northern European countries (except Estonia 4% and Belarus 5,6%). Moreover, the carrier frequency in Belarus is one of the highest in Europe. The average frequency of *35delG* among the European countries is 2%. The origin and spread of *35delG* among Europeans is attributed to the 'founder effect' and possible heterozygote advantage. It is considered that the *35delG* mutation appeared about 14 000 years ago in the territory of ancient Greece and then spread to Europe.

The goal of our study was the reconstruction of the possible ancestor haplotype linked to the *35delG* mutation among Belarus patients.

We genotyped 96 Belarus patients with HL homozygous for the *35delG* mutation, as well as 144 residents of the country with normal hearing for 4 single nucleotide polymorphisms (SNP) and 2 microsatellite markers (STR) inside or flanking the GJB2 (D13S175 and D13S141).

According to the obtained results, the most frequent 2 STR and 1 SNP variants in studied patient cohort is: 105 (90%) for the marker D13S175, 127 (76%) for the marker D13S141 and (96%) for SNP 1. The frequency of other STR and SNP variants among Belarusian patients is insignificant. The most wide–spread haplotype linked to the *35delG* mutation among Belarus patients is the following: 105–127– SNP 1 (D13S175– D13S141– SNP 1). This haplotype is also frequent among other European populations, as well as in Greece– estimated «homeland» of *35delG* mutation. The data obtained shows that *35delG* mutation was introduced on the territory of Belarus with Neolithic migration from Mediterranean area and spread with high frequency throughout the territory of the country. The hypothesis about the common founder of GJB2 mutation for Europeans was confirmed.

Keywords: 35delG mutation, Belarus, haplotype.

MON-250
Ovarian follicle culture in 2D and 3D systems

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Search for methods to maintain and restore the ovarian reserve is one of the most important directions of research in the field of experimental embryology due to the fact that the occurence of a cancer in women of reproductive age and girls who have not reached puberty increases. Currently systems and methods of the ovarian tissue and the individual follicles culture in order to obtain mature follicles competent for fertilization and further development are evolved actively. We have compared the results of primary ovarian follicles obtained from the ovaries of C57BL/6J × CBA/J F1 hybrid mice, cultivation in 2D and 3D-systems during 12–14 days. To create the 3D-environment follicles have been encapsulated in alginate hydrogel droplets of different densities or cultured in the 'hanging drop' (both individually and co-culturing with stromal cells of the cortical layer of the ovary). The latter model have been used to study the influence of the presence of ovarian stromal cells in the cultural system on the growth of primary follicles. During the ovarian follicle cultivation on the adhesive Petri dishes the abnormality of follicle structure took place due to theca cells eviction and following migration of granulosa cells on the surface of the theca cells. Follicle structure abnormality has led to a loss of contacts between oocyte and corona radiata cells and the further death of oocyte. Thus, the follicle culture in 2D-system can not be used to obtain oocytes competent for further *in vitro* fertilization. In 3D-culture system an increase of follicle diameter was observed, sometimes *in vitro* ovulation took place, but no antral cavity formation has been observed. In both cultivation systems there were 3 groups of ovulated oocytes: MII – the polar body fully formed, AI – early stage of the polar body formation, MI – no polar body. It was turned out that during *in vivo* ovulation all three types of oocytes were observed. Oocytes from the culture had smaller diameter and more grained cytoplasm than oocytes in natural cycle. Cultivation of the follicles during 7–14 days did not influence on the follicles viability. Staining with the fluorescent dyes that detect live and dead cells showed that dead cells were only in peripheral zone of the follicles. Possibly it was occurred due to a mechanical injury during the follicle isolation. Co-cultivation of the ovarian stromal cells with the follicles in the 'hanging drop' led to a formation of aggregates consisted of the stromal cells and the follicle. Time-lapse microscopy visualization was used to precise the results. In the cases when follicle was dead stromal cells did not form aggregates. Thereby it is possible to get the viable oocytes in the 3D-cultural systems, but their functional condition is the aim of further studies.

Keywords: None.

MON-251
Phylogenetic relationships of some *Teucrium* (Lamiaceae) taxa growing in Turkey based on ITS region

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Teucrium L. (Lamiaceae) is a cosmopolitan genus and most of the members of the genus distribute around the Mediterranean basin (almost 95% of all the taxa). In Turkey, *Teucrium* has about 34 species or 47 taxa with sixteen of these being endemic to Turkey. The genus *Teucrium* has traditionally been divided into the sections distinguishable by the calyx shape and the inflorescence structure in Turkey. The species growing in Turkey are classified into eight sections: *Teucrium* Benth., *Chamaedrys* (Mill.) Schreb., *Polium* (Mill.) Schreb., *Isotriodon* Boiss., *Scorodonia* (Hill) Schreb., *Stachyobotrys* Benth., *Scordium* (Mill.) Benth. and *Spinularia* Boiss. ITS4 and ITS5A primers were used for the amplification of ITS region. Standard PCR techniques were applied for this study. After the visualization of amplified samples on an agarose gel electrophoresis, they were sequenced using automated methods. In this study, some members of the eight sections were studied. Some *Ajuga, Nepeta, Satureja,* and *Origanum* taxa were chosen as different outgroups as well. Molecular data resulted in support of some morphological findings. The Sect. *Scordium* and the Sect. *Chamaedrys* displayed a close relationship to each other than the other sections of *Teucrium*. Besides, the members of the Sections *Scordium, Chamaedrys*, and *Teucrium* formed monophyletic groups. On the other hand, Sect. *Stachyobotrys* seems polyphyletic. Our data may solve some sys-

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tematic problems of the genus. In addition, this study will contribute to the revision of the genus in Turkey.
Keywords: ITS, Teucrium, Turkey.

MON-252
Pou3f4 is required for normal development of otic capsule and modiolus of mouse inner ear

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Pou3f4, encoding a POU domain transcription factor, is the major gene responsible for DFNX2 (DFN3), an X-chromosome linked nonsyndromic deafness in human. It has been shown that *Pou3f4* is expressed in the peri-otic mesenchyme during embryonic inner ear development and consistently, the lack of Pou3f4 function led to defects in bony structures surrounding and within the inner ear such as otic capsule and modiolus, respectively. Inner ear bones are formed by two distinct processes; otic capsule by endochondral ossification and modiolus by intramembranous ossification. To better understand the role of *Pou3f4* in inner ear bone development, we examined the inner ears of *Pou3f4del-J* mouse, a spontaneous mutant with a deletion in the *Pou3f4* locus.

The *Pou3f4*-deficient cochlea displayed abnormalities in the otic capsule and a loss of modiolus. Spatial and temporal expression patterns of chondrocyte markers such as *Sox9* and *Aggrecan* were relatively normal in the developing mutant cochlea. However, expression of hypertrophic chondrocyte marker such as *Col10a1* was slightly delayed, and subsequent bone maturation in the otic capsule appeared to be defective assessed by abnormal expression patterns of *Col1a1* and *Osteocalcin*. These results suggest that Pou3f4 function is required for normal hypertrophic chondrogenesis and bony maturation, but not for early chondrogenesis, during otic capsule formation. Consistent with the lack of modiolus in the mutant cochlea, a bone marker *Osteocalcin* was absent, but instead, *E-cadherin*, which is known to play a role in mesenchymal to epithelial transition, was ectopically expressed. This result suggests that the loss of modiolus in the *Pou3f4*-deficient cochlea is due to an epithelial transition of the mesenchymal tissues in the modiolar region.

Together, our observations suggest that mesenchymal expression of *Pou3f4* during inner ear development is crucial for normal development of both otic capsule and modiolus.
Keywords: bone, inner ear, pou3f4.

MON-253
Primary characteristics of cytochromes P450 of the CYP74 family of *Selaginella moellendorfii*

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Enzymes of lypoxygenase signaling system (including the members of the CYP74 family of cytochrome P450 – allene oxide synthases (AOSs), hydroperoxide lyases (HPLs) and divinyl ether synthases (DESs)) play important role in the regulation of response to stress. Among these groups of enzymes DES are less studied. Previously characterized enzymes are mainly found in Solanaceae plants. They utilize 9-hydroperoxides of fatty acids. There are only two characterized 13-specific DESs of garlic and flax. However, divinyl ethers were found in plants of the different taxons: for example, Ranunculaceae – Dicotyledonous, Iridáceae and Asparagaceae – Monocotylidonous.

Selaginella moellendorfii is the unique object, because there are a lot of various divinyl ethers in tissues of this plant. There are six genes in its genome, which possibly encode DESs. They have a low degree of similarity with the CYP74 enzymes of other subfamilies, that's why they were organized into the new subfamilies – CYP74L and CYP74M. Genes *CYP74M1*, *CYP74M2* and *CYP74M3* were cloned and corresponding enzymes were characterized. These enzymes are not similar isoforms, which catalyze the formation of one group of products, but clearly different enzymes which are responsible for formation of extended diversity of oxylipins. Structures of these compounds have been determined by GC-MS, NMR and UV-spectroscopy. Meaning of such variability of formed oxylipins for plants's life is still unknown.

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Keywords: Cytochrome P450, Selaginella moellendorfii, The CYP74 family.

MON-254
Regional difference of gene expression in bluefin tuna musculature

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Bluefin tuna *Thunnus Olientaris* has anatomically and biochemically a unique muscle composition. In fish myotomal muscle, fast and slow muscles are anatomically separated, where fast muscle occupies the majority of trunk region and slow muscle forms thin layer beneath the skin (superficial slow muscle). In addition to these muscles, bluefin tuna has a large slow muscle lying near the vertebrae, called deep slow muscle. This characteristic muscle is involved in endothermy, the most representative biological feature of tunas. However, the molecular basis on the development of such unique musculature system is completely unknown. Myosin heavy chain (MYH) is a key molecule in the muscle contraction. MYH is encoded by the multi-gene family and spatial expression of MYH genes (*MYHs*) determines morphological, functional and metabolic features of muscle. In various fish species, the composition of *MYHs* shows a clear segregation between fast and slow muscles, although expressional patterns of *MYHs* in tuna muscle has not been proven yet. The present study aims to reveal molecular mechanisms involved in development of bluefin tuna musculature by analyzing spatial differences in the expression of *MYHs* and related genes.

One *MYH*, named *myhf1*, was cloned from fast muscle and its full-length cDNA sequence determined. Phylogenetic analysis based on deduced amino acid sequences showed that *myhf1* belongs to an adult fast-type. Interestingly, functionally important surface loops of myhf1 had characters to those of MYHs from tropical fish, suggesting the adaptation of bluefin tuna muscle to its endothermy. Quantitative real-time PCR and *in situ* hybridization showed a dominant expression of *myhf1* in fast muscle, whereas it was also expressed marginally in superficial and deep slow muscles. Two paralogues of *Sox6*, a known transcriptional repressor of slow muscle-specific genes, were cloned from fast muscle. Unexpectedly, two paralogues were expressed not only in fast but also in slow muscle. Taken together with expression of the adult fast-type *myhf1* in slow muscle, fast muscle fibers in slow muscle of bluefin tuna.

We also characterized the whole transcripts in bluefin tuna muscle by RNAseq analysis. cDNA libraries were constructed from fast, superficial slow, deep slow, and cardiac muscles, and sequenced using an Ion PGM sequencer. A total of 445 Mb

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sequences data were obtained from 4 libraries. Among the obtained 118,259 contigs, 32, 275, 106, and 233 contigs were specifically detected in deep slow, superficial slow, fast, and cardiac muscles, respectively. Especially, deep slow muscle-specific contigs are interesting, since they are possibly involved in the formation of this unique musculature.

Keywords: MYH, transcriptome, tuna.

MON-255

Regulation of WD40-repeat protein 62 by Aurora A- Insights into the maintenance of the mitotic spindle during neurogenesis

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Mammalian neurogenesis occurs during embryogenesis and persists in specific regions of the adult nervous system. Neurogenesis is multidimensional - waves of cell proliferation interspersed with lineage determination and migration events. Understanding such a complex process can be facilitated by dissecting the mechanisms leading to disease that arise during neurodevelopment.

Autosomal recessive primary microcephaly (MCPH) is a neurodevelopmental disorder characterised by decreased brain volume and severe cerebral defects. Recently, mutations in *WD-repeat protein 62 (WDR62)* were found to underlie MCPH. The resultant clinical manifestations suggest that WDR62 is key to cell proliferation and migration in the developing brain. Recent studies showed that WDR62 localised to the spindle pole during cell division. Knocking down WDR62 led to abnormal spindle formation, and premature differentiation of neuroprogenitor cells led to reduced brain volume in mice.

Containing WD-repeats structurally favourable for important scaffolding functions, we hypothesise that WDR62 complexes act as important nodes conferring signalling specificity or provide platforms mediating the subcellular localisation of its binding partners.

Here, the composition of immunoprecipitated WDR62 complexes was elucidated by high performance liquid chromatography-tandem mass spectrometry, revealing distinctive interaction partners of WDR62 in interphase and mitosis. Upon mitotic entry, WDR62 was found to interact with the mitotic regulator, Aurora Kinase A (AURKA). AURKA plays key roles in mitosis - regulating spindle formation and architecture, and mitotic progression. Depleting WDR62 resulted in mitotic spindle deformities, consistent with the phenotype observed upon AURKA inhibition. Strikingly, the inhibition of AURKA led to the loss of WDR62 localisation at the spindle pole, and the biochemical basis of this relationship was further characterised in cultured cell models. While the association of WDR62 with microtubules was regulated by AURKA activity, WDR62 was in turn important for the localisation of AURKA to microtubules. Loss of WDR62 localisation at the spindle pole consequentially results in the impediment of its mitotic functions.

The revelation of WDR62-AURKA interaction and the further characterisation of their relationship will provide important insights into mitosis and mammalian neurogenesis. Interestingly, WDR62 was recently found upregulated in certain cancers. As an inhibitor of AURKA is in clinical trials for cancer, the characterisation of this mitotic interaction will further improve molecular understanding of their contribution to cancer progression.

Keywords: Mitotic spindle, Neurogenesis, Signalling.

MON-256

Retrortansposon-based molecular markers for genetic diversity analysis in genus *Linum*

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SSAP (Sequence-Specific Amplified Polymorphism) method was used to study the genetic diversity of 22 *Linum* species from sections *Linum*, *Adenolinum*, *Dasylinum*, *Stellerolinum*. The SSAP method is based on DNA restriction, ligation of adapters to the restriction sites, and amplification of the DNA fragment with primers complementary to the adapter sequences and the selected conservative region of the retrotransposon.

For investigation of species of genus *Linum* we chose retrotansposons *FL1a*, *FL1b* and *Cassandra*. In total 223 polymorphic bands were scored. Nine groups could be distinguished on the basis of SSAP fingerprint similarity: group ‘A’ included different species of sect. *Adenolinum* (syn. *L. perenne* group); group ‘B’ consisted of *L. hirsutum* subsp. *hirsutum* accessions and group ‘C’ – of *L. hirsutum* subsp. *pseudoanatolicum* and *L. hirsutum* subsp. *anatolicum*; Groups ‘D’, ‘E’, ‘F’ and ‘G’ included species accessions of sect. *Linum*: *L. marginale; L. narbonense, L. decumbens* and *L. grandiflorum* respectively. Group ‘H’ included accessions of *L. angustifolium* and *L usitatissimum* (sect. *Linum*); group ‘I’ – *L. stelleroides* accessions (Figure Neighbor-joining dendrogram for SSAP markers. 1, 2, 5, 6, 7, 8 *L. perenne*; 3 *L. altaicum*,; 4 *L.* komarovii; 9 *L. leonii*; 10, 11 *L. pallescens*; 12, 13 *L. mesostylum*; 14,15 *L. lewisii*; 16, 17, 18, 19, 20 *L. austriacum*; 21 *L. amurense*; 22, 23, 24, 25 *L. hirsutum*; 26 L. *marginale*; 27, 28 *L. narbonense*; 29, 30 *L. decumbens*; 31, 32 *L. grandiflorum*; 33, 34, 35, 36, 37, 38 *L. angustifolium*; 39 *L. usitatissimum* subsp. *biene*; 40, 41 *L. usitatissimum* convar. *crepitans*; 42, 43, 44, 45 *L usitatissimum*; 46, 47 - *L. stelleroides*.). Each group contained at least one group-specific marker. Species clusterization on the bases of

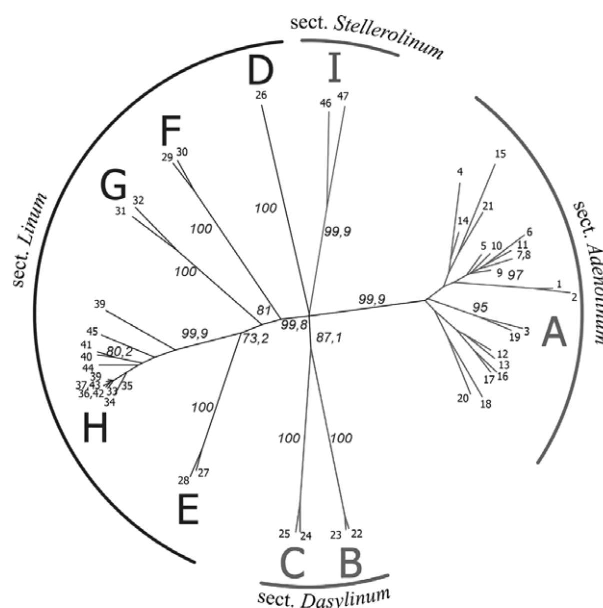

Fig. 1.

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SSAP markers was in concordance with their taxonomic division into sections *Dasylinum, Stellerolinum, Adenolinum* and *Linum*. All species of sect. *Adenolinum* clustered apart from species of sect. *Linum*. The data confirmed the accuracy of the separation in these sections. Members of section *Linum* are not as closely related as members of other sections, so taxonomic revision of this section is desirable. *L. usitatissimum* accessions genetically distant from the modern flax cultivars were revealed in our work. These accessions are of particular interest for flax breeding and introduction of new resistances and other useful traits into flax cultivars.

The work was financially supported by grants MK-6205.2012.4, RFBR 12-04-01469 and RFBR 13-04-01770.
Keywords: flax, retrotransposon, SSAP.

MON-257
SCN1A gene polymorphisms in patients with drug-resistant epilepsy

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Epilepsy is a life-shortening neurological disorder affecting approximately 1–2% of the worldwide population. Among the genes known to be involved in epilepsy, the SCN1A gene represents one of the most commonly mutated human epilepsy genes. The SCN1A gene codes for the a-subunit of the voltage-gated sodium ion channel. To date about one thousand mutations a reported at SCN1A gene. SCN1A has 26 exons and elucidations for polymorphisms that are most associated with epilepsy is actual task.

An intronic SCN1A IVS5-91G>A polymorphism (rs3812718) is associated with susceptibility to epilepsy [1,2]. Across the studies the strongest association with epilepsy was was found for rs3812718: odds ratio (OR) = 0.85 for allele G (p = 0.0009) and 0.73 for genotype GG versus AA (p = 0.003) [1]. rs2298771 is also associated with susceptibility to epilepsy [1].

Using 454 GS Junior we have performed high-throughput sequencing for exon and important intron regions of SCN1A gene for 42 patients with drug-resistant epilepsy and controls older than one year. For coupled samples the results was verified by Sanger sequencing.

Excluding a few rare mutations of SCN1A gene from this analysis, we have identified and analysed four polymorphisms: rs2298771 (c.3166G>A, exon 16), rs6432860 (c.2259T>C, exon 13), rs7580482 (c.1212A>G, exon 9), rs3812718 (c.603-91G>A, IVS5-91G>A).

We first identified the strong correlations between genotypes for rs2298771, rs6432860 and rs7580482 polymorphisms. Finally, we have found the next associations for polymorphisms:

rs2298771 GG / rs6432860 TT / rs7580482 AA / rs3812718 GG - 5 cases

rs2298771 GA / rs6432860 TC / rs7580482 AG / rs3812718 GA -16 cases

rs2298771 GA / rs6432860 TC / rs7580482 AG / rs3812718 GG - 1 cases

rs2298771 AA / rs6432860 CC / rs7580482 GG / rs3812718 AA - 14 cases

rs2298771 AA / rs6432860 CC / rs7580482 GG / rs3812718 GA - 5 cases

rs2298771 AA / rs6432860 CC / rs7580482 GG / rs3812718 GG - 1 cases

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Keywords: Epilepsy, High-throughput sequencing, SCN1A.

MON-258
Semi-rational engineering of *Neisseria polysaccharea* amylosucrase towards the synthesis of novel oligosaccharides for carbohydrate-based vaccines

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Chemical synthesis of complex oligosaccharides still remains critical. Enzymes have emerged as powerful tools to circumvent chemical boundaries of glycochemistry. However, natural enzymes do not necessarily display the required properties and need to be optimized by molecular engineering. Combined use of chemistry and tailored bio-catalysts may thus be attractive for exploring novel synthetic routes.

Within the frame of the development of synthetic routes to provide antigenic oligosaccharides entering in the composition of multivalent vaccines to prevent shigellosis, we have developped a semi-rational engineering strategy to create enzymes displaying

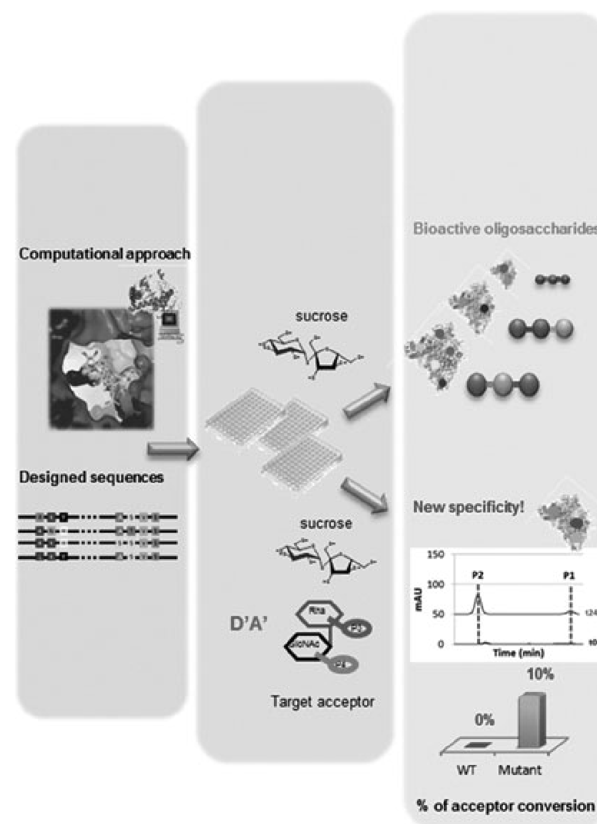

Fig. 1.

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the requested specificity toward a non-natural substrate, a chemically protected disaccharide which is a key intermediate in the synthesis of *S. flexneri* serotype 1b repeating unit.[1]

Following a computer-aided design strategy, a focused library of ~16.8 x10^4 mutants of amylosucrase from *Neisseria polysaccharea* (*Np*AS), a sucrose-utilizing a-transglucosidase, was constructed. Upon screening for their ability to utilize sucrose as donor substrate, 55 improved variants were identified. They were subsequently assayed for their aptitude to glucosylate the target non-natural acceptor.[2, 3, 4]

As a result, one mutant containing 7 mutations introduced in the catalytic site, was isolated with a totally novel ability to recognize and glucosylate with the requisite regiospecificity a disaccharide acceptor, which is not recognized at all by the parental wild-type enzyme. Moreover, 4 additional mutants displaying original product profiles or enhanced sucrose activity compared to the wild-type enzyme were identified and characterized in more details at both biochemical and structural levels.

Altogether, these results will hopefully help to improve our comprehension of the sequence-structure-function relationships of this class of enzymes with the view of providing new insights to guide the design of tailor-made enzyme libraries.

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Keywords: antigenic oligosaccharides, computer-aided strategy.

MON-259

Serum protein profile analysis by SELDI-TOF in caveolin-1 transgenic mice

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Introduction: A caveolin-1 null (CAV-1$^{-/-}$) mouse model was created, to assess the role of caveolin-1 in biogenesis, endocytosis, cell proliferation, signaling pathways and other molecular mechanisms of diseases. Recent studies highlight an important role of caveolin-1 in the regulation of different protein expression. In this content, the aim of our study was to estabilish the serum protein profile for cav-1 transgenic mice versus control, using SELDI TOF – MS (1).

Methods: Serum from transgenic mice Cav-1$^{-/-}$ (Cav-1 KO: Cav1^{tm1Mls}/J) and Cav-1$^{+/+}$ (B6129PF2/J) (The Jackson Laboratory) as control were analyzed. After optimization of protocol selection, the serum protein profiling study was performed on CM10 protein chips using SPA (sinapinic acid) as matrix on a SELDI-TOF personal edition system (Bio-Rad).

Results: Experimental parameters specific to the ProteinChip, including spot protocols (laser intensity and detector sensitivity) were optimized to peak detection variance. Optimal instrument settings, regular calibration along with controlled sample handling and processing nearly doubled the number of peaks detected and intensity variance. The protein profiles were analyzed with the ProteinChip Data Manager Software 3.0.7. The proteomic spectra obtained were compiled, normalized, and mass peaks identified with mass-to-charge ratios between 2000–6000 Da, 10000–19000 Da and between 25.000–40.000 Da. Comparative analysis of protein profile revealed that there were a total of 18 different expressed peptide peaks with statistical significance in the molecular range of 2000–40000 Da. Among which, the expressions of 8 proteins were up-regulated and that of 10 proteins were down regulated in transgenic mice.

Conclusions: The monitoring of protein profiles in caveolin-1 transgenic mice, may allow us to understand the involvement of these mutations in the molecular mechanisms of diseases. SELDI-TOF MS technology represents a proteomics approach that can rapidly identify tens of thousands of proteins. However, it is challenging work to validate, prioritize, and select the best targets from those candidate proteins.

Acknowledgment: PN 09.33-04.15 and POSDRU 141531/2014.

Reference

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Keywords: caveolin-1, mass spectrometry, Proteomics.

MON-261

Site-specific genome mutagenesis using the CRISPR/Cas9 system in *C. elegans*

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Background: The CRISPR/Cas9 system has become an efficient technique to generate mutations in the genome of a variety of model organisms. The system consists of a nuclease (Cas9) which is guided by a single guide RNA (sgRNA). The Cas9-induced targeted double strand breaks are subsequently repaired by non-homologous end joining (NHEJ) or homology-directed repair (HDR). Specifically designed donor templates can trigger HDR and thereby lead to insertions of transgenes at specific locations of the genome. Consequently, the CRISPR/Cas9 system is a precise and effective tool for tailored genome editing.

Observations: We applied the CRISPR/Cas9 system to introduce point mutations into the *C. elegans* genome by using the Cas9 nuclease expressed under different germline promoters. In addition, we used varying concentrations of the nuclease, sgRNA and donor template to optimize the efficiency of the system. Furthermore, we developed a single-step approach to clone sgRNAs. After gonadal injection of young adult hermaphrodites, we tested a variety of screening techniques to detect NHEJ- and HDR-events with a special emphasis to identify single amino acid substitutions.

Conclusions: We optimized the sgRNA cloning procedures and screening techniques to identify animals containing engineered inserts in the genome. Our data show that the CRISPR/Cas9 system can be used in combination with a donor template to insert tailored changes into the genome of *C. elegans* in a fast manner and at low cost.

Keywords: CRISPR/Cas9, Genomic engineering, Site directed mutagenesis.

MON-262

Structural and signalling function of b-catenin during cardiogenesis

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Beta-catenin is an armadillo protein family member and has dual function being an essential component of the Adherence junction mediating cell-cell contact and acting as a transcriptional co-activator of the T-cell factor/lymphoid enhancer factor complex. In this study we have addressed the role of β-catenin during cardiogenesis, namely we have analyzed how b-catenin haploinsufficiency reflected on embryonically heart development. For this

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purpose we induced embryonic β-catenin in heart using Cre-mediated technique. To generate cardiac-specific deletion of β-catenin β catflox/+, a-myosin heavy chain (aMHC)-Cre mice were mated with β catflox/flox mice and embryos were recovered from timed pregnancies. Yolk sac tissues after embryo recovery were used for genotyping. Morphological analysis of embryos hearts were performed with HE and IM staining using. Signalling function of β-catenin during cardiogenesis was analyzed with qPCR using. Gene expression was represented by the ΔC_T value normalized to the reference gene GAPDH. The $\Delta\Delta C_T$ value of each target gene was then calculated by subtraction of the average ΔC_T from the control group. Finally, the n-fold difference was calculated by using the formula $2\Delta\Delta^{CT}$. In our experiment we studied the level of TCF-4, β-catenin, β-MHC, α-MHC, ANP, BNP, cFos and cMyc. For all morphological and molecular biological analysis we have used newborn mice and embryos at E10.5, E12.5 and E14.5. When mice harboring a Cre recombinase transgene under control of an aMHC-Cre promoter were crossed with mice containing a floxed β catenin allele, the embryos appeared morphologically normal at embryonic stages E10.5 and E14.5. To confirm that Cre-mediated deletion of β-catenin gene produces a null phenotype. Immunohistochemical analysis confirmed the absence of β-catenin in the developing myocardium of mutant mice and also demonstrated that N-cadherin was not affected in β-catenin-deficient cardiomyocytes. Thus, the absence of β-catenin does not perturb N-cadherin expression in the embryonic heart, probably due to functional redundancy between β-catenin and plakoglobin in the formation of AJ formation. Interesting that genotyping of β-catenin newborn mice after similar crosses revealed that aMHC-Cre+, β-cateninflox/flox mice were under-represented, suggesting that some mice were dying in utero, probably at late gestation. Thus, only 10% of mice born had a genotype of aMHC-Cre+, β-cateninflox/flox, and one of the mutants died shortly after birth. Additionally in our work we registered some violations of β-catenin signalling and perturbation of β-catenin target genes expression at all analyzed embryos and newborn mice.

Keywords: None.

MON-263
Studies on subcellular localization of the germ cell-expressed protein from *Drosophila melanogaster*

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Juvenile hormone (JH) and 20-hydroxyecdysone have prominent roles in regulating insect development. There is an accumulating evidence that the antimetamorphic function of JH involves Methoprene-tolerant (MET), so MET becomes a putative JH receptor protein. MET belongs to the family of bHLH-PAS transcription factors. The bHLH-PAS proteins are critical regulators of the gene expression networks that are responsible for many essential physiological and developmental processes. In contrast to other insects that depend on MET for survival, *Drosophilids* posses a MET paralog Germ cell-expressed (GCE). While neither mutation of single protein is lethal, mutants missing both paralogs die as prepupae, suggesting that MET and GCE have some redundant functions in vivo. The bHLH-PAS transcription factors functional activity is often correlated with shuttling between nucleus and cytoplasm, according to the masking and unmasking by interacting partners, signals directing these proteins to nuclear or cytoplasmic compartment of the cell.

Recently we identified Nuclear Localization Signals (NLSs) and Nuclear Export Signals (NESs) for MET (Greb-Markiewicz *et al.*, 2011). In order to determine the sequences of NLSs and NESs in GCE, series of deletion and point mutants tagged by yellow fluorescence protein (YFP) were prepared by cloning into EYFP-C1 vector, expressed in mammalian cells and observed with confocal microscopy system.

Contrary to nuclear localization of MET, full lenght GCE we could observe both in nucleus and cytoplasm of the cell. Our results show that dominant NLS from MET is lacking in GCE. We have detected presence of active NLSs in bHLH, hinge region, PAS B and C-terminal fragment of GCE. Active NESs motifs are present in PAS A and PAS B. We found additional factors influenzing localization pattern of deletion mutants and full lenght GCE in absence and presence of JH. We conclude that GCE similarly to MET is a cytoplasm-nucleus shuttling protein with a complicated control system of localization, which vary between proteins.

This work was supported by a statutory activity subsidy from the Polish Ministry of Science and High Education for the Faculty of Chemistry of Wroclaw University of Technology.
Keywords: juvenile hormone, methoprene tolerant protein, NLS.

MON-264
Study of genetic diversity of green toads (*Pseudepidalea viridis*) populations in Warsaw using msDNA

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In the structure of urban agglomeration, small artificial lakes in parks and squares are often inhabited by amphibians like smooth newts and green toads. These habitats are separated and confined by the busy roads and lacking of green areas housing estates. It may affect a high degree of populations isolation, interrupt functional connectivity and genetic structure of species.

The high level of isolation could lead to such negative phenomena as inbreeding depression and decreased in genetic diversity. It may also increase the probability of extinction risk of the local populations. All of these effects could be the result of random population size fluctuations and environmental changes including climate changes. On the other hand, occurrence of common alleles and similar frequency in subpopulations from different study sides may suggest the presence of certain rate of the gene flow.

The aim of the project is to investigate the genetic and demographic structure of the green toad (*Pseudepidalea viridis*) population, species present inter alia in the urban environment of Warsaw, the capital city of Poland. Samples were collected in 2012 and 2013 from five neighboring districts. DNA was extracted directly from eggs and fragments of dead individuals collected near each of studied reservoirs. We used 13 polymorphic microsatellite markers developed for the European green toad. We assessed genotypes for 72 individuals and thus described the genetic characteristic of local populations.

Our results shows high level of inbreeding in studied populations, however some gene flow between close populations can be observed. Those results indicate that migration in urban environment is very difficult for amphibians, nevertheless it is not impossible. That's suggest, a little help applied from humans could significantly increased genetic diversity and population fitness

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through facilitating migration. We also confirmed that using 13 polymorphic microsatellite markers is enough to study landscape genetics of green toad populations.
Keywords: genetic diversity, landscape genetic, msDNA.

MON-265
System analysis of cells peptidome of the Phiscomitrella patens

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Cells within an organism must communicate over both short and long physiological ranges to ensure proper patterning and functional connections. There are several ways to achieve this in plants, including phytohormones, mobile transcription factors, noncoding RNAs, and small signaling peptides.

Peptides are key regulators of many physiological processes in animals, including defense reactions and hormonal, neurohumoral and signaling functions.

As in animals, peptide signals regulating plant growth and development act as ligands of receptor-like kinases.

Peptide pools of the two life forms as well as of the protoplasts of Physcomitrella patents moss were studied by tandem mass spectrometry analysis.

Using tandem mass spectrometry analysis we identified about 4000 gametophore peptides that were fragments of 761 precursor proteins. In protonema cells, we identified about 4000 peptides derived from 855 precursor proteinsthe pool of endogenous peptides and carried out transcriptional profiling of gametophores, protonemata and protoplasts of the P. patens moss.

Transcriptome profiling of the three moss cell types showed that genes of peptide precursors are expressed at higher levels that the rest of the genes We demonstrated significant differences between the peptidomes of protoplasts, protonemata and gametophores and found that the transcriptional level of many gametophore- or protonema- specific peptide precursor proteins tends to be higher in the corresponding tissue.
Keywords: None.

MON-266
Telocytes versus fibroblasts and endothelial cells: a proteomic approach

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Telocytes (TCs) are newly described interstitial cells characterized by the presence of small cellular bodies and a variable number of very long and thin extensions named telopodes. Telopodes are defined by a length of ~100 micrometers and an uneven caliber given by a succession of very thin segments - podomers and dilations named podoms. TCs were morphologically characterized by light, fluorescence, transmission and scanning electron microscopy, but their proteome was not analyzed. Therefore, we examined by 2D Nano-ESI LC-MS/MS the differentially expressed proteins in lung TCs by comparison with fibroblasts and microvascular endothelial cells. Based on the bioinformatics analysis, proteins extracted from primary cultures of these cells were analyzed and screened by two-sample t-test ($P < 0.05$) and fold change (>2). We identified hundreds of proteins up- or down-regulated, respectively, in TCs as compared with fibroblasts and microvascular endothelial cells. TCs proteins were classified into different categories based on their molecular functions and biological processes. While the proteins identified in TCs are mainly involved in catalytic activity and as structural molecular activity, the proteins in FBs are involved particularly in the synthesis of collagen and other extracellular matrix components. The up-regulated ECs proteins e.g. cell surface glycoprotein MUC18 and von Willebrand factor are considered as hallmarks for these cells. In conclusion, we report here the first extensive identification of proteins from TCs using a quantitative proteomics approach, suggesting that TCs might play specific roles in: a) intercellular signaling b) mechanical sensing and mechanochemical conversion task, c) tissue homoeostasis and remodeling/renewal, d) anti-oxidative stress and anti-cellular aging mechanisms; e) cancer cell proliferation through the inhibition of apoptosis.
Acknowledgments: This study was supported (for DC) by the Sectorial Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/159/1.5/S/141531. The work was partially supported by a grant of the Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number 82/2012 (PN-II-PT-PCCA-2011-3.1-0553). We thank the Institutes of Biomedical Sciences and Department of Chemistry, Fudan University, for automated 2-D nano-ESI LC-MS/MS analysis of peptides.
Keywords: Endothelial cells, Fibroblasts, Telocytes.

MON-267
The CYP74 family in evolution of cytochromes P450

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Lipoxygenase cascade is one of the most important signaling cascades. Its functioning results in formation of physiologically active derivatives of polyenoic fatty acids – oxylipins. Oxylipins have been found in anim als, proteobacteria, brown and red algae and all land plants. Crucial enzymes of lipoxygenase cascade providing diversity of oxylipins are lipoxygenases and cytochromes P450 of the CYP74 family. Lipoxygenases catalyze oxygenation of fatty acids into hydroperoxides, and the CYP74 enzymes are responsible for their further conversions.

Based on phylogenetic analysis of the CYP74 family and the P450 superfamily one could conclude that the CYP74 enzymes are the most ancient among all P450s. It is confirmed by the following facts. Unlike classic cytochromes P450 which are monooxygenases, the CYP74 enzymes don't need redox partners or molecular oxygen for functioning. Thereby sites conservative for monooxygenases P450 are absent in the CYP74 sequences. Besides, the CYP74s' oxygen-binding domain is substituted by the I-helix central domain involving in catalytic action. And haem-binding domain of the CYP74s contains additional nine amino acids. Evolutionally deletion of motif and simple reaction are more likely than insertion and complex reaction, respectively.

Appearance of the CYP74 enzymes correlates with accumulation of molecular oxygen in the atmosphere. It results in necessity to save cell from membrane fatty acids oxygenated by active forms of oxygen. We suppose that the CYP74 enzymes catalyzed

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conversion of oxygenated fatty acids into various compounds – oxylipins which later began to function as signal molecules. It is confirmed by the fact that structure of the CYP74 enzymes is similar to the structure of some catalases. And there are some catalases which possess activities similar to the CYP74s. Thus, one could conclude that the CYP74 enzymes are ancestral for the whole P450 superfamily.

This work is partly supported by Russian Foundation of Basic Research (13-04-40103-H, 14-04-01532-a, 12-04-97087-r, 12-04-97059-r), MK-4886.2013.4 and SS-1890.2014.4.
Keywords: Cytochrome P450, Molecular evolution, The CYP74 family.

MON-268

The diatom-derived aldehyde decadienal affects ERK signaling during metamorphosis of the ascidian *Ciona intestinalis*

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Diatoms are photosynthetic unicellular organisms playing key roles in the transfer of energy through marine food chains. During grazing by their predators, diatoms can produce polyunsaturated aldehydes, such as decadienal, which have been shown to induce deleterious effects on embryonic and larval development of benthic organisms [1,2]. These effects are mediated by the physiological messenger nitric oxide in the sea urchin *Paracentotus lividus* [3]. Here, we investigate the effect of decadienal on larval development and metamorphosis of the ascidian *Ciona intestinalis*. After hatching, the mobile larva is transformed into a fixed juvenile through a remarkable regression of the tail. At the molecular level, *C. intestinalis* development is regulated by different events, sometimes cross-talking together. These events include: NO production/diffusion, ERK and JNK activation and apoptosis mediated by capsase 3 activation [4,5]. In a previous study we reported that NO mediates a nitro-oxidative stress pathway during *Ciona* larval development resulting in ERK nitration and that increasing NO levels induce the acceleration of metamorphosis [6]. Recently, we have also shown that the increase of NO levels contributes to maintain ERK activation through the down regulation of the MAPK phosphatases, MKP1 and MKP3 [7]. Now we show that larvae exposed to increased levels of decadienal encounter a significant reduction in NO levels during development, resulting in a delay in metamorphosis. Moreover, we show that this event is related to ERK inactivation as demonstrated also by the effect on the downstream ERK gene transcription. Finally, we analyzed the expression of several genes involved in redox homeostasis and we observed that some genes involved in glutathione metabolism are up-regulated upon treatment with decadienal, thus suggesting a fine regulation of glutathione levels. We hypothesize that larval developmental delay induced by decadienal may represent a defense mechanism of the organism to overcome the negative effects of the toxin until the larva attaches to the substrate for metamorphosis into the adult. Overall these results give new insights into the molecular pathways targeted by decadienal, affecting key decisions in biphasic life cycle transition from vegetative to reproductive stages.

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Keywords: development, ERK signaling, nitric oxide.

MON-269

The effect of transferrin sialylation on foetal development during pregnancy

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Transferrin (Tf) is glycoprotein which sialylation is considered to take part in iron transporting. Changes in number of sialic acid residues are thought to have an impact on iron transport and foetal development.

Capillary electrophoresis (Beckman Coulter PA 800plus) was used to separate glycosylated subfractions of transferrin. Electrophoresis was conducted by commercial test for Carbohydrate-deficient Transferrin analyzing with modification. Exposure to tobacco smoke was measured by cotinine level using ELISA test. Iron parameters were determined by colorimetric methods.

Patients were divided in terms of: stage of pregnancy (1st, 2nd and 3rd trimester of pregnancy), health status (ID-iron deficiency and women with proper iron stores as control group) and smoking status (smoking and non-smoking). Relative level of 5-sialoTf during pregnancy was rising while 3-sialo and 4-sialoTf relative level were decreasing in both: ID and control (no-ID and non-smoking) group. 6-sialoTf relative level was rising during pregnancy only in the control group. In the group with ID positive correlation between: 5-sialoTf level and AC (abdominal circumference) ($r = 0.431$; $p < 0.05$) 6-sialoTf level and BPD (biparietal diameter), HC (head circumference), AC and FL (femur length) ($r = 0.450$, $r = 0.496$, $r = 0.508$, $r = 0.423$; $p < 0.05$ respectively) was shown and negative correlation between 3-sialoTf level and BPD, HC, AC and FL ($r = -0.514$, $r = 0.579$, $r = -0.538$, $r = 0.590$; $p < 0.05$ respectively) occurred. Similar observation in the control group was detected.

More-sialylated Tf isoforms positively correlated with foetal biometric parameters and lowered iron stores what may suggest that high content of Tf sialic acid residues protect foetus from lower iron delivery.

Keywords: foetal development, sialylation, transferrin.

MON-270

The evolutionary conserved LIM homeodomain protein LIM-4/LHX6 impose dual neurotransmitter identity of a *C. elegans* motor neuron-type

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The expression of specific transcription factors dictates the differentiated features of postmitotic neurons. However, the mechanism of how specific molecules determine or specify neuronal cell fate during development is not fully understood. In *C. elegans*, the cholinergic and peptidergic SMB neurons consist of four motor neurons that innervate muscle quadrants in the head, send processes posterior down the sub-lateral cords (White et al., 1986), and monitor the amplitude of sinusoidal movement (Gray et al., 2005). To identify the factors that specify the neuronal cell fate of SMB, we performed genetic screens and isolated several *lim-4* mutant alleles, in which *flp-12* neuropeptide gene expression was completely abolished only in the SMB neurons. Previously, it was shown that the LIM-4 LIM homeobox protein has a major role in specification of

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AWB chemosensory neuron identity (Sagasti et al., 1999). We found that the expression of other SMB markers, *odr-2* (GPI-Anchored protein), *cho-1* (choline transporter), and *unc-17* (synaptic vesicle acetycholine transporter), were also abolished in *lim-4* mutants and LIM-4 maintains its own expression by autoregulation in the SMB neurons. To investigate the molecular mechanism of *lim-4*, we did promoter analyses and bioinformatics searches with the SMB marker genes and identified several *cis*-regulatory motifs including putative LIM-4 binding sites. We confirmed that these regulatory elements were sufficient to drive the expression of a non-SMB marker gene in the SMB neurons. In addition, we expressed *lim-4* cDNA under the control of the heat shock promoter which not only fully restored *flp-12* gene expression in *lim-4* mutants, but induced the ectopic expression of *flp-12* in other cell-types. Since *lim-4* is evolutionary well-conserved throughout different species, we also expressed the LIM-4 human orthologue, LHX6 and LHX8 cDNA under the control of heat shock promoter in *lim-4* mutants, and found that *lim-4* mutant phenotypes were fully rescued. Furthermore, we are currently expressing the human LHX6 or *C. elegans* LIM-4 in the human neuroblastoma cells to test if expression of these genes could induce the cholinergic cell fate. Taken together, LIM-4 appears to be necessary and sufficient to promote specification of the cholinergic and peptidergic SMB motor neuron fate.

Keywords: C. elegans, homeodomain, motor neuron.

MON-271
The influence of biological and environmental factors on MT1/MT2 ratio

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Metallothionein (MT) is a protein presents mainly in 4 isoforms: MT1, MT2, MT3, MT4. MT1 and MT2 are most prevalent in the human body. The concentrations of these two isoforms, and consequently MT1/MT2 ratio, are regulated by many factors, including metals, cytokines, glucocorticoides and free radicals. These factors are determined by such aspects of human biology as gender, pregnancy, as well as by environmental factors including the use of oral contraceptives and cigarette smoking, all which may affect the MT1/MT2 ratio in the body.

The investigations were performed in healthy subjects ($n = 128$), who were in similar age (26 ± 5 years). The concentrations of MT1 and MT2 were determined in erythrocyte lysate by ELISA Kit (ref. No. E91119Hu for MT1, E91868Hu for MT2, Uscn Life Science Inc., China). The MT1/MT2 ratio was calculated. The data concerning cigarette smoking were verified by determination of plasma cotinine, metabolite of nicotine, using the commercial Cotinine ELISA test (ref. No.: EIA-3242, DRG International, Inc., USA). MT1 and MT2 concentrations were analyzed in the following groups: group A (female non-smokers not taking oral contraceptives, $n = 36$), group B (female non-smokers taking oral contraceptives, $n = 26$), group C (female smokers not taking oral contraceptives, $n = 15$) group D (pregnant women in third trimester, $n = 30$) and group E (male non-smokers, $n = 21$).

The groups B and E had significantly higher MT1/MT2 ratio (3.75 ± 1.52 and 4.23 ± 1.58, respectively) when compared to the group A (2.65 ± 0.95), while the differences in MT1/MT2 ratio between groups A and C (2.63 ± 1.08) and between groups A and D (3.27 ± 1.34) were not observed.

The gender and oral contraceptives can cause higher MT1/MT2 ratio.

Keywords: biological factors, environmental factors, isoforms of metallothionein.

MON-272
The lipoxygenase cascade in several species of brown algae

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Plants lack an immune system in the sense that it exists in animals, but they possess mechanisms that recognize potential pathogens and initiate defense responses. It has become evident that various types of oxygenated fatty acids, collectively termed «oxylipins», are involved in responses to biotic and abiotic stress factors. These compounds are similar to the eicosanoids formed from arachidonate in animals, which have so many various functions, especially in the inflammatory processes. The existing diversity of oxylipins is provided by nonclassic cytochromes P450 of the CYP74 clan. The CYP74 clan includes hydroperoxide lyases (HPLs), allene oxide synthases (AOSs), divinyl ether synthases (DESs) and the most poorly studied epoxy alcohol synthases (EASs). Despite differences in product specificities, they all metabolize 9- and/or 13-hydroperoxides as substrates, which are produced as a result of the oxygenation of polyunsaturated fatty acids by the action of lipoxygenases (LOXs).

The CYP74 clan members have been found in a wide variety of organisms including animals, plants, bacteria but not algae. Despite this fact oxylipins were detected in different algae species. We analyzed patterns of endogenous oxylipins in several species of brown algae: *Undaria pinnatifida, Sargassum miyabei, Sargassum pallidum* and *Saccharina cichorioides*. Different isomers of epoxy alcohols, ketols, hydroxy and trihydroxy acids were detected. The structures of all compounds have been resolved using GC-MS, HPLC and NMR.

Analysis of genome data of brown alga *Ectocarpus siliculosus* with open reading frames revealed 12 sequences which have homology with cytochromes P450 of higher plants. One of them has similarity with the CYP74 enzymes. We obtained corresponding recombinant protein using *E. coli* expression system. Affinity-purified protein EsCYP74 was incubated with model substrates – 9- and 13-hydroperoxides of linoleic acid. Incubation resulted in formation of a number of epoxy alcohols and dihydroxy acids. Thus, the described enzyme EsCYP74 is the first member of the CYP74 clan detected in algae, and it belongs to one of the most poorly studied groups of the CYP74 enzymes – epoxy alcohol synthase. Detection of the CYP74 enzyme in brown alga complements the common scheme of the CYP74 catalysis and confirms our hypothesis of the CYP74 clan origin and evolutional history of the P450 superfamily.

This work is partly supported by Russian Foundation of Basic Research (13-04-40103-H, 14-04-01532-a, 12-04-97087-r, 12-04-97059-r), MK-4886.2013.4 and SS-1890.2014.4.
Keywords: epoxy alcohol, lipoxygenase pathway, oxylipins.

MON-273
The role of Otx2 in the correct positioning of the midbrain-hindbrain barrier

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During embryonic development, the neural plate is regionalized anteroposteriorly into several domains with their own individual identities and fates. A correct positioning of the boundaries between those domains is crucial for a normal development of the nervous system. In order to study the mechanisms underlying this boundary positioning, we focused on the establishment of

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the midbrain-hindbrain barrier (MHB), an already well-studied structure in which a small number of genes have been shown to play a role (Wurst, 2011). During normal embryonic development, the interplay between these genes, most notably the Otx2 and Gbx2 homeogenes, leads to the correct expression and positioning of the signaling molecule Fgf8. Fgf8 in turns confers to the MHB a role as a self-regulating organizing center for the surrounding domains, known as the isthmic organizer. The regionalization of the midbrain and hindbrain therefore depends on the correct location of the MHB. Mutual repression between the anteriorly expressed Otx2 and the posteriory expressed Gbx2 was thought to be the earliest phenomenon determining the position of the MHB. The expression of those two genes indeed abut each other at the MHB, and experiments where the limit of expression of either Otx2 or Gbx2 was artificially shifted led to a global shift of the MHB gene expression network towards the new limit (Broccoli, 1999). However, using a conditional knock-in strategy for Otx2, we were able to shed a light on the shortcomings of this model: the position of the MHB and the correct development of the midbrain and hindbrain do not only depend on a mutual repression between Otx2 and Gbx2. When we triggered an ubiquitous expression of Otx2 in mutant mice, we observed that, contrary to the posterior shift forecasted by the current model, the MHB was set in place in an anterior position, and that it lacked in sharpness. We are now trying to understand what functions as yet unexplored those homeogenes could control that could further explain their role in the regionalization of the midbrain and hindbrain. One possibility is that Otx2 and Gbx2 may drive the expression of other adhesions molecules, thus giving rise to two cell populations with different adhesive properties, which would then segregate according to the homeogene they are expressing. Another possibility is that the ectopic Otx2 expression modifies the retinoic acid signalling pathway activity through direct interaction with some of its actors, which would result in a modification of hindbrain patterning.

Wurst, W., Bally-Cuif, L., Neural plate patterning: upstream and downstream of the isthmic organizer, 2001, *Nature Reviews: Neurosciences* 2:99–108.

Broccoli, V., et al, The caudal limit of Otx2 expression positions the isthmic organizer,1999, *Nature* 401:164–168.

Keywords: isthmic organizer, midbrain development, neural plate segmentation.

MON-274
The *Schistosoma mansoni* protein SmShb interacts with and regulates SmVKR1 signalling

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Venus Kinase Receptors (VKRs) are invertebrate Receptor Tyrosine Kinases (RTKs) formed by an extracellular Venus Fly Trap (VFT) ligand binding domain and an intracellular tyrosine kinase domain. These receptors were initially discovered in the parasitic platyhelminth *Schistosoma mansoni,* then identified in many invertebrates (Ahier *et al*., 2009; Vanderstraete *et al*., 2013).

Quantitative RT-PCR on various stages and isolated organs of the sea urchin and various insects (Ahier *et al*., 2009), together with recent studies in the parasite *S. mansoni* (Vanderstraete *et al.,* 2014), argued for a role of VKRs in embryonic development and in reproduction. To better understand the cellular functions of VKRs, *S. mansoni* interacting partners of SmVKRs were identified by yeast-two-hybrid (Y2H) screening and SmVKR signalling pathways were characterized in *Xenopus* oocytes (Vanderstraete *et al.,* 2014).

The protein SmShb was shown to interact specifically with the phosphorylated form of SmVKR1. SmShb is an SH2 domain-containing protein, homologous to members of the Shb adaptor family known to transduce signals induced by activated RTKs. Its interaction with phospho-SmVKR1 occurs between its SH2 domain and the phosphotyrosine residue (pY979) located in the juxtamembrane region of SmVKR1. This interaction activates in *Xenopus* oocytes specifically the JNK signaling pathway. *SmShb* and *SmVKR1* transcripts were detected by *in situ* hybridization in mature oocytes and testes of adult schistosomes.

To further analyze the potential importance of SmShb/SmVKR1 signalling in germinal cells, we focussed on the determination of SmShb interacting partners by Y2H screening of a *S. mansoni* adult cDNA library using SmShb as a bait. Among the various potential SmShb partners, we identified two homologs of RhoU and TcTex-1, already known for their respective functions in cell migration and sperm motility.

Preliminary data have shown that the knockdown of SmShb by RNA interference in adult *S. mansoni* results in an accumulation of mature sperm in testes.

Together, these data suggest a role of SmVKR1/SmShb pathway in maturation and migration of germinal cells.

Keywords: signaling, Tyrosine kinase receptor.

MON-275
What changes matter? A genomic approach to human evolution

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We humans and our closest living relatives, the chimpanzees differ only in 1–2% of our genomes. Despite this genetic similarity we vary in many anatomical and behavioral traits. Upright walking and larger brains are just two prominent examples amongst many others that allowed us to adapt to new environments (compare image). Although full genome sequences are now available for humans, chimpanzees and other primates, surprisingly little is known about the genetic basis underlying these traits. One reason being, that even within the 1–2% difference lie many genetic changes potentially driving human evolution. Because open reading frames of genes tend to be very similar between the great apes, it has been argued that the majority of significant evolutionary changes affect cis-regulatory sequences. To identify the regulatory changes specific to the human lineage we undertook a whole genome approach focusing on the potential regulatory regions that are altered in the human lineage. We aligned human, chimp, macaque, and mouse genomes and concentrated on human-specific drastic changes in otherwise conserved non-coding regions. For simplicity reasons we limited our search criteria to deletions larger than a 100 bp and unique to the human genome. We identified 298 such human- specific deletions, that potentially alter regulatory functions in the human genome. We used a mouse transgenic approach to test if the deletions affect enhancer activity *in vivo*. Out of the 12 tested elements, 4 showed tissue-specific expression at diverse developmental stages. We focused on two human-specific deletions for further study. The first removes an enhancer element near the gene *OSR2,* and the observed expression pattern argues for a role in craniofacial development. The second deletion removes a regulatory element in the gene *ACVR2A*. The transgene expression and the phenotype of a full knockout of *ACVR2A*, suggest a role in the

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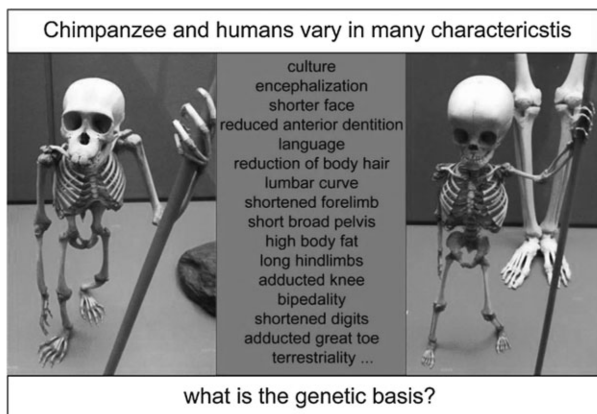

Fig. 1.

human-specific shortening of digit 2–5 and smaller size of upper incisors. Importantly, and in contrast with previous studies, we have mirrored the human situation in a mouse model by removing the enhancer element from the mouse genome, which is causing a down regulation of the nearby gene. We are currently analyzing the mouse model for human-like phenotypes using 3D reconstruction of the skull and the cranial base on micro-computer-tomography generated images.

Keywords: Genomics, Human Evolution, Mouse models.

MON-276
Zelda: an essential transcription factor in the *Drosophila* development

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The *Drosophila* zinc finger transcription factor Zelda (Zld)-Zygotic early *Drosophila* activator- appears to play a major role as a regulator of genome activation in the earliest stages of *Drosophila* development. Zld binds to a sequence motif referred to as a TAGteam site and functions as a key transcriptional activator during the maternal-to-zygotic transition – MZT. Zld has been shown to mark regions that are later bound by zygotically expressed transcription factors, suggesting that it can also be part of the machinery that controls the sequence of events following the MZT. It has also been proposed that Zelda ensures coordinated gene expression during embryonic development, either by increasing chromatin accessibility for several other transcription factors, or by being involved in the RNA polymerase II pausing mechanism. Zelda has been shown to be expressed in larval, pupal and adult stages, indicating a possible involvement in the regulation of molecular events throughout the developmental process. However, no data exist to date regarding its potential function in post embryonic development.

Our studies show that overexpression or knock down of Zld in larval wing discs, resulted in strong impaired phenotypes, an indication of its essential role in wing growth. Zld's study of expression in the developing larval wing discs and other larval tissues revealed the presence of novel alternatively spliced transcripts and a protein isoform with possibly different molecular function. Moreover, ChIP assays using larval wing discs revealed that Zelda binds to an intronic region of the optomotor-blind gene, a downstream target of the Dpp pathway. The immunohistochemical stainings of larval wing discs and the expression levels of several selected genes and protein products, showed altered expression of target molecules with key roles in more than one signaling pathways, when Zld was either knocked down or overexpressed. In these terms, the paradigm of Zelda function during embryogenesis where it coordinates the activity of many genes could also apply later in development. Furthermore, recent reports suggest that a prominent link exists between zygotic gene activation and pluripotency control in vertebrates. Thus, the elucidation of the exact role of a transcription factor with distinct functions in zygotic gene activation and patterning processes throughout the development of a simple model organism, could prove extremely valuable for any future study concerning similar and related mechanisms in higher organisms.

Keywords: development, *Drosophila*, wing.

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CSIII-03 – Mitochondria & mitochondrial disorders

MON-278

3-mercaptopyruvate sulfurtransferase-derived hydrogen sulfide regulates calcium-induced mPTP opening in adult and old rat hearts

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Hydrogen sulfide (H_2S) is a biologically active gasotransmitter, which regulates a lot of important physiological functions in the body. In the cardiovascular system H_2S de novo synthesis in belongs to pyridoxal-5-phosphate-dependent enzymes – cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (MPST). The modulation of endogenous H_2S generation in the cardiovascular system and other tissues and the functional consequence of this modulation are not clear. The formation of non-selective calcium-dependent cyclosporin-sensitive permeability transition pore (mPTP) between the outer and inner mitochondrial membranes is the basis for induction of the cell death by apoptosis. The aims of this study were to elucidate the mechanisms underlying the cardioprotective effects of H_2S in aging rats. Exogenous hydrogen sulfide in physiological concentrations depressed Ca^{2+}-induced mPTP opening in a dose-dependent fashion in adult and rat heartsl. Preincubation of isolated mitochondria with the inhibitor of mitochondrial ATP-dependent potassium channels (K_{ATP}-channels) 5-hydroxydecanoate (10^{-4} mol/l) reduced the protective effect of NaHS (10^{-5} mol/l), indicating the possible involvement of mitochondrial K_{ATP}-channels in inhibition by hydrogen sulfide of Ca^{2+}-dependent pore opening. To clarify the role of endogenous H_2S in the regulation of mPTP opening in the adult and old rats heart, in vitro experiments were carried out using propargylglycine (PG), a specific blocker of the enzyme CSE, and o-(carboxymethyl)hydroxylamine (O-CMH) (10^{-5} to 10^{-3} mol/l), a blocker of MPST. It is interesting to note that only inhibition of MPST by O-CMH causes significantly increase of Ca^{2+}-dependent mitochondrial swelling in adult and old rat heart. PG at any concentration, when added directly to the medium, had no effect on the swelling dynamics. We observed up regulation of mRNA expression of MPST in 2,3-fold in old rat heart compared with adult animals. Under condition of both PG and L-cysteine introduction to adult and old rats was shown that mRNA expression of MPST increased in 8,2-fold and 2,5-fold respectively compared to control. It has been shown that: 1) endogenous H_2S synthesized by enzyme MPST in mitochondria can be involved in the regulation of Ca^{2+}-induced mPTP opening in both adult and old rat hearts; 2) dramatic decrease in H_2S synthesis in old rat heart mitochondria was followed by an increased sensitivity of mPTP opening to Ca^{2+}; 3) the opening of K_{ATP}-channels in the heart muscles plays a significant role in the protective mechanisms of H_2S. These data provide clear evidence for the cytoprotective actions of MPST-derived H_2S in cardiac pathologies.

Keywords: heart, hydrogen sulfide, mitochondrial permeability transition pore.

MON-279

A 'Floating Boat Bridge' of cytochrome c molecules in plant respirasome

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In plants, cytochrome *c* participates in channeling electrons rather than in simply shuttling them between complexes III and IV [1]. However, the mode cytochrome *c* behaves inside a supercomplex such as the respirasome, formed by complexes I, III and IV, remains veiled from a structural point of view. Here, we report *ab-initio* Brownian Dynamics calculations and NMR-driven docking computations showing two well-defined binding sites for cytochrome *c* at the head soluble domain of cytochrome c_1, namely a non-productive (or *distal*) site with a long heme-to-heme distance and a functional (or *proximal*) site with the two heme groups close enough as to allow electron transfer. As inferred from Isothermal Titration Calorimetry experiments, the two binding sites exhibit different equilibrium dissociation constants, for both reduced and oxidized species, that are all within the micromolar range, so revealing the transient nature of such a respiratory complex. Although the docking of cytochrome *c* at the *distal* site occurs at the proximity to the interface between cytochrome c_1 and the Rieske subunit, it is fully compatible onto the complex III structure. In our model, the extra *distal* site in complex III could indeed facilitate the functional channeling of electrons towards complex IV by building a 'floating boat bridge' of cytochrome *c* molecules (between complexes III and IV) in plant respirasome.

Reference

1. Genova M.L. and Lenaz G. (2013) A critical appraisal of the role of respiratory supercomplexes in mitochondria. *Biol Chem*, 394, 631–639.

Keywords: Cytochrome c, Respirasome, Supercomplexes.

MON-280

Assessing PAT-induced mitochondrial redox status and apoptosis via mitochondria-mediated pathway

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Patulin (PAT) is a toxic metabolite produced by several filamentous fungi of the genera of *Penicillium*, *Aspergillus* and *Byssochlamys*, principally by *Penicillium expansum*. Several studies indicate there is a risk associated to the PAT intake, through the consumption of purees and apple juices. The gastrointestinal lumen is thus directly exposed to PAT. In this study, we tried to understand cell-death pathway on human colon carcinoma cells (HCT-116).

Using specific probes in flow cytometry, we assess mitochondrial redox status in terms of mitochondrial transmembrane potential (ΔΨm) and mitochondrial superoxide (mtO2·) production.

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Treated cells by PAT exhibit a loss of DYm and a downstream generation of O2·. We also demonstrated that PAT induces apoptosis in HCT-116. There has an increase in caspase3 activity following the use of NucView caspase3 substrate. The resistance of cells deficient in pro-apoptotic proteins (Bax and Bak) to treatment by PAT showed that this mycotoxin promotes the apoptosis through a mitochondria-dependent pathway. Then, to understand whether PAT-induced stress is a cause or consequence, we pretreated cells with N-acetylcysteine (Nac) and we showed that Nac restores cell viability and reduce significantly PAT-induced damages for all tested markers. It be concluded that PAT-induced oxidative stress is the major cause of cell death.

Keywords: apoptosis, Mitochondria membrane potential loss, mycotoxin.

MON-281

Beneficial metabolic changes induced by glycogen phosphorylase inhibitor treatment

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Glycogen metabolism is regulated by two antagonistic enzymes: glycogen phosphorylase (GP) and glycogen synthase. Glycogen phosphorylase is responsible for the breakdown of glycogen, hence it has major role in hepatic glucose production (HGP). Consequently, it is likely that GP inhibition would reduce blood glucose levels in type II. diabetes.

We evaluated the metabolic properties of novel glucose-based GP inhibitors KB228 (N-(3,5-dimethyl-benzoyl)-N'-(β-D-glucopyranosyl)urea, Ki = 937 nM) and BEVA335 (3-β-D-glucopyranosyl-5-(2-naphtyl)-1,2,4-triazole, K_i = 0.411 nM). In vivo, single i.p. injection of KB228 enhanced glucose sensitivity in chow-fed and high fat diet-fed, insulin resistant (C57/Bl6J mice on 60% fat hypercaloric diet for 3 months) mice that depended on glucose excursion to the liver. KB228 increased oxygen consumption suggesting enhanced mitochondrial function which was presumably linked to the overexpression of uncoupling protein-2 (UCP2). These unexpected changes were observed in animal and cellular models (HepG2) under both normoglycemic and hyperglycemic conditions. In addition, KB228 induced mammalian target of rapamycin complex 2 (mTORC2) which may take an active part in glucose influx and increased glycogen deposition in the cells.

Furthermore, our data demonstrate that such inhibitors do not only reduce glucose levels but enhance glucose-induced insulin release in mice, therefore we investigated these effects on cellular model of pancreatic β-cells, MIN6 cells. KB228 and BEVA335 induced mitochondrial activity, insulin production and facilitated insulin secretion in MIN6 β-cells after two-day treatment. Simiarly to the liver, mTORC2 activity was enhanced in MIN6 cells too by GP inhibitor treatment. KB228 and BEVA335 treatment induced MIN6 proliferation which mirrored in the pancreas of C57/Bl6J mice (chow-and HFD-fed) treated with KB228 (90 mg/kg).

We have described hereby several unexpected, though beneficial metabolic effects of glucose-based GP inhibitors that can be exploited in the future.

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Keywords: Glycogen phosphorylase inhibitors, mitochondria, mTORC2.

MON-282

Ca2+-dependent fusion pore induced by α,ω-dioic acids as a possible mechanism of the mitochondrial permeability transition

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Recently we found that the α,ω-dioic acids (among them α,ω-hexadecanedioic acid (HDA) was most effective) are able to induce the opening of the 'non-classical' cyclosporin A (CsA)-insensitive pore in the liver mitochondria loaded with Ca^{2+} or Sr^{2+}. It has been suggested that such permeability is based on the formation of a pore in the membrane of organelles which has lipid nature. In this paper, in order to clarify the molecular mechanism of this process, we studied the effect of HDA on the Ca^{2+}-dependent permeability of artificial membranes using the model of large unilamellar liposomes (LUV) loaded with the fluorescent dye sulforhodamine B (SRB). It was shown that HDA, like palmitic acid (PC), is able to induce permeability of LUV for SRB in the presence of Ca^{2+}. However, the kinetics of SRB release upon the induction of Ca^{2+}-dependent permeability of LUV by HDA and PC is different. Furthermore, it was found that HDA is inferior to PC in ability to induce LUV permeability for SRB. Under the same conditions, α, ω -tetradecanedioic acid which acyl chain is 2 carbon atoms shorter than HDA is less effective. Consequently, the effectiveness of these α,ω-dioic acids as inducers of pore opening is sharply reduced with decreasing number of carbon atoms in their acyl chain, which may be associated with a decrease in their ability to dissolve in lipids. It was established that HDA/Ca^{2+}-induced SRB release from LUV is also dependent on the pH of the surrounding solution. The maximum release of SRB from LUV induced by HDA/Ca^{2+} complex formation was observed at alkaline pH (pH 9.0–10.0). Using the method of ultrasonic interferometry it's shown that HDA, unlike PC, doesn't induce a chemotropic phase transition in the lipid bilayer of membrane in the presence of Ca^{2+}, which as was shown earlier underlies the permeability of lipid membranes induced by Ca^{2+} and PC. Thus, it can be assumed that the Ca^{2+}-dependent permeability of the LUV, induced by HDA and PC occurs by different mechanisms. The method of dynamic light scattering showed that the addition of Ca^{2+} to HDA-modified LUV induces their fusion. We believe that the process of LUV fusion may be accompanied by partial SRB release from LUV due to the formation of fusion pores.

We assumed that the process of membrane fusion may underlie the previously described HDA/Ca^{2+}-induced nonspecific permeability of the inner membrane of liver mitochondria. It is possible that HDA and Ca^{2+} induce fusion of inner and outer mitochondrial membranes in the field of contact sites where the membranes are close together, with subsequent formation of fusion pore resulting in swelling of the mitochondria. This study was supported by the Ministry of Education and Science of Russia (No 1365).

Keywords: Fusion pore, Mitochondrial permeability transition, α,ω-Dioic Acids.

MON-283

CDK4 and CDK5 inhibition prevents the Drp1-dependent mitochondrial fission and neuron death induced by MPP+

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Mitochondrial dysfunction is an early event of cell death in the neurodegenerative diseases, such as Parkinson's disease (PD).

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Mitochondrial function is closely linked to its morphological dynamics, fusion and fission (fragmentation). Aberrant mitochondrial fission controlled by mitochondrial fission protein of dynamin-related protein 1 (Drp1) may result in cell death. Our previous results have showed the involvement of Drp1-dependent mitochondrial fission in a 1-methyl-4-phenylpyridinium (MPP^+)-induced parkinsonian model. Recent studies also demonstrated that mitochondrial dynamics change at different stage of cell cycle and cyclin-dependent kinase 5 (CDK5) is involved in the regulation of mitochondrial fission during neuron apoptosis. Herein, we investigated whether CDK inhibition protects postmitotic and terminally differentiated neurons from MPP^+ intoxication by reducing the Drp1-dependent mitochondrial fission. We found that neurons overexpressed dominant negative $Drp1^{K38A}$ or the treatment of selective inhibitor of CDK4 (CDK4I) or CDK5 (roscovitin) inhibit MPP^+-induced mitochondrial fission as early as 4 h after the treatments in primary cortical neurons. There is no nuclear fragmentation at 4 h after MPP^+ treatment; however, the co-treatment of CDK4I or roscovitin significantly reduced the number of neurons with condensed nucleus and neuron death. The MPP^+-induced upregulation of mitochondrial Drp1 protein expression was significantly attenuated with more pronounced after the treatment of CDK4I than roscovitin. In the cortical neurons overexpressed wild type Drp1, the prevention of CDK4/5 inhibition in MPP^+-induced mitochondrial fission was blunted. These results reveal that CDK4 and CDK5 inhibition prevents Drp1-dependent mitochondrial fission to protect the neurons against MPP^+ toxicity.

Keywords: CDK4, mitochondrial dynamics, Parkinson's disease.

MON-284
Characterization of Mcl-1 isoforms and their impacts on cell growth and survival

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The Bcl-2 family proteins, which regulate many cell survival and death pathways, play a critical role in a variety of cellular processes under physiological as well as pathological conditions. Mcl-1 belongs to the anti-apoptotic subfamily of the Bcl-1 family protein, which is widely expressed in many cell types in the vertebrate. Mcl-1 has long been noticed to exist as two major isoforms with different gel-mobility. By extensive biochemical characterization, we demonstrated that the slow mobility (SM) isoform is a full-length protein targeted to the outer membrane of mitochondria (MOM), whereas the fast-mobility (FM) isoform is an N-terminally truncated product generated inside the mitochondrial matrix via an MPP-mediated cleavage reaction. The 33 amino acid residues at the N-terminus of the Mcl-1 protein turn out to serve both as a mitochondrial targeting and processing signal. Interestingly, unlike the full-length protein located at MOM, the mitochondrial matrix-localized isoform failed to interact with BH3-only proteins and manifested a greatly attenuated anti-apoptotic activity. To gain further insights of the Mcl-1 functions in the mitochondrial matrix, we generated Mcl-1 knock-in mutant mice (the 3RG mutant) that are defective in producing the matrix isoform. The phenotype of the 3RG mutant mice will be presented at this meeting.

Keywords: cell survival, mitochondria.

MON-285
Comparison of the effects of riboflavin and ascorbic acid on orofacial and plantar formalin induced pain

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Background: There are evidences that mitochondria play an important role in pain processing by modulating radical oxide species production as well as ATP. It is also documented that vitamin B_2 (riboflavin), a prophylactic drug in migraine therapy, is an ultimate precursor in the biosynthesis of flavin mononucleotide (cofactor in mitochondrial complex I) and that the vitamin C (ascorbic acid) is a vitamin with mitochondrial transport and functions acting as antioxidant as well as pro-oxidant.

Aim of the Study: To investigate if a single dose of the above mentioned vitamins, modulate pain perception from orofacial (OFT) and plantar (PFT) formalin tests in mice.

Methods: The experiments were done in BALBc mice and each group had at least 8 animals per group. Mice received intraperitoneally riboflavin-vitB2 (100 mg/kg), ascorbic acid-vitC (500 mg/kg) or saline-NaCl (0.1 ml/10 g b.w.) and were thereafter divided into 6 groups: OFT_{vitB2}, $OFT_{vitC,}$ $OFT_{NaCl,}$ $PFT_{vitB2,}$ $PFT_{vitC,}$ $PFT_{NaCl.}$ The nociceptive score was determined by recording the number of seconds animals spent grooming the injected area and expressed as mean ±SEM separately for the 2 phase of the formalin test, the neurogenic (first) respective inflammatory (second) phase. Data was analyzed using ANOVA and the Post-hoc comparisons between groups was determined using the Bonferroni-test.

Results: The B_2 and C vitamins significantly modified pain perception in both phases of OFT and the second phase from PFT. When compared with control group (148 ± 9s), only the riboflavin had analgesic effect on the second phase of OFT (81 ± 15s, $p = 0.025$). By contrary both riboflavin (36 ± 9s, $p < 0.001$) and ascorbic acid (81 ± 18s, $p = 0.013$) had analgesic effect on the second phase of PFT when compared with saline group (145 ± 12s). No significant statistical differences between the vitamins analgesic effect were recorded in PFT.

Discussions: By the use of riboflavin and ascorbic acid we expect to transitory modulate mitochondrial function and thus the ATP and/or ROS production. In our study a single dose of B_2 and C vitamin modulate pain perception in both models of pain. Although B2 vitamin acts on both OFT and PFT, vitamin C modifies only the PFT. Thus, we can hypothesize, that mitochondrial ATP is more important in pain modulation then ROS production or that the pain from OFT and PFT have different mechanisms.

Taken together, our study demonstrates that ascorbic acid and riboflavin modulate pain perception after a single dose, but the mechanisms that could be linked to mitochondrion modulation require further investigation.

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Keywords: free radicals, inflammatory pain, mitochondria.

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MON-286

Cox26 – a novel component of the cytochrome c oxidase required for proper enzymatic function

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Cellular respiration is a basic mechanism for energy conversion. Oxidative phosphorylation (OXPHOS), which is carried out in the mitochondria of eukaryotic cells, provides energy in the form of ATP. The protein complexes constituting the OXPHOS machinery are located in the inner mitochondrial membrane. Complexes I to IV comprise a respiratory chain, transferring electrons in a series of redox reactions. The electron transfer is coupled to proton pumping across the inner membrane, and thus establishes a proton gradient, which is then used by complex V (ATP synthase) to generate ATP. The respiratory chain complexes form higher oligomeric structures called supercomplexes or respirasomes to promote efficient electron transport.

Cox26, a protein of a yet unknown function, is a constituent of respiratory chain supercomplexes in yeast *Saccharomyces cerevisiae*. We have identified Cox26 as a novel subunit of the cytochrome c oxidase (COX), the terminal enzyme of the mitochondrial respiratory chain. Yeast cells lacking Cox26 are affected in cytochrome c oxidase function and exhibit a decrease in COX enzymatic activity. Furthermore, the absence of Cox26 influences respirasomes formation and leads to a decreased association of the cytochrome c oxidase with supercomplexes.

Keywords: cellular respiration, cytochrome c oxidase, mitochondrial electron transport chain.

MON-287

Cross-talk between the PINK1/Parkin pathway and the ER-mitochondria interface: role in the regulation of mitochondrial physiology

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The kinase PINK1 and the E3 ubiquitin ligase Parkin, products of two genes responsible for familial Parkinsonism, participate in the same mitochondrial quality control mechanism. Upon depolarization of the mitochondrial membrane, the PINK1/Parkin pathway gets activated, leading to increased fission, outer membrane rupture and finally complete elimination of damaged organelles by mitophagy. Our team has identified the TOM (Translocase of the Outer Membrane) complex as a molecular switch in the pathway. Upon $\Delta\psi$ collapse, Parkin is recruited by PINK1 to the TOM machinery and preferentially interacts with the TOMM70 receptor. Further proteolytic destabilization of TOM core components primes mitochondria for mitophagy.

There is growing evidence that ER-mitochondria contacts, responsible for lipid and Ca^{2+} exchanges between the two compartments, play a crucial role in Parkin-dependent mitochondrial clearance. Furthermore, our own recent results suggest that the ER-mitochondria interface is regulated by the PINK1/Parkin pathway.

More precisely, we have observed both by confocal and electron microscopy that the ER-mitochondria interface is significantly enhanced in Parkin-deficient cells. This alteration is most likely at the origin of the mitochondrial Ca^{2+} overload observed in these cells. Though we were not able to detect Parkin at ER-mitochondria contacts, using a subcellular fractionation-based approach, we demonstrated the presence of PINK1 and TOM subunits, including TOMM70, at this interface. In parallel, we showed that the ER-mitochondria tether Mfn2 is increased in abundance in tissue from Parkin-deficient mice, specifically at the ER-mitochondria interface, suggesting that it is a central target of Parkin at this interface, possibly responsible for the observed perturbations in Parkin-deficient cells.

We are currently using fluorescence microscopy and Ca^{2+} imaging in order to elucidate the involvement of the PINK1/Parkin pathway in the regulation of ER-mitochondria contacts and their function. These studies should give new insight into how PINK1 and Parkin regulate mitochondrial physiology and how loss of their function contributes to the development of Parkinsonism.

Keywords: Calcium homeostasis, Mitochondrial dysfunction, Parkin.

MON-288

Cytochrome c6 binds cytochrome f to form an oriented yet unspecific ensemble

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Fast redox exchange within the photosynthetic electron transport chain relies on short-lived nature of the complexes between cytochrome b_6f and its acceptors, namely plastocyanin and cytochrome c_6. This complex must conciliate its ability for a swift intermolecular electron transfer and a rapid dissociation and, hence, a limited specificity. To dig into this issue, we have studied the interaction between cytochrome c_6 and the soluble fragment of cytochrome f from the cyanobacterium Nostoc sp. PCC 7119 by NMR spectroscopy. To avert redox exchange, the low potential M58C variant of cytochrome c_6 was used instead of the wild-type protein to characterize the complex with cytochrome f by chemical shift perturbation and paramagnetic relaxation NMR experiments. Notably, the interaction is highly dynamic, as expected from a pure encounter complex, without any dominant conformation. Ensemble docking computations and Monte-Carlo simulations suggest a model in which charge–charge interactions pre-orient cytochrome c_6 with its haem edge facing cytochrome f, still yielding a wide assortment of conformations. Hydrophobic contacts between both cytochromes bring the two haem groups close enough to allow a fast electron transfer. The resulting balance between non-polar and electrostatic interactions along the lifetime of the complex is responsible for its dynamics and avoids any high-energy transition state barrier to slow down the dissociation process.

[1]Díaz-Moreno I., *et al. Biochim. Biophys. Acta-Bioenergetics* (2014) 1837, 1305–1315.

[2]Díaz-Moreno I., *et al. FEBS Lett*. (2005) 579, 2891–2896.

Keywords: Electron transfer, Paramagnetic NMR, Transient complex.

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MON-289
Cytoprotective role of TASK-3 in the mitochondria of human keratinocytes

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The activation of mitochondrial potassium channels induces cytoprotection in various cell types.

Inner membrane mitochondrial ion channels of the human keratinocyte HaCaT cell line were investigated using a patch-clamp technique, reverse transcriptase–PCR and immunochemistry to confirm presence of TASK-3 channel. We showed that TASK-3 knockdown HaCaT cells markedly decreased viability after UVB radiation exposure compared with control cells.

Keywords: mitochondrial potassium channel, TASK-3 channel, cytoprotection.

MON-290
Cytosolic and intramitochondrial cAMP produced by soluble adenylyl cyclase control in mammalian cells the subunits turnover and activity of the respiratory chain complex I

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In mammalian cells the nuclear-encoded subunits of complex I are continuously imported into mitochondria, where are incorporated together with mitochondrial-encoded subunits to form the mature complex or separately exchanges with pre-existing copies in the complex. The results of our work show that both the cytosolic cAMP, produced by plasma membrane adenylyl cyclase, and intramitochondrial cAMP, produced by the soluble adenylyl cyclase, modulate at post-translational level the concentration of nuclear-encoded subunits of the mitochondrial exposed catalytic moiety of the complex and consequently its NADH-ubiquinone oxidoreductase. The cytosolic cAMP/PKA promote the mitochondrial import/maturation of these subunits, the intramitochondrial cAMP stabilizes the mitochondrial level of the subunits adequate for their assembly in the functional complex by preventing their degradation by mitochondrial Lon protease.

Keywords: cAMP, mitochondria, sAC.

MON-291
Diagnostic value of activity of mitochondrial enzymes activity in children with the hepatic form of glycogen storage disease

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Glycogen storage disease (GSD) are a group of inherited diseases, accompanies with carbohydrate metabolism disorders, when the type of disease I, III and VI, enzyme deficiency implements in glycogen accumulation in liver. This errors leads to others metabolic ways errors, which lead to disturbance in mitochondria function.

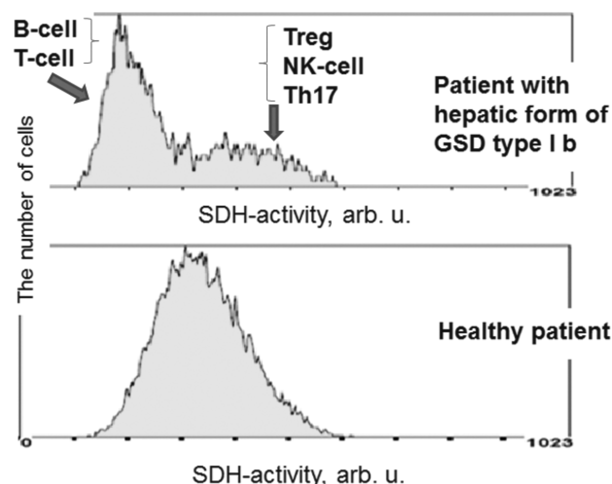

Fig. 1. The heterogeneity of the SDH activity in the whole lymphocyte populations.

Aim: To evaluate the diagnostic value of the mitochondrial enzymes activity in children with the hepatic form of GSD.

Patients and methods: We examined 106 children with hepatic form of GSD at age from 1 to 17 years. The distribution by sex was 36 girls, 70 boys. Control group consisted of 34 healthy children.

Lymphocytes enzymes activities (succinatedehydrogenase (SDH) and NADH-dehydrogenase(NADH-H) are respiratory chain enzymes (complex I and complex II respectively), moreover SDH is also the Krebs cycle enzymes, that catalyzed the oxidation succinate to fumarate) were measured using the quantitative cytochemical method with a help cytodensitometry (Videotest, Russia) and SDH activity in lymphocytes subsets was measured by flow cytometry (Beckman Coulter FC500 (USA)).

Results: We found SDH activity decrease, along with NADH-D activity increase in children with hepatic form of GSD. SDH/NAH-D index was decrease in GSD-patients compared with the control group. The most significant alterations were found in type-I of GSD.

We found the heterogeneity of the SDH activity in the whole lymphocyte populations. SDH activity was decreased in B-cells and T-cells along with SDH activity was increased of NK-cells, Treg and TH17-lymphocytes (Fig 1).

High correlation between base excess, hepatomegaly and lymphocytes enzymes activitywas found RBE = 0.99 and R hepatomegaly = 0.87 respectively in GSD patients.

Conclusion: Our investigation revealed features of mitochondrial dysfunction in children with GSD. We found changes of the lymphocytes subsets number and their functional activity, this findings correspond to increased infectious diseases rate in children with GSD. Dehydrogenases activity of lymphocytes correlates with the main clinical - laboratory parameters, and can be used as additional diagnostic criteria of energy metabolism disorders and for the assessment of the severity of children with GSD.

Keywords: Glycogen storage disease, mitochondrial dysfunction, mitochondrial enzymes activity.

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MON-292
DNA helicases involved in the maintenance of the plant organellar genomes

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The dynamic state of the plant mtDNA is due to recombination processes involving repeated sequences that modulate its structure. Large repeats are interconvertible by homologous recombination (HR) and contribute to the multipartite structure of mtDNA, while rare ectopic recombination occurs among small repeats and generate alternative mtDNA configurations at substoechiometric levels, which contribute to the rapid evolution of the mtDNA structure in plants. HR is thus important in mtDNA maintenance and evolution but is also involved in repair and replication mechanisms.

Several organellar proteins have been identified as implicated in the control of ectopic HR or in mechanisms of HR-dependent repair. But many additional factors remain to be identified, including the DNA helicases involved in recombination surveillance which are postulated to be required for rejection of ectopic HR intermediates .

We identified an Arabidopsis DNA helicase homologous to the bacterial DNA helicase RecG, that we called RECG1. Bacterial RecG acts as a translocase in DNA recombination, repair and replication. It promotes holliday junctions translocation, the regression of replication forks and avoiding replication re-initiation when replication forks collide. GFP fusion showed that Arabidopsis RECG1 is dually targeted to mitochondria and plastids. Promoter-GUS fusion showed that the gene is expressed in most plant tissues, in particular in leafs and root vascular systems. The Arabidopsis RECG1 is able to complement its bacterial orthologue for the repair of UV-induced DNA repair, but not the additional bacterial DNA translocase RuvAB. Using T-DNA insertion mutants we tested the role of RECG1 in the surveillance of ectopic HR and the repair of DNA damage induced by ciprofloxacine. We found that RecG1 affects the efficiency of mtDNA repair by recombination. In addition, loss of RECG1 results in increased ectopic HR of the mtDNA having as consequence specific changes in the segregation of mtDNA sequence.

Keywords: mitochondrial DNA, Plants, recombination.

MON-293
Evaluation of the action of a new dihydropyridine derivative, YV-241, in head and neck squamous cell carcinoma

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Mitochondria are organelles responsible for both ATP production and maintenance of cellular life. Transformed cells present genetic, molecular and biochemical alterations in mitochondria, which may be either causal or contributing factor for cancer development. Mitochondrial dysfunction has been associated with defects in the apoptosis pathway in solid tumors, including head and neck squamous cell carcinoma (HNSCC). Therefore, it is believed that anticancer agents targeted to mitochondria of cancer cells present reduced effects in normal cells. In the present study, we assessed a new dihydropyridine derivative YV-241 with respect to cell viability and mitochondrial membrane potential ($\Delta\Psi_m$), and also the type of cell death promoted by the compound. Cell viability assay was employed in a human HNSCC cell line (HN13) and a normal cell line (primary culture of human fibroblast). The reduction of resazurin (blue and nonfluorescent) to resorufin (pink and highly fluorescent) indicates cell proliferation and viability. The HN13 cancer cell line was more sensitive ($IC_{50} = 7.55$ μM) than the normal human fibroblast, which presented an IC_{50} of 23.76 μM. The JC-1, a cationic carbocyanine dye that accumulates in mitochondria, is a $\Delta\Psi_m$ marker. The HN13 cell line showed a concentration-dependent decrease of $\Delta\Psi_m$ in the presence of the compound, while fibroblast was not significantly affected. The cellular alterations in the presence of the compound were assessed by transmission electron microscopy and cell death signaling by analysis of apoptosis markers such as PARP, caspase-3, caspase-8, as well as cytochrome *c* release from mitochondria. YV-241 presented a cytotoxic effect apparently via apoptosis, with mitochondrial impairment involvement. In this context, YV-241 presents a potential action against cancer cells and mitochondria are the probable target.

Financial Support: CNPq, CAPES and FAPESP, Brazil.
Keywords: antitumor drug, mitochondria, oral cancer.

MON-294
Exercise training decreased the sensitivity of calcium-induced mitochondrial permeability transition pore opening via NO-dependent mechanism in rat heart

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Aging is characterized by a decline of cardiac function and an increase of oxidative stress, which are major contributors to cell death through mitochondrial dysfunction. Mitochondrial permeability transition pore (MPTP) opening plays a significant role in the transition of mitochondria from a physiological condition to induction of cell death. In contrast, exercise training not only improves cardiac function, but also reduces the risk of heart disease. However, the mechanisms by which exercise training improves heart function and cardiovascular disease risk profile are not understood. The purpose of the present investigation was to study the effect of long-term exercise training on the sensitivity of calcium-induced MPTP opening in adult and old rats including the activity and gene expressions of isoforms of nitric oxide synthases (NOS) such as constitutive NOS (cNOS), which including neuronal NOS (nNOS) and endothelial NOS (eNOS), and also inducible NOS (iNOS)) in this process. Exercise training animals were subjected to dosed physical load carried by the forced swimming five days a week for six weeks for adult rats (5 month) and four weeks for old rats (24 month). We found that mitochondria isolated from hearts of the adult and old trained rats demonstrated the similar decrease in sensitivity to Ca^{2+} of the MPTP-opening in range concentration 10^{-7} to 10^{-4} mol/l compare to untrained. We hypothetized that such decrease in sensitivity of the MPTP-opening occurred due to increased production of NO by cNOS during exercise. Our results demonstrated that administration of NO syhnthase inhibitor N(omega)-nitro-L-argi-

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nine methyl ester (L-NAME) in dose of 10 mg/kg to trained rats increased the sensitivity of MPTP-opening to Ca^{2+} in range concentration 10^{-7} to 10^{-4} mol/l. Analysis of NOS isoforms responsible for NO production during exercise revealed significant age differences. In adult trained rats we observed significant stimulation of the cNOS activity and noticable elevation in the iNOS activity. Moreover, the gene expressions of nNOS and iNOS were increased by 5-fold and 24-fold respectively while eNOS was decreased by 3-fold. Alternatively, in old trained rats, the activity of cNOS was modestly increased while the activity of iNOS was decreased. Correspondently, the gene expressions nNOS and iNOS were decreased by 4-fold and 2-fold respectively while eNOS was increased by 3-fold. Taken together, our data suggest that of long-term exercise training reduces the sensitivity of the MPTP opening to the action of calcium ions by increasing the synthesis of nitric oxide - an endogenous inhibitor of MPTP opening.

Keywords: exercise training, mitochondrial permeability transition pore, nitric oxide.

MON-295
Functional roles of nitric oxide in roots and mitochondria

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Nitric oxide (NO) has emerged as an important signal molecule in plants. Reductive and oxidative pathways are involved in NO production. Nitrite is produced by cytosolic nitrate reductase (NR) and then converted to NO by the same enzyme with nitrite reductase activity. Mitochondrial complex III and IV are the sites for nitrite to NO reduction. Nitrite reduction to NO increases as oxygen availability decreases. The reaction leads to ATP synthesis. NO generated in mitochondria converted to nitrate by the hypoxically-induced haemoglobin in cytosol. Under hypoxia plant mitochondrion serves as a nitrite: NO reductase and becomes a major component in anoxic nitrogen cycling where it directly contributes to a decrease of cell reduction level and to limited ATP synthesis. We found that NO inhibits aconitase in mitochondria and increases citrate which then acts as a potent inducer of AOX expression. NO production, inhibition of aconitase, and induction of AOX also leads to a shift of plant metabolism towards amino acid biosynthesis, since citrate is a precursor for the 2-oxoglutarate required for glutamate synthesis.

Keywords: alternative oxidase, mitochondria, nitric oxide.

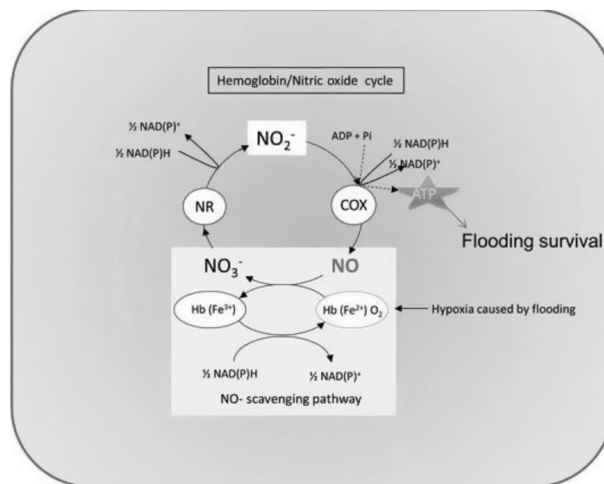

Fig. 1.

MON-296
Functional study of mitochondrial UQCRB, a terpestacin-binding protein, in angiogenesis

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Ubiquinol-cytochrome c reductase binding protein (UQCRB), one of the subunits of mitochondrial Complex III, is a target protein of anti-angiogenic natural small molecule, terpestacin. Previously, the biological role of UQCRB was limited to the maintenance of Complex III, however, identification and validation of the protein target of terpestacin have enabled to uncover the role of UQCRB in oxygen-sensing and angiogenesis. To explore the biological role of UQCRB, UQCRB mutant cell lines was generated on the basis of human case report. In this case, the UQCRB mutant implies gain-of-function phenotype of UQCRB. UQCRB mutant stable cell lines exhibit pro-angiogenic activity via mitochondrial ROS-mediated HIF1 signal transduction. Also, morphological abnormality of mitochondria is detected in UQCRB mutant stable cell lines. In addition, proliferative effect of UQCRB mutant is significantly regulated by terpestacin. Further studies including analysis of mitochondrial function of UQCRB mutant stable cells will be presented. Collectively, this study can provide molecular basis underlying UQCRB-related biological process and reveal potential key roles of UQCRB in various cancers and metabolic diseases.

Keywords: angiogenesis, mitochondria, ROS.

MON-297
Hydrogen peroxide-induced gain of peroxidase activity of cytochrome c upon phosphate-mediated binding to zwitterionic lipids: implications for apoptosis

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Cytochrome c (Cyt-c) has been shown to participate in cardiolipin (CL) oxidation and, therefore, in mitochondrial membrane permeabilization during the early events of apoptosis. The gain of peroxidatic activity has been ascribed to CL/Cyt-c interactions which, nevertheless, are also ubiquitous in healthy cells, thereby posing the question of which is the triggering event.

We observed that the cationic protein Cyt-c is able to interact electrostatically with the main lipid components of the mitochondrial membranes, the zwitterionic lipids phosphatidylcholine and phosphatidylethanolamine, through mediating phosphate anions that interact specifically with amino groups in the surfaces of the protein and the membrane models[1]. We have investigated the chemical reactivity of Cyt-*c* associated to model systems and in complexes with PE- and PC-liposomes using a combination of electrochemistry, Raman, fluorescence and mass spectroscopy. In these complexes Cyt-c reacts efficiently with H_2O_2 at submillimolar levels, which specifically sulphoxides the axial ligand Met80

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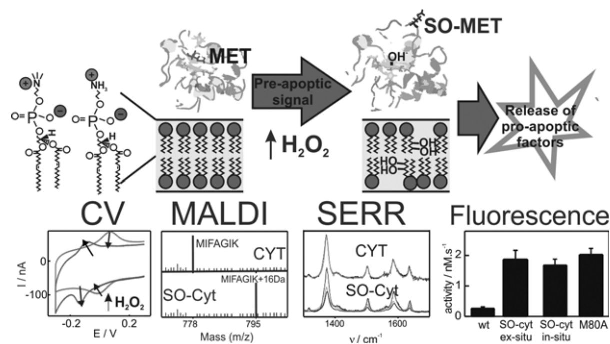

Fig. 1.

(SO-Cyt). The modified protein is stable and presents significantly enhanced peroxidatic activity.

Based on these results, we postulate that the rise of H_2O_2 levels from submicromolar to millimolar registered during the initiation of the apoptotic program may represent one signaling event that triggers the gain of peroxidatic function of Cyt-c.

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Keywords: ageing signals, apoptosis, Cytochrome c.

MON-298
Hyperpolarization of isolated mitochondria triggers mitophagy in OPA1-mutated fibroblasts

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Mitochondria integrity is regulated by mitophagy, a process which subjects damaged mitochondria to degradation. Mitophagy was shown to be monitored by dynamic events involving fission and fusion of mitochondrial network subsequently engaging mitochondrial membranes remodeling. Mutations on actors of this dynamism among which Optic Atrophy 1 (OPA1) lead to a heterogeneous group of human neurodegenerative disorders. Human studies on OPA1-mutated fibroblasts from patients have shown bioenergetics deficiency and a more or less fragmented mitochondrial network associated with a drop in mitochondrial mass. These data suggest a regulatory role of these dynamic events and their actors in mitochondrial biogenesis, elimination and turnover. Our objective was, combining biomolecular strategies and standardized-imaging approaches using micropatterned coverslips, to highlight an impaired mitophagy in a pathological context affecting mitochondrial dynamics. Our studies have shown that OPA1 mutations associated with high mitochondrial network fragmentation are characterized by a drop in mitochondrial volume per cell, whereas mtDNA content remain rather constant if not increased. Single-cell imaging coupled with 3D-image analysis showed an increased mitochondria-autophagolysosmes contact and immunoblotting analysis comfort these findings with increased autophagy biomarkers activation; thus reflecting an active basal degradation rate of cellular components. Unexpectedly, as opposed to studies correlating drop of mitochondria potential membrane to mitophagy level, imaging approaches showed that isolated mitochondria displayed a higher membrane potential despite a high degradation level in our model. This is concomitant to OXPHOS subunits' expressions which remain rather constant despite an enhanced degradation. These findings led us to the following hypothesis in which OPA1

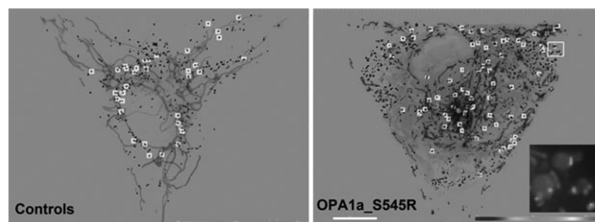

Fig. 1.

mutations associated with fragmentation and cristae disorganization lead to unsuitable OXPHOS repartition. The enhanced energetic activity relative to OXPHOS density seems to correlate with mitochondria elimination; thus highlighting a new stimulus to mitophagy activation. Moreover, studies show that this boosted mitophagy is associated with an impaired biogenesis, resulting in mitochondria turnover imbalance; thus placing mitochondrial degradation machinery as a potential target for future therapeutic strategies.

Keywords: Autophagy, Mitochondrial dynamics,3-D image analysis.

MON-299
Identification and functional reconstitution of an *Aspergillus fumigatus* uncoupling protein

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Aspergillus fumigatus is an opportunistic pathogen that causes invasive infections in immunocompromised hosts. Previous studies suggested the presence of alternative compounds on its electron transport chain (ETC), such as uncoupling protein (UCP). UCPs are mitochondrial carriers, activated by fatty acids and sensitive to purine nucleotides, which dissipate electrochemical proton gradient generated by ETC during respiration. Here, coding sequences of three mitochondrial carriers [*Af*DC: dicarboxylate carrier (XP_755633), *Af*OAC: oxaloacetate transporter (XP_753416) and *Af*UCP (XP_755831)] were functional reconstituted for assessment of their linoleic acid-induced uncoupling activity. Full-length cDNAs were cloned into pET28a vector. Heterologous expression in *Escherichia coli* was carried out for 4 hours at 37°C after 1 mM IPTG induction. Recombinant proteins were purified from inclusion bodies and solubilized in medium containing 1.67% sodium lauroylsarcosinate and 4% decylpolyoxyethylene at 4°C for 2.5 h. Lipid films were prepared from ethyl ether solution of lecithin, cardiolipin and phosphatidic acid by a stream of nitrogen. Proteoliposomes were prepared with a lipid/protein ratio of 410:1. H^+ fluxes were monitored by a fluorimetric assay based on the quenching of SPQ probe by TES^-. Vesicles containing *Af*UCP and *Af*OAC were able to transport H^+ induced by linoleic acid. However, only proteoliposomes containing *Af*UCP had that H^+ efflux inhibited by ATP, which characterize a UCP-like activity. Furthermore, we performed the heterologous expression of *Af*UCP in *Saccharomyces cerevisiae* and its uncoupling activity was demonstrated by poten-

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tial membrane measurements using the safranine O method. The differences in yeasts mitochondrial coupling were analyzed by monitoring $\Delta\psi$ changes during ADP phosphorylation supported by NADH oxidation. Our results showed that, compared with control cells, mitochondrial electrical transmembrane potential of transformant spheroplasts was slightly smaller and the transient $\Delta\psi$ decrease associated with ADP phosphorylation was longer, indicating an uncoupling activity of respiration. The results presented in this work provide evidence that *Af*UCP (XP_755831) protein, expressed in prokaryote and eukaryote system, exhibited functional features of an uncoupling protein, transporting H^+ in a process stimulated by fatty acids and inhibited by purine nucleotide.

Keywords: Aspergillus fumigatus, mitochondria, uncoupling protein.

MON-300
Impact of miR-378* and its target desmin intermediate filament on mitochondria distribution and respiration in cardiomyocytes

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Background: MiR-378 and miR-378* microRNAs are derived from an intron of the PGC-1β gene, a regulator of mitochondrial biogenesis. Their expression is either repressed or increased during heart failure depending on the model. Through proteomics approaches, we identified new targets of these miRs in H9c2 fetal cardiomyoblasts, among which mRNAs coding proteins involved energy metabolism, endoreticulum stress response and cytoskeletal proteins for miR-378* (1).

Aims: To better assess its role in energy metabolism and differentiation; we overexpressed miR-378* in primary neonate rat cardiomyocytes (NRC) that are more differentiated than H9c2 cardiomyoblasts.

Results: We identified desmin as a new target of miR-378* in NRC. Desmin is a muscle-specific type III intermediate filament that forms a cytoskeletal lattice, which has been proposed to play a key role as a structural integrator of myofibrils and mitochondria positioning. Confocal microscopy analysis showed that miR-378* overexpression altered desmin filaments organization in NRC. It also decreased the basal-to-maximal respiration ratio and ATP synthase coupled respiration as assessed by Seahorse mitochondria stress test assay. Confocal microscopy analysis of NRC stained with the mitochondrial dye MitoTracker revealed that miR-378* overexpression alters mitochondria distribution in the cell and western blot analysis revealed a down-regulation of COX1 and COX4 components of the respiratory chain. AAV-mediated rescue of desmin expression in presence of miR-378* preserved mitochondria distribution.

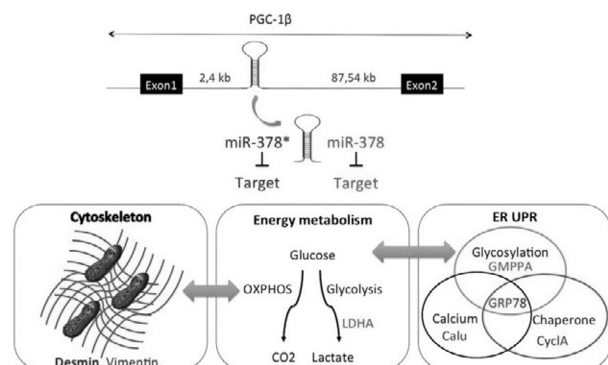

Fig. 1.

Conclusion and perspectives: These results show that miR-378* overexpression in cardiomyocytes alters their oxidative capacities suggesting that miR-378* has an antagonistic action to its host gene PGC-1β. Originally this repressive action could be mediated in part by the alteration of the coupling between desmin filaments cytoskeletal network and mitochondria, two cellular components whose organization is severely perturbated in the context of heart failure.

(1) Proteome Modulation in H9c2 Cardiac Cells by microRNAs miR-378 and miR-378. Mallat Y et al. Mol Cell Proteomics. 2014; 13:18–29.

Keywords: desmin, microRNA, mitochondria.

MON-301
Influence of p53 on ISR gene expression under mitochondrial dysfunction

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Tumor suppressor p53 is activated in response to different cellular stresses, particularly as a result of mitochondrial dysfunction induced by the inhibition of mitochondrial respiratory chain complex III. ISR (Integrated Stress Response) is a program of gene expression regulated by transcription factor ATF4 and aimed to increase cell survival. The interrelation of p53 activation and ISR gene expression under mitochondrial dysfunction has been studied in this work.

Gene expression in human colon cancer cells exposed to mitochondrial respiratory chain complex III inhibitor myxothiazol has been analyzed by RT qPCR. Expression of ATF4 and several its target genes has been found to be induced at early time points (5 h), but drop at later time points (13 h) after activation of p53 tumor suppressor. Prevention of myxothiazol-induced p53 activation by uridin supplementation resulted in recovery of increased expression of ISR-related genes.

In contrast, preliminary p53 activation by nutlin-3 before addition of complex III inhibitor has led to the abolishment of ATF4 gene induction in response to short incubation with myxothiazol. The findings indicate that p53 activation negatively regulates expression of ISR genes induced at early stage of III complex inhibition, probably due to suppression of transcriptional factor ATF4.

This conclusion is consistent with the fact that in contrast to complex III inhibition mitochondrial respiratory chain complex I inhibition by piericidine did not result in p53 activation and led to the long-term enhanced expression of ATF4 and its target genes.

Work was supported by Russian Foundation for Basic Research grant 12-04-01444.

Keywords: Mitochondrial dysfunction, Tumor suppressor p53.

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MON-302
Interaction between VDAC1 and ALS-linked SOD1 mutants: implications for mitochondria health

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Mutations in superoxide dismutase 1 gene (hSOD1) represent the first cause of familial form of Amyotrophic Lateral Sclerosis. Most of ALS-linked SOD1 mutants still show enzymatic activity, suggesting that the SOD1-mediated disease is caused by the gain of some unknown toxic properties, such as impairment of mitochondrial function. As observed in affected cells, mitochondria appear enlarged, vacuolated and disorganized [1]; in addition, the most diffused mutants SOD1, G93A and G85R, have been found associated with the outer mitochondrial membranes (OMM) [2], suggesting that SOD1 aggregates may be responsible of the mitochondrial degeneration. As recently demonstrated, this SOD1 accumulation on OMM is made possible by the presence of the Voltage-Dependent Anion Channel isoform 1 (VDAC1) [3]. VDAC1 is the main pore-forming protein of OMM, directly involved in metabolic cross-talk between mitochondria and cytoplasm [4]. VDAC1 sequence is highly conserved in eukaryotes: e.g., a high level of similarity was found between VDAC1 and por1, its homologous in yeast *S. cerevisiae*. It has been shown that expression of corresponding ALS-linked yeast SOD1 mutant G93A, in ΔSod1 strain, promotes accumulation of this protein on OMM [5], underlining the suitability of yeast to study the distribution of mutants SOD1 between cytosol and mitochondria. To better understand the role of VDAC in mitochondrial impairment SOD1-mediated, the yeast strain lacking of endogenous porin (Δpor1) was used in this work. As reported, Δpor1 is not able to grow in a not fermentable carbon source (glycerol) at 37°C, while VDAC1 and 2 complement the growth defect. Exploiting this feature, we analyzed the effect of co-presence of human forms of VDAC and SOD1 proteins on yeast phenotype. Our preliminary data show that no effect on yeast growth was observed with fermentable glucose source; on the contrary, with glycerol, the overexpression of human wild-type SOD1 or misfolded G85R in presence of VDAC1 strongly inhibits yeast growth. Surprisingly, the human SOD1 mutants G93A and G37R, are able to restore and ameliorate yeast growth. These data highlight how the activity of SOD1 proteins are linked to the mitochondrial metabolism and are mediated by VDAC. Use of VDAC1 mutants or chimeras as in [6] has allowed us to propose the VDAC regions involved in the process.

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Authors acknowledge ARISLA and PRIN 2010 funding.
Keywords: ALS-linked SOD1 mutants, Mitochondrial metabolism, VDAC.

MON-303
Interaction of the mitochondrial ISC proteins Yah1 and Isu1 studied by NMR spectroscopy

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Mitochondria contain the complex Iron-Sulfur-Cluster Assembly (ISC) machinery which is crucial for the maturation of both mitochondrial and cytosolic iron-sulfur (Fe/S) proteins in eukaryotes. Here, we determined the 3D structure of the mitochondrial [2Fe-2S] ferredoxin Yah1 in both redox states through NMR spectroscopy performing classical and non-conventional NMR experiments. In the latter approach, paramagnetic tailored NMR experiments were used to overcome fast relaxation of nuclei close to the Fe/S cluster which makes their detection impossible with standard approaches. Thereby, backbone resonance assignments for 92 and 88 out of 115 amino acid residues were obtained for oxidized and reduced Yah1, respectively. The undetected residues (stretches 96–108 and 143–145 in oxidized Yah1; 95–109 and 142–146 in reduced Yah1) were all located close to the [2Fe-2S] cluster. The NH signals in the C-terminal region (160–169), i.e. far from the cluster, were also extensively broadened suggesting an unstructured polypeptide chain. For better insight into the biochemical nature of the protein interaction, we used NMR spectroscopy combined with several biophysical and biochemical methods to structurally determine the contact interface between Yah1 and Isu1. To explore the regions of interaction between Yah1 and Isu1 we performed NMR titrations through ^{1}H-^{15}N HSQC spectra under anaerobic conditions. In the presence of Isu1, spectral changes were observed only for reduced but not for oxidized Yah1, consistent with the affinity measurements by the equilibrium interaction method thermophoresis. Residues of Yah1 with largest variation were Ala133, Tyr134, Gly135, Gln88, Glu95, and Ile110. These residues are located in a region surrounding the [2Fe-2S] cluster on the α3 helix side, and likely represent the Yah1-Isu1 interaction region. Conspicuously, a group of acidic residues (Gln88, Glu95, Asp128, and Asp131) in helix α3 is present in the interaction region, suggesting a key role of these electrostatic residues in electron transfer to Isu1. The final goal of this study is the understanding of the role of Yah1 as an electron donor in Fe-S protein biosynthesis.

Keywords: Iron Sulfur Cluster, NMR Spectroscopy, Paramagnetic NMR.

MON-304
Interaction of the mitochondrial ISC proteins Yah1 and Isu1 studied by NMR spectroscopy

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Mitochondria contain the complex Iron-Sulfur-Cluster Assembly (ISC) machinery which is crucial for the maturation of both mitochondrial and cytosolic iron-sulfur (Fe/S) proteins in eukaryotes. Here, we determined the 3D structure of the mitochondrial [2Fe-2S] ferredoxin Yah1 in both redox states through NMR spectroscopy performing classical and non-conventional NMR experiments. In the latter approach, paramagnetic tailored NMR experiments were used to overcome fast relaxation of nuclei close

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to the Fe/S cluster which makes their detection impossible with standard approaches. Thereby, backbone resonance assignments for 92 and 88 out of 115 amino acid residues were obtained for oxidized and reduced Yah1, respectively. The undetected residues (stretches 96–108 and 143–145 in oxidized Yah1; 95–109 and 142–146 in reduced Yah1) were all located close to the [2Fe-2S] cluster. The NH signals in the C-terminal region (160–169), i.e. far from the cluster, were also extensively broadened suggesting an unstructured polypeptide chain. For better insight into the biochemical nature of the protein interaction, we used NMR spectroscopy combined with several biophysical and biochemical methods to structurally determine the contact interface between Yah1 and Isu1. To explore the regions of interaction between Yah1 and Isu1 we performed NMR titrations through ^{1}H-^{15}N HSQC spectra under anaerobic conditions. In the presence of Isu1, spectral changes were observed only for reduced but not for oxidized Yah1, consistent with the affinity measurements by the equilibrium interaction method thermophoresis. Residues of Yah1 with largest variation were Ala133, Tyr134, Gly135, Gln88, Glu95, and Ile110. These residues are located in a region surrounding the [2Fe-2S] cluster on the a3 helix side, and likely represent the Yah1-Isu1 interaction region. Conspicuously, a group of acidic residues (Gln88, Glu95, Asp128, and Asp131) in helix a3 is present in the interaction region, suggesting a key role of these electrostatic residues in electron transfer to Isu1. The final goal of this study is the understanding of the role of Yah1 as an electron donor in Fe-S protein biosynthesis.

Keywords: Iron Sulfur Cluster, NMR characterization, Protein - protein interactions.

MON-305
Interplay between mitochondrial membrane potential loss, microglia, and neuron causes massive neuronal loss

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Depolarization in mitochondrial membrane potential (ΔΨm loss) have been closely associated with many neurodegenerative disorders. However this relationship has not been clearly demonstrated. By co-culture with neuron and microglia and CCCP treatmentm, we established simple and biological relevant in vitro neurophathological mimic system for neurodegeneration caused by ΔΨm loss. Using this system, we have demonstrated that neuronal loss is triggered by phagocytosis-enhancing effect of microglia to neighboring neuron with ΔΨm loss. Notably, at cell morphological and molecular levels, we show that microglia more vlunerable to ΔΨm loss than neuron, which is correlate with microglial activation. Our results provide important evidence that it is necessary to microglia activated- as well as neuron damaged by ΔΨm loss for ΔΨm loss-associated with neurodegenerative progression. Furthermore, these findings will contribute to new strategy for targeted therapeutics regulated microglial ΔΨm in progressive neurodegenerative disorder. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (NRF-2010-0027963).
Keywords: microglial activation, mitochondrial depolarization, neuron loss.

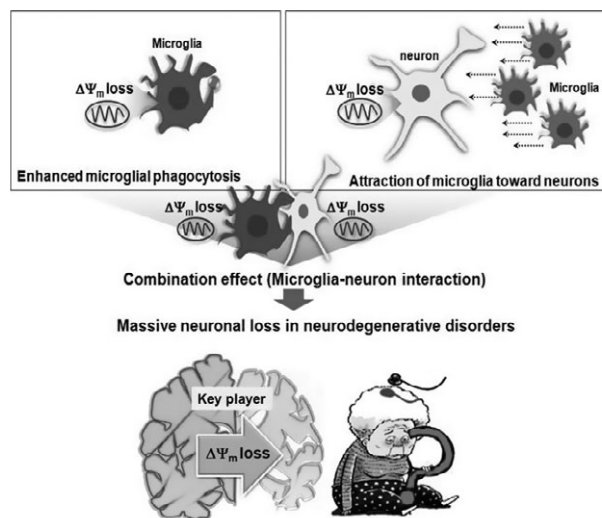

Fig. 1.

MON-306
Intrarenal angiotensin-II crosstalk with mitochondrial dysfunction in nephrotoxicity-induced by adriamycin in rats

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Adriamycin (ADR) is commonly used for many of solid tumors treatment. Its clinical utility is, however, largely limited by the adverse reactions, are known to be nephrotoxic. But the mechanism by which it induce kidney damage is still not completly understood. But its nephrotoxicity might relate to increase reactive oxidant status (ROS), mitochondrial dysfunction. Until now, neurohormonal activation of its is unclear. ADR might activate the renin angiotensin system. Angiotensin-II also induced ROS and mitochondrial dysfunction. The aim of this study was to investigate whether angiotensin-II production inhibition has the protective effect on attenuation of mitochondrial function in rats with acute ADR-nephrotocixity or not. Rats were divided into five groups as a control, ADR, co-treated ADR with captopril, co-treated ADR with Aliskren, co-treated ADR with both captopril and Aliskren groups. Creatinin kinase (CK) levels were measuremented at the end of treatment period. The kidneys were homogenized and biochemical measurements were made in mitochondria, cytosol. Mitochondria membrane potential (MMP) and ATP levels were determined. ADR incresead CK levels and oxidative stress in mitochondria too ($p < 0.05$). ADR significantly decreased MMP and ATP level in kidney mitochondria ($p < 0.05$). Co-administration with ADR and Aliskren and captopril improved the dissipation of MMP ($p < 0.05$). The decreased in ATP level was restored by treatment with inhibitors of ACE and renin. We concluded that inhibitors of angiotensin-II are effective against acute ADR induced nephrotoxicity via the restoration of MMP and ATP production and prevention of mitochondrial damage in vivo.

Keywords: Adriamycin induced nephrotoxicity, Mitochondrial ATP.

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MON-307
Investigating quality control in human mitochondrial translation using ribosome profiling

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The ability of mammalian mitochondria to conduct intra-organellar protein synthesis is a well-known phenomenon. Disruptions to this process are very often linked to serious disorders, making it an extremely important area of study. Unfortunately many aspects of mitochondrial (mt-) translation remain undefined, especially quality control mechanisms that recycle stalled ribosomes. We lack this detailed knowledge due to a lack of tools to study mt-protein synthesis in vivo. We believe that ribosome profiling is an approach to overcome these limitations.

Ribosome profiling reveals mRNA fragments protected by ribosomes. Deep sequencing then provides a ribosome profile that represents their distribution throughout the whole transcriptome. This project aims to apply a modified ribosome profiling protocol to human mitochondria. This will help elucidate previously undescribed mechanisms that rescue stalled mitoribosomes during organellar translation. I will focus specifically on investigating potential candidates for quality control and rescue factors, such as protein C12orf65. The importance of C12orf65 in mt-translation was confirmed in a patient who harbours a mutation in *C12orf65* gene. Disruption in assembly of OXPHOS complexes I, IV and V was shown by BN-PAGE, in agreement with published data of patients with similar genetic defects. However the exact role of this protein remains unknown and can only be determined by more advanced techniques like ribosome profiling.

To validate the adapted ribosome profiling protocol in detecting stalled mitoribosomes I applied it to analyse a cell line containing a homoplasmic mutation in mitochondrial tRNA valine. This mutation causes lower levels of mt-tRNA^{Val} that I expected would cause mitoribosome stalling at valine codons compared to control cell lines. Preliminary results show differences in distribution of mitoribosomes compared to a control cell line. However more detailed statistical analysis is being conducted to determine whether this is exclusively due to stalling at mitochondrial valine codons.

In conclusion the modified ribosome profiling protocol optimised for mitoribosomes will be applied to analyse cell lines with mutated or depleted of C12orf65. Increase and position of mitoribosome stalling in the absence of C12orf65 will reveal if this protein plays a role of mitoribosome rescue factor.

Keywords: mitochondria, ribosome, translation regulation.

MON-308
Involvement of mitochondria-mediated apoptosis in α and β-Zearalenol cytotoxicity, prevention by Crocin

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Zearalenone (ZEN) and its metabolites are commonly found in many food commodities and harmfully affect the gastrointestinal tract. The major ZEN metabolites are α-zearalenol (α-ZOL) and β-zearalenol (β-ZOL). Since it is well known that mitochondria play a central role in apoptosis triggered by many stimuli, an effort was made to examine whether α /β-Zearalenol -induced cytotoxicity occurs through mitochondria-mediated apoptotic pathway. The intestinal system being one of the primary targets of mycotoxins, the human colon carcinoma cell line HCT116 was used in this study. Using flow cytometric analyses we showed that α-ZOL and β-ZOL induced a loss of cell viability by inducing apoptosis. α and β -ZOL-induced apoptosis was mediated through a mitochondria-dependent pathway, characterized by the loss of mitochondrial transmembrane potential (DYm), a downstream generation of O_2 and caspase 3 activation. Besides, deficiency of the pro-apoptotic proteins Bax and Bak partially protected cells against α and β –ZOL-induced mitochondrial alterations.

Therefore, we showed that treatment by α and β –ZOL combined to the Crocin (CRO), a common dietary carotenoid with well-known antioxidant activity, showed a significant reduction of α and β –ZOL induced mitochondrial damages for all tested markers. It could be concluded that *CRO* is effective in the protection against α and β –ZOL hazards. This could be relevant, particularly with the emergent demand for natural products which may prevent multiple human diseases.

Keywords: crocin, Mitochondrial dysfunction, zearalenone.

MON-309
Kinetic characterization of recombinant alternative oxidase from *Aspergillus fumigatus*

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Aspergillus fumigatus is a saprophytic fungus, thermotolerant and able to grow up to 55°C. This characteristic is important to stress resistance during the development of aspergillosis in immunosuppressed patients which is associated the alternative mitochondrial pathway. Previous studies in our laboratory showed the presence of this pathway in *A. fumigatus* and identified the protein alternative oxidase (AOX). AOX is an ubiquinol oxidase cyanide resistant, inhibited by salicylhydroxamic acid (SHAM) and molecular mass of 40.80 kDa. Furthermore, the alternative pathway maintains the ubiquinone pool to avoid auto-oxidation of the reduced ubiquinone when the flow of electrons from the cytochrome pathway is limited. Consequently prevents the formation of reactive oxygen species. The objective of this study was to purify active recombinant AOX (rAOX) for enzymatic characterization. The AOX cDNA was cloned into pTZ57R vector, subcloned into pET28a vector and the sequences were confirmed by DNA sequencing. Heterologous expression was optimized in *Escherichia coli* BL21(DE3) at 37°C with 0.5 μM isopropylthio-β-galactoside following 4 hours of induction and it was validated by mass spectrometry (MS-MS) and western blot using anti-AOX. The rAOX of inclusion bodies (IB) were purified from bacterial cell of expression after lysis by sonication and successive washes with buffer A [50 mM Tris-HCl, 50 mM NaCl, 1 mM Tris2-carboxyethylphosphine hydrochloride (TCEP), 5% glycerol, 1% Triton X-100]. The rAOX of IB were denatured with buffer B [5 mM TCEP, 5,25% N-Lauroylsarcosine] and refolded at 4°C for 48 hours by dilution of rAOX into buffer C [12.5 mM methyl-beta-D-cyclodextrin, 1 mM EDTA]. The circular dichroism of rAOX refolded showed α-helix secondary structure predominantly and when it was incubated with SHAM showed loss of

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structure. The AOX activity was performed by ubiquinol oxidase assay which measures the absorbance change of ubiquinol-1 or decyl-ubiquinol at 278 nm. The AOX refolded showed activity in the presence of substrate ubiquinol-1 (V_{max} = 1421.28 µmol/min/mg) and decyl-ubiquinol (V_{max} = 1261 µmol/min/mg). The inhibitor SHAM decreased the activity of AOX refolded in the presence of both substrates respectively (V_{max} = 355 µmol/min/mg and V_{max} = 710 µmol/min/mg). The purification of active rAOX of IB was effective because it showed ubiquinol oxidation in both conditions of the assay. Further studies of AOX structure and interaction with inhibitors will enable the development of drugs for treatment of aspergillosis.

Supported by: CNPQ, CAPES, FAPESP.
Keywords: AOX, Refolding, Ubiquinol.

MON-310
Mapping the critical residues for hVDAC3 activity: electrophysiological study and phenotypic assays in yeast cells of hVDAC3 cysteine mutants

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VDAC3 is the most ancient member of the family of mitochondrial porins [1]. It shares 60–70% sequence identity with VDAC1 and VDAC2, but the mitochondrial function of VDAC3 is not clear. In literature, it is reported that VDAC3 is involved in apoptotic pathways in synovial fibroblast, respiratory electron transport, ATP synthesis by chemiosmotic coupling and heat production by uncoupling proteins (Parkinson's disease and Huntinghton's disease), toll-like receptor signaling pathway (Hepatitis B), viral carcinogenesis, calcium signaling and HTLV-I infection [2] but any rationale is still lacking. Differently from VDAC1 and VDAC2, recombinant VDAC3 is not able to form channels in an artificial membrane and its heterologous expression in Δpor1 yeast cells can only partially complement the growth defect in glycerol at 37°C. In a previous work, we demonstrated that the replacement of the first 20 amino acids of hVDAC3 N-terminal region with the corresponding residues of hVDAC1 (hN1-VDAC3) allows VDAC3 to fully restore the wild type phenotype [3]. In a more recent work [4], we characterized the chimera hN1-VDAC3 also electrophysiologically. Unlike hVDAC3, hN1-VDAC3 forms channels with a conductance comparable to that of hVDAC1. The sequence analysis of the VDAC isoforms reveals that a significant difference is the number of cysteines, 7 in hVDAC3 and only 2 in VDAC1. Bioinformatic studies made us to hypothesize that the hVDAC3 N-terminus Cys oxidation may cause formation of disulfide bridges or other adducts. It is tempting to speculate that such Cys oxidation may cause problems for the channel formation. By site-directed mutagenesis we replaced the VDAC3 Cys with Ala, (as in hVDAC1). hVDAC3 mutants were cloned into pET21a, expressed in *E. coli* together with proper controls. The mutated proteins were refolded by dialysis and their electrophysiological activity studied upon reconstitution in planar lipid bilayer (PLB). The mutant proteins were also cloned in a yeast shuttle vector and expressed in *Δpor1* yeast cells to study their effects on phenotype, apoptosis and ROS production. The main result from this study is that, both in vitro and in cellulo, the mutagenesis of cysteine provokes a drastic change in the pore-forming activity of VDAC3 and in the ability to restore a normal oxidative function of mitochondria. This result highlights the role of Cys in VDAC3 and explains the presence of different isoforms in the cell. From the structural and mechanistical point of view a model is presented of the activity of this pore-forming protien, completely neglected till now.

De Pinto V. et al BBA 2010; Messina A. et al BBA 2012; Reina S: et al. FEBS Lett. 2010;Reina S. et al. BBA 2013
Keywords: Cysteine, Electrophysiology, VDAC3.

MON-311
Mitochondria of rat myometrium possess ATP-sensitive channels, which can be activated by Ca ions

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Isolated mitochondria were loaded with K^+-sensitive probe PBFI for 10 min. under room temperature in K^+-free medium. After dilution with isolation medium and final centrifugation at 12000 g pellets were resuspended in isolation medium without EDTA and stored on ice. PBFI fluorescence was measured using an excitation ratio technique (λ_{exc} 340/380, λ_{em} 480) after addition of mitochondrial aliquots into K^+ medium. For the measurements of mitochondrial side scattering pellets after second centrifugation were resuspended in isolation buffer without EDTA and stored on ice.

Results: Accumulation of K^+ in mitochondrial matrix resulted in fast increase of PBFI fluorescence, which was effectively suppressed by addition of ATP (200 µM). Nevertheless, no suppression was observed when the activator of K^+_{ATP}-channel diazoxide (50 µM) was added along with ATP These results provide reason to assume the existence of the functional of K^+_{ATP}-channel in mitochondria of the rat myometrium. Measurements of the side scattering of mitochondrial suspension at 520 nm confirmed our assumption. Fast swelling of mitochondrial matrix as a result of K^+ accumulation was slowed down significantly under presence of ATP (200 µM). In contrast to this, diazoxide addition (50 µM) along with ATP caused restoration of the rate and the steady-state volume of mitochondrial swelling to the levels of ATP- and diazoxide-free control.

Addition of $CaCl_2$ (100 µM) into the incubation medium resulted in acceleration of the swelling rate of mitochondria as well as in lowering of steady-state volume in comparison to the level of Ca^{2+}-free control. These effects of high Ca^{2+} concentration were suppressed either by 1 µM CsA or 10 µM RuR. Thus Ca^{2+}-induced mitochondrial swelling that we observed was mediated by Ca^{2+} accumulation through the mitochondrial Ca^{2+} uniporter and the permeability transition pore (PTP) opening. Further, blockers of K^+_{ATP}-channel glybenclamide (2 µM) and 5-HD (200 µM) partially blocked mitochondrial swelling rate. Effect of K^+_{ATP}-channel blockers was observed only in K^+ medium and did not observed when K ions were substituted equimolarly for Na^+.

Conclusions: These results allow to assume the existence of functional K^+_{ATP}-channels on the mitochondria isolated from rat uterus. High concentrations of Ca^{2+}, which evoke the PTP opening, can induce the opening of K^+_{ATP}-channel either.

Keywords: mitochondria, mitoKatp, PTP.

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MON-312
Mitochondrial DNA import and the TIM22 channel

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We previously demonstrated that isolated plant, mammalian and *Saccharomyces cerevisiae* mitochondria are able to import double-stranded DNA through an active mechanism [1–3]. This imported DNA can be transcribed or repaired *in organello* [4–5]. For plant and yeast organelles, the voltage-dependent anion channel (VDAC) seems to be involved in DNA translocation through the outer membrane. We took *S. cerevisiae* as a model to identify the still elusive inner membrane proteins participating in mitochondrial DNA import. Using native yeast mitochondrial membranes for patch-clamp experiments, we showed that the TIM22 complex can form a channel [6]. By BN-PAGE and co-immunoprecipitation, we also found that VDAC, which is involved in DNA transport through the mitochondrial outer membrane, interacts with the TIM22 complex. DNA import assays were thus performed with mitochondria from yeast strains with different expression levels of Tim22p and Tim18p. It turned out that the absence of TIM22 complex significantly impairs DNA import into the organelles. Taken together, our data raise the possibility that the channel formed by the TIM22 complex could be a way for the DNA to cross the inner membrane of yeast mitochondria.

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Keywords: DNA import, mitochondria, TIM22.

MON-313
Mitochondrial network and respiration in primary fibroblasts derived from ALS patients

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder characterized by progressive, gradual degeneration of motor neurons responsible for controlling voluntary muscles. Most of the cases of ALS are sporadic with only 10% of recognized inherited (familial) forms. Among the familial forms the most common are diseases caused by mutations in *SOD1* gene. A growing body of evidence suggests that impaired mitochondrial functions are involved in the pathophysiology of many neurodegenerative diseases. Early cellular symptoms in ALS development are defects in mitochondrial energy production and mitochondrial dynamics. Understanding the relationship between mitochondrial malfunctions and progress of ALS may provide a useful tool for early diagnosis and potential pharmacological targets for treatment of this disease.

In order to verify presence of mitochondrial stress in fibroblasts derived from patients diagnosed with ALS (familial and sporadic forms) we studied mitochondrial membrane potential, cytosolic [Ca^{2+}], ROS production as well as respiration rates and activity of complex I and IV of electron transport chain. The mitochondrial structure and organization were visualized by confocal microscopy.

We found decreased mitochondrial membrane potential and increased cytosolic [Ca^{2+}] in cells with both forms of ALS. Control flux coefficient calculated on the basis of titration with specific respiratory inhibitor (sodium amytal) was higher in fibroblasts with sporadic form of ALS while maximal respiration rate on complex I substrates was slightly lowered which may suggest abnormalities in functioning of the complex. Organization of mitochondrial network in ALS cells was different than in control cells.

Keywords: ALS, mitochondrial dynamics, Neurodegeneration.

MON-315
MtDNA genetic comparison between Chinese and domestic diving beetles, *Cybister (Cybister) japonicus* (Coleoptera: Dytiscidae)

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Diving beetles (Coleoptera: Dytiscidae) have been known to about 3800 species in the world (Nilsson, 2001) that the hind legs are flattened and setose, and set way back, separating the anterior abdominal sternites spiracles open up under the elytra and many taxa use ‘physical gills’ (air bubbles brought down from the surface), the larvae are also predacious, sometimes called ‘water tigers’; can crawl, paddle, or use serpentine movement in swimming, grooved mandibles for inserting enzymes and removing liquids from prey.

The population of *Cybister* (*Cybister*) *japonicus* are more and more decreasing, so are extinct species in Korea. *Cybister* (*Cybister*) *japonicus* imported from China are being reared and selling by merchant in these days. Therefore, geophylogenetic relationships among 14 districts of domestic species and the genetic comparison between the Chinese and domestic diving beetles was accomplished by mitochondrial gene Cytochrome Oxidase I(COI) and 16S rRNA partial sequencing. The phylogenetic tree based on the nucleotide sequence of COI(567 bp) and 16S rRNA (547 bp) was divided into 3 groups, and the similarity rate of intra-group was more than 98% and inter-group was 75% from COI and 64% from 16S rRNA gene. Also, Chinese diving beetles belonged to one of the 3 groups.

Keywords: MtDNA, genetic comparison, diving beetles, *Cybister* (*Cybister*) *japonicus*, Coleoptera, Dytiscidae.

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MON-316
Multiparametric redox imaging in different cellular compartments

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Mitochondrial reactive oxygen species (ROS) production is often linked to cellular Ca^{2+} signaling. There is a general notion that Ca^{2+} load stimulates mitochondrial ROS increase. There is a large body of evidence showing either increase, or decrease in mitochondrial ROS production upon Ca^{2+} load. However, these data were obtained using isolated mitochondria and it remains unknown to what extent these observations can be extrapolated to mitochondrial ROS production *in situ*. Can this ROS production be detected using genetically encoded redox probes targeted to mitochondrial matrix? Would H_2O_2 production in the matrix be associated with concomitant changes in reduced-oxidized glutathione (GSH/GSSG) ratio? Will the changes in the mitochondrial ROS production, if any, spread to the cytoplasm? To address these issues, we took an advantage of simultaneous imaging of red H_2O_2 sensor and green fluorescent sensors for H_2O_2, pH and GSH/GSSG.

We designed HyPerRed, red genetically encoded fluorescent probe for hydrogen peroxide detection, targeted it to mitochondrial matrix and co-expressed HyPerRed-mito in HEK-293 cells with a variety of green redox biosensors targeted to either mitochondria or cytoplasm in order to detect changes in H_2O_2, GSH/GSSG and pH simultaneously. Although both ATP and thapsigargin (TG), an inhibitor of SERCA Ca^{2+} pump, were able to increase cellular Ca^{2+}, only TG induced small but well defined peak of H_2O_2 production in the mitochondrial matrix.

To localize mitochondrial H_2O_2 production we co-transfected cells with H_2O_2 probes targeted to the matrix (HyPerRed-mito) and to the mitochondrial intermembrane space (HyPer2-IMS). Addition of TG induced an increase of HyPerRed-mito fluorescence, but did not affect HyPer2-IMS signal. This is indicative of the absence of IMS-specific ROS generation or the spread of H_2O_2 from the matrix to the IMS. The GSH/GSSG and pH levels remained stable in both mitochondrial matrix and cytoplasm. Mitochondrial H_2O_2 generation coincided with Ca^{2+} increase in the matrix. Ca^{2+} increase started 10–20 seconds before H_2O_2 elevation.

Taken together, our results show that inhibition of Ca^{2+} reuptake into the ER induces small, local and transient increase in matrix H_2O_2 production which is not accompanied with detectable GSH oxidation. Furthermore, they indicate that H_2O_2 does not spread from the matrix to the IMS and cytoplasm.

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Keywords: ROS, hydrogen peroxide.

MON-317
Neuroprotective effects of Tauroursodeoxycholic acid upon mitochondrial dysfunction

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Mitochondrial dysfunction and oxidative stress play a crucial role in Parkinson's disease (PD) progression. Nuclear factor E2-related factor 2 (Nrf2) is the master regulator of redox homeostasis, binding to the antioxidant responsive element in the promoter of several phase II antioxidant enzymes. Another protective mechanism is mitophagy, which is mainly regulated by PTEN-induced putative kinase 1 (PINK1) and Parkin. This involves the voltage-dependent cleavage of full-length PINK1 in polarized healthy mitochondria to a shorter fragment that has no affinity for mitochondrial membrane. Loss of mitochondrial membrane potential inhibits PINK1 cleavage, which accumulates in the outer mitochondrial membrane, leading to recruitment of Parkin. Once in the mitochondria, Parkin polyubiquitinates mitofusin and voltage-dependent anion channel 1, leading to mitochondria clearance and prevention of fusion of dysfunctional mitochondria. Tauroursodeoxycholic acid (TUDCA), is an endogenous bile acid with neuroprotective properties in different models of neuropathologic conditions. Importantly, we have recently showed that TUDCA exerts a neuroprotective activity in a mice model of PD, but the mechanisms involved are still unidentified.

This work aims to characterize the neuroprotective effect of TUDCA upon mitochondrial depolarization triggered by the mitochondrial uncoupler carbonyl cyanide m-chlorophelyhydrazone (CCCP). SH-SY5Y cells were treated with CCCP in the presence or absence of TUDCA. TUDCA increases LC3II/LC3I ratio, mitochondrial full-length PINK1 and the recruitment of Parkin to mitochondria, indicating an up-regulation of mitophagy. Furthermore, CCCP leads to the activation of Nrf2 and the up-regulation of antioxidative enzymes. However, in cells pre-treated with TUDCA, CCCP administration no longer triggers the activation of Nrf2, suggesting that this bile acid is modulating the threshold of oxidative stress, so that the redox environment, even in the presence of CCCP, is not sufficient to elicit its activation.

Overall, our results show that TUDCA protects against CCCP-induced cell death. The characterization of the mechanisms through which TUDCA modulates oxidative stress and neurodegeneration should provide evidences to validate and extend its future clinical application to neurodegenerative diseases like PD.

Supported by FCT PTDC/NEU-NMC/0248/2012.
Keywords: mitophagy, oxidative stress, Tauroursodeoxycholic acid.

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MON-318
New insights on the molecular mechanisms underlying the medium-chain fatty acid acyl-CoA deficiency (MCADD)

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The MCADD is the most common genetic disorder of the mitochondrial fatty acid ß-oxidation (mFAO) pathway. The MCAD enzyme catalyzes the first step of mFAO, a dehydrogenation reaction of the fatty acyl substrates in the C2-C3 carbon atoms, yielding an enoyl-CoA derivative. The MCAD is a homotetramer being each monomer composed by 396 amino acids (mature form). The most common mutation found in MCADD patients is translated in the substitution of lysine 304 residue by a glutamic acid (p.K304E) being associated with protein conformational changes. To better understand the molecular mechanisms underlying MCADD an *in silico* assessment of structural features of the wild-type MCAD and the p.K304E mutant form were performed through Molecular Dynamics simulations. The enzyme's coordinates were obtained from the crystal structure of *Sus scrofa* (pig) MCAD complexed with the FAD cofactor and 3-thiaoctanoyl-CoA as substrate. Our results show that the protein surface is the most flexible region while the interface between the two dimers (protein core) is the most stable. Interestingly, the p.K304E mutation is located at the protein core, being involved in helix-helix interactions, essential for the tetramer assembly. The catalytic pockets formed by the two monomers of the same dimer, are highly dynamic and flexible. Upon substrate binding the number of water molecules inside the pocket is considerably reduced and the conformation of the E99 and Y375 residues' side-chains show a significant change to better accommodate the substrate. Unlike the crystallographic structure, the side-chain of the catalytic residue E376, switches towards the opposite side of the lipid's C2-C3 bound which may suggest an indirect interaction between them. Our simulations also reveal that the R256 residue may play an important role on MCAD catalysis through the stabilization of the intermediate enolate form. To our best knowledge, this is the first *in silico* study of MCAD dynamics behavior. The MCAD structural data gathered are currently being applied in the study of other MCAD mutants aiming the discovery of potential therapeutic drugs for the treatment of MCADD patients.

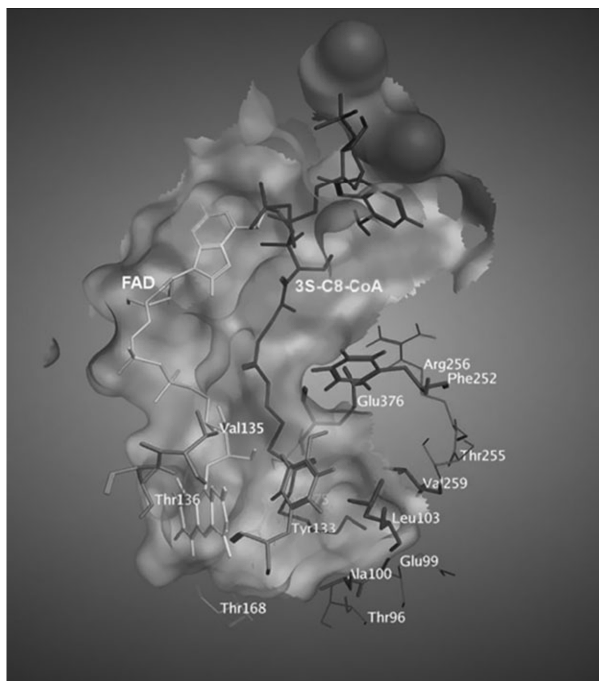

Fig. 1.

Acknowledgement: FCT (PEst-OE/SAU/UI4013/2011 to iMed.UL).

Keywords: Acyl-CoA Dehydrogenase, Mitochondrial Fatty Acid Beta-oxidation, Molecular Dynamics simulations.

MON-319
New role of cytochrome c in programmed cell death

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Programmed Cell Death (PCD) is a fundamental event for the development of living organisms. In mammalian cells, early events in PCD involve the release of cytochrome *c* (C*c*) from mitochondria to the cytoplasm to act at the first stages of the apoptotic process, playing a key role in assembling the apoptosome. In plants, PCD is part of a general process – the so-called hypersensitive response - that also involves the release of C*c* to the cytoplasm but its role in cell death remains veiled. Such a highly conserved cytoplasmic location of C*c* upon apoptotic stimuli made us to think of a common link for PCD in evolutionarily distant species.

To better understand the role of C*c* in the onset of PCD in humans and plants, a proteomic approach based on affinity chromatography with C*c* as bait was used. Upon combining this approach and Bimolecular Fluorescence Complementation (BIFC), a total of 8 human and 9 plant novel proteins interacting with C*c* under PCD were found [1,2,3]. These C*c*-partners are involved in protein folding, translational regulation, oxidative stress and DNA damage, as well as in energetic and mRNA metabolism. Strikingly, some of the novel human C*c*-targets are closely related to those of plant C*c*, indicating that the evolutionarily well-conserved cytosolic C*c* – from plants to mammals - interact with a wide range of targets under PCD conditions.

Computational modeling of the complexes formed by human and plant C*c* with their counterparts shows how the heme crevice of C*c* is located at the complex interface, as is it at the vast majority of known redox adducts of C*c*. However, in contrast to the high turnover of the redox complexes formed by C*c* with its mitochondrial partners, those occurring under PCD conditions lead to the formation of rather stable nucleo-cytoplasmic ensembles, as inferred from Surface Plasmon Resonance (SPR) and Nuclear Magnetic Resonance (NMR) measurements. On the basis of these findings, we suggest that human and plant C*c* interacts with pro-survival, anti-apoptotic proteins after its release into the cytoplasm. Then, C*c* may interfere with cell survival pathways and unlock PCD in order to prevent the spatial and temporal co-existence of antagonist signals.

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Keywords: Apoptosis, programmed cell death, Protein - protein interactions.

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MON-320
NO-dependent prevention of mitochondrial permeability transition pore opening by hydrogen sulfide in old rat heart

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Hydrogen sulfide (H_2S) is known as the gaseous signaling molecule like nitric oxide (NO) and carbon monoxide (CO) that participates in variety of cellular functions in cardiovascular system. H_2S is produced enzymatically by three enzymes: cystathionine
γ-lyase (CSE), cystathionine ß-synthase (CBS), and 3-mercatopyruvate sulfurtransferase (3-MST). But there is limited evidence of cross-talk between H_2S and NO. In aging heart mitochondria permeability transition pore (mPTP) opening causes membrane potential collapse leading to mitochondrial dysfunction and apoptosis. The aim of our research was to investigate the impact of H_2S on the sensitivity of the mPTP to Ca^{2+} and mRNA expression of CSE, eNOS, nNOS and iNOS in heart tissue. It was shown that in physiological concentrations, NaHS inhibits calcium-induced mPTP opening, which testifies H_2S protective effect on pore formation in the heart of adult and old rats. It has been shown in experiments *in vivo* that a single administration of either NaHS or L-cysteine leads to decreasing the mPTP sensitivity to Ca^{2+} in adult and old rat hearts, but a single administration of PG leads to its significant increasing only in old rat hearts. At the same time in combination with L-cysteine, this increased sensitivity was restored to the control level in old animals mitochondria. In the heart of adult rats under condition of both PG and L-cysteine introduction it was shown that mRNA expression of CSE as well as nNOS and iNOS increased in comparing to control, but eNOS mRNA expression was in 2,7-fold decreased. In the heart of old rats mRNA expression levels of CSE and eNOS were reduced in 12,7- and 2,4-fold compared with adult animals. Thus, the results obtained suggest that both exogenous and endogenous H_2S participate in the modulation of mitochondrial membrane permeability changes, in particular through inhibition of calcium-induced mPTP opening in the heart of adult and old rats. Therefore, H_2S can be an important regulatory factor in the cardiovascular system under physiological and pathological conditions. The findings of this study revealed that cytoprotection elicited by CSE-derived H_2S is eNOS-dependent. Hereby, it could be suggested that mitochondrial eNOS activity is normally affected by H_2S, so that the protective effect of H_2S on Ca^{2+}-dependent mPTP opening can be mediated by constitutive NO *de novo* synthesis. Future studies will aim to more fully understand the mechanisms related to crosstalk between H_2S and eNOS-derived NO in the context of cardiovascular diseases protection.

Keywords: hydrogen sulfide, mitochondrial permeability transition pore, nitric oxide.

MON-321
NRH:quinoneoxidoreductase 2 and its role in p53 tumor suppressor stabilization in response to mitochondrial electron transport chain dysfunction

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NRH:quinoneoxidoreductase 2 (NQO2) is a cytosolic protein that catalyzes metabolism of quinones. NQO2 is ubiquitously present in all tissues and induced along with a battery of defensive genes in response to different stresses. It is concerned that NQO2 plays an important role in development of neurodegenerative disorders and cancer, but the mechanism of its action in disease initiation and progression is unknown. The same is definitely true for p53 and mitochondria being the most important regulatory protein and organelle in human cells and playing crucial roles in cancer, neurodegenaration, inflammation and ageing. Perhaps, NQO2, p53, and mitochondria could act in cooperation.

We previously found that NQO2 contributes to the p53 stabilization and activation after mitochondrial electron transport chain complex III inhibition. But the mechanism of its action is unknown.

To study the possible interaction of NQO2 and p53 in different cancer cell lines under normal conditions and after mitochondrial electron transport chain inhibition we used Bimolecular Fluorescence Complementation (BiFC) method. N-terminal (N-YFP) and C-terminal (C-YFP) fragments of YFP were fused to p53 and NQO2 proteins and the fusion proteins were expressed in HeLa or RKO cells. Expression of fusion proteins was verified by Western-blot analysis with NQO2 and p53 antibody. Recovery of the whole YFP fluorescence was detected in the cells expressing C-YFP-NQO2 and N-YFP-p53 by fluorescent microscopy (picture below).

Thus, using BiFC we received rather strong experimental evidence that p53 forms a complex with NQO2 in cells under normal conditions and after mitochondrial complex III inhibition. According to immunofluorescent images under normal conditions NQO2-p53 complexes are localized predominantly in the cytoplasm, and after complex III inhibition they can be seen mainly in cell nuclei, where p53 performs its function. This result is a good start for investigation of the role and detailed mechanism of p53-NQO2 interaction in cancer cells under normal conditions and mitochondrial dysfunction.

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Keywords: mitochondrial electron transport chain, NQO2, p53 tumor suppressor.

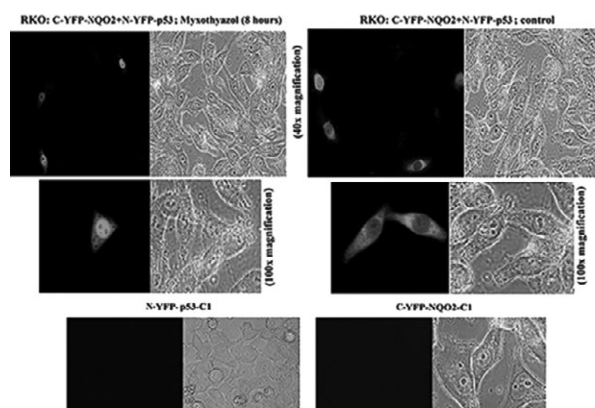

Fig. 1.

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MON-322
p53 interacts with VDAC1 to regulate apoptosis

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p53, a tumor suppressor and transcription factor, functions as a stress sensor, responding to a myriad of signals, including DNA damage, oxidative stress and ischemia, by arresting the cell cycle, initiating DNA repair, or initiating cell death. Inactivation of p53 function is an almost universal feature of human cancer cells. While the mechanism by which p53 mediates apoptosis remains unclear, evidence indicates that p53 encodes a sequence-specific transcription factor that controls the expression of genes whose products mediate apoptosis. However, sub-organellar localization by various methods shows that p53 also localizes to the surface of the mitochondria and induces apoptosis without requiring further DNA damage. p53 protein can directly promote mitochondrial outer membrane (OMM) permeabilization (MOMP) to trigger apoptosis. Upon stress, a cytoplasmic pool of p53 rapidly translocate to the mitochondrial surface, where it physically interacts with both anti- and pro-apoptotic Bcl-2 family members to inhibit or activate their respective functions, leading to MOMP and apoptosis. Recently, p53 was demonstrated to drive the mitochondrial protein voltage-dependent anion channel (VDAC1) into high molecular weight complexes. VDAC1, at the OMM, regulates cellular energy production and cell metabolism by controlling the traffic of metabolites including ATP, ADP and other ions, between mitochondria and the rest of cell, thereby regulating cell survival. VDAC1 has been proposed to be a critical player in apoptosis. Studies in our lab have demonstrated that VDAC1 oligomerization, as induced by various apoptosis stimuli, mediates the formation of a large, flexible pore between individual subunits of VDAC1, serving as a channel for Cytochrome c (Cyto c) crossing the OMM leading to apoptotic cell death. In this study, we demonstrate the direct interaction of p53 with VDAC1 and the regulation of VDAC1 oligomerization-mediated apoptosis by p53. We found that purified p53 interacts with purified VDAC1, as revealed by microscale thermophoresis and as reflected in the decrease in channel conductance of bilayer-reconstituted VDAC1. Moreover, overexpressing p53 in p53-null cells enhanced the level of VDAC1 oligomeric state as revealed by chemical cross-linking and immunobloting and apoptosis in the absence of apoptotic stimuli. Furthermore p53 also promotes VDAC1 over-expression by an as yet unknown signaling pathway. Our proposed model suggests that p53 increases VDAC1 monomeric levels which shift the equilibrium towards VDAC1 oligomerization allowing Cyto c release, and subsequently apoptosis. These findings point to VDAC1 as a new target of p53 and further support VDAC1 oligomerization as being a key step in the induction of apoptosis.

Keywords: apoptosis, p53, VDAC1.

MON-323
Proteomic studies of plasma membrane-mitochondrial proteins involved in recognition memory of visual imprinting in chicks

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Visual imprinting is a learning process through which young, visually naïve animals come to recognize a visual object by being exposed to it (training) and subsequently approach the object in preference to other objects. A large body of evidence implicates a restricted part of the forebrain, the intermediate medial mesopallium (IMM), as a memory storage site for visual imprinting in the domestic chick [1]. A number of learning-related, time-dependent molecular changes have been identified in the IMM. Most of these changes were found in plasma membrane and mitochondrial proteins [2]. Therefore we have undertaken a proteomic investigation of the plasma membrane – mitochondrial P2 fraction to identify proteins involved in visual imprinting. Two-dimensional gel electrophoresis with subsequent mass spectrometry was employed to identify differentially expressed proteins across chicks with different estimated levels of imprinting 24 h after training. We further inquired whether the amounts of those proteins in the IMM were correlated with memory strength. The amounts of the following proteins in the left IMM increased significantly with memory strength: membrane cognin, voltage dependent anionic channel, dynamin, a protein similar to the p32 subunit of splicing factor SF2, heterogeneous nuclear ribonucleoproteins A2/B1. The learning-related increase of mitochondrial proteins observed in the present study and previously [2] could be mediated by an increase in the number of mitochondria. However, levels of transcription factors involved in mitochondrial biogenesis and copy number of mitochondrial DNA did not change significantly in the left IMM. We suggest that the mitochondrial proteome rather than the number of organelles is changed in a learning-related manner 24 h after training and is involved in long-term memory associated with imprinting.

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Keywords: Memory, mitochondria, proteome.

MON-324
Recovery of adriamycin induced mitochondrial dysfunction in liver by selenium

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Background: Adriamycin (ADR) is a chemotherapeutic drug. Its toxicities may associate with mitochondriopathy. Selenium (Se) is a trace element for essential intracellular antioxidant enzymes. However, there is lack of data related to the effect of selenium on the liver tissue of ADR-induced mitochondrial dysfunction.

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Objective: The study was to investigate whether Se could restore mitochondrial dysfunction of liver-exposed ADR.

Methods: Rats were divided into four groups as a control, ADR, Se, co-treated ADR with Se groups. The livers biochemical measurements were made in mitochondria, cytosol. ATP level and mitochondria membrane potential (MMP) were measured. Total oxidant (TOS), total antioxidant (TAS) status were determined and oxidative stress index (OSI) was calculated by using TOS and TAS.

Results: ADR incresead TOS in mitochondria and also oxidative stress in mitochondria. ADR sligtly decreased MMP, and ATP level. Even if co-administration with ADR and Se partially restorated the dissipation of MMP, but this partial restoration resulted in a significant elevation of ATP level. TOS in mitochondria and cytosol was diminished, as well as OSI.

Conclusion: We concluded that selenium can prevent against to ADR induced oxidative stress in liver by the restoration of MMP and ATP production and prevention of mitochondrial damage in vivo.

Keywords: Adriamycin induced hepatotoxicity,, Mitochondrial ATP, Mitochondrial membrane potential.

MON-325
Reduced tryptophan and NAD synthesis pathway is associated with valproate treatment: a novel mechanism underlying mitochondrial dysfunction and potential drug-induced liver injury

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Valproic acid (VPA) is an important anticonvulsive drug with potential adverse hepatotoxic effects and lysine deacetylase inhibitor (KDACi) with established anti-cancer properties. The effects of VPA on Zinc-dependent lysine deacetylases and histone acetylation have been reported. The links between VPA, non-histone acetylation and NAD metabolism have yet to be elucidated. This work aims to characterize the *in vivo* effects of VPA on amino-acid metabolism and to quantify the crucial cofactor NAD^+ and its biosynthetic precursors in biological samples.

Plasma and liver samples were obtained from Wistar rats subjected to administration of VPA under different regimens (acute and sub-chronic) as two dosages (n = 10/group). Controls were treated similarly with vehicle. Human plasma samples were collected from individuals under chronic treatment with valproate (n = 33) and controls (n = 39). Analyses of individual amino acids in plasma samples and rat liver tissues were performed using GC-FID and MS/MS, respectively. Novel sensitive UHPLC-MS/MS-based methodologies were optimized and validated for the analyses of $NAD(P)^+$, NAD(P)H and nicotinic acid.

Plasma profiling of amino acids in rats, revealed a significant depletion of tryptophan (Trp) associated with VPA treatment, with a reduction of up to 30% during acute and subchronic treatment with 100 mg/Kg regimen ($p < 0.05$). Trp levels in rat liver tissues were not significantly different from controls. Human plasma samples also revealed a significant reduction of Trp levels (up to 30%; $p < 0.005$). Quantification of NAD-related compounds in rat liver revealed a significant decrease of up to 30% of NAD^+ and $NADP^+$ levels after a single drug administration (100 mg/Kg) ($p < 0.001$). No significant reduction was observed after sub-chronic treatment (100 mg/Kg/day for two weeks).

The present work reveals a significant effect of VPA treatment on the levels *in vivo* of the amino acid tryptophan, with downstream effects on the hepatic availability of the essential cofactor NAD^+ at onset of therapy (animal study). NAD levels reduction *in vivo* may affect mitochondrial energy metabolism as well as disturb post-translational modification of proteins involved in these pathways. The rescuing of NAD levels during sub-chronic treatment hints a probable compensatory mechanism, resulting in reduced tryptophan levels during this regimen.

These novel findings, correlating tryptophan depletion and NAD reduction during VPA treatment *in vivo*, will contribute to gain new insights on the molecular mechanisms underlying the adverse or therapeutic properties of VPA.

Keywords: Hepatotoxicity, Mitochondrial metabolism, NAD metabolism.

MON-328
Role of the mitochondrial import complex TOM and the PINK1/Parkin pathway in mitochondrial quality control in neurons

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Mutations in genes encoding Parkin and PINK1 cause autosomal recessive forms of Parkinson's disease. These proteins regulate jointly several processes relevant to maintenance of mitochondrial quality. Using FRET microscopy to explore local physical proximities between proteins, we showed that Parkin interacts with PINK1 at the TOM machinery, a protein complex responsible for the mitochondrial import of the vast majority of the mitochondrial proteins. The data accumulated suggest that after massive mitochondrial depolarization, the degradation of key subunits of TOM initiates Parkin-dependent mitophagy. These results were obtained in cell lines, such as COS7 and HEK293.

Here, we explored the relevance of these findings in primary cortical neurons from wild type and Parkin-deficient mice. By confocal and FRET microscopy, we show that following mitochondrial depolarization triggered by the protonophore CCCP, PINK1 accumulates on the outer mitochondrial membrane (OMM) and recruits Parkin in proximity of TOM. These events are impaired in Parkin-deficient cells, suggesting that Parkin plays a role in stabilizing PINK1 on the OMM. Using an image-based quantitative analysis of different mitochondrial subcompartment markers at different time points after CCCP treatment, we confirm significant mitochondrial loss in wild type but not Parkin-deficient cells. Components of the TOM machinery are lost earlier than other mitochondrial markers, confirming that this machinery is an early target for Parkin-depedent degradation. In parallel, we investigated the relationship between PINK1/Parkin-mediated mitochondrial degradation and mitochondrial biogenesis by exploring the expression of master genes of this process, as well as nuclear and mitochondrial genes encoding key mitochondrial components. Our results indicate that mitochondrial biogenesis is slowly activated following CCCP treatment in neurons, and that this process is compromised in the absence of Parkin.

Altogether our results confirm that the TOM machinery acts as a molecular switch in the PINK1/Parkin pathway, coupling loss of mitochondrial protein import efficiency with different quality control mechanisms, including mitochondrial degradation and biogenesis.

Keywords: Mitochondria, Neurons, Parkinson Disease.

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MON-329
Structure, dynamics and function of phosphomimetic mutants of respiratory cytochrome c

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Post-translational modifications of proteins are relevant regulatory mechanisms to control an ample number of cell metabolic processes. One of the most usual modifications is phosphorylation, which can alter the electrostatic and structural features of proteins thereby affecting their interactions with other proteins.

Cytochrome *c* (C*c*) stands out as a target for post-translational modification. Indeed, C*c* phosphorylation appears in some pathological situations, such as ischemia or cancer [1]. C*c* has a double role, transferring electrons from the cytochrome bc_1 complex to cytochrome *c* oxidase and triggering Programmed Cell Death (PCD) under oxidative stress conditions. Both functions are regulated by phosphorylation of the residues Thr, Ser and Tyr at positions 28, 47 and 48, respectively [2–4].

Since the specific C*c*-phosphorylating kinases are still unknown, we have constructed three phosphomimetic mutants. Two of them correspond to substitutions of Thr28 and Ser47 by the canonical amino acid Asp. To mimic Tyr48 phosphorylation, we resorted to the evolved tRNA technique. Thus, the Tyr48-encoding triplet was replaced by an AMBER codon. Then, we incorporated a non-canonical amino acid (*p*-carboxymethyl-L-phenylalanine, *p*CMF) that emulates phosphotyrosine, with the help of an evolved tRNA targeting the AMBER stop codon. Such a mutation has drastic consequences not only on the structure and dynamics of C*c* (3D conformation, thermal stability, redox potential), but also on its biological function.

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Keywords: Cytochrome c, Post-translational modifications, Programmed Cell Death.

MON-330
Sumoylation of human mitochondrial NADH dehydrogenase (ubiquinone) Fe-S protein 7 (NDUFS7) by SUMO-1

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Human NADH dehydrogenase (ubiquinone) Fe-S protein 7 (NDUFS7) is one of the most conserved core subunits of mitochondrial complex I. It has a bound iron-sulfur cluster N2 (tetranuclear) which is the terminal redox center in the electron transport chain (ETC) of complex I. NDUFS7 protein is encoded by the nuclear genome and is incorporated in the peripheral segment of complex I. Most mitochondrial matrix proteins are synthesized in the cytosol and imported into mitochondria by mitochondrial targeting sequences (MTSs). We previously defined the N terminus of the first 60 amino acids of NDUFS7 is an effective MTS. We also identified that there is a nuclear localization signal (NLS) and a nuclear export signal (NES) located in the C-terminus of NDUFS7.

Sumoylation has been recognized to play an important role in protein localization, function and turnover. Small ubiquitin-related modifiers (SUMOs) can be conjugated to target proteins by E1 (SAE1/SAE2), E2 (UBC9) and E3 enzymes after translation. Using *in silico* analysis, NDUFS7 protein was found to have several potential sites for sumoylation. In this study, we co-transfected plasmids expressed NDUFS7, SUMO-1 and UBC9 into HEK293 cells, and detected the conjugation of NDUFS7 with SUMO-1 by immunoblotting analysis with various antibodies. The results indicated that NDUFS7 could indeed be sumoylated *in vivo*. To confirm this finding, SUMO-specific protease SENP was co-expressed with NDUFS7, SUMO-1 and UBC9 proteins in some experiments. The results showed that overexpression of SENP could reduce the level of interaction between NDUFS7 and SUMO-1. These results suggested that NDUFS7 can be sumoylated by SUMO-1 in cells. In addition, by using site-directed mutagenesis, we also identified that lysine 202 in NDUFS7 is the major site for sumoylation, which is consistent with the consensus sumoylation motif. Moreover, sumoylation of NDUFS7 was found to be associated with protein stability and thus its turnover. Further studies are on the way to explore the functional details of NDUFS7 sumoylation.

Keywords: mitochondrial complex I, NDUFS7, sumoylation.

MON-331
The 18 kDa protein TSPO interacts with VDAC1 and limits mitochondrial quality control

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Selective autophagic removal of mitochondria (mitophagy) is critical for maintaining normal cell homeostasis but our knowledge of its regulatory pathways is limited. The mitochondrial Translocator Protein (TSPO) is an 18 kDa multi-drug binding protein with a canonical role in mitochondrial cholesterol transport. Dysregulation of mitophagy and differential expression of TSPO, are both associated with pathological conditions characterized by cumulative damage to mitochondria. We therefore explored the role of TSPO mitophagy. Here, we report that TSPO inhibits mitochondrial autophagy downstream of the PINK1/Parkin pathway, preventing essential ubiquitination of proteins. TSPO abolishes mitochondrial relocation of P62/SQTSM1, and consequently that of the autophagic marker LC3, thus leading to an accumulation of dysfunctional mitochondria. Independent of cholesterol regulation, the modulation of mitophagy by TSPO is instead dependent on the Voltage Dependent Anion Channel 1 (VDAC1), to which TSPO binds, reducing mitochondrial coupling and promoting an overproduction of the Reactive Oxygen Species (ROS) that counteracts Parkin mediated ubiquitination of proteins. This set of data identifies TSPO as a novel element in the regulation of mitochondrial quality control by autophagy, and demonstrates the importance of its expression ratio with VDAC1 in mitochondrial and cell homeostasis making TSPO an

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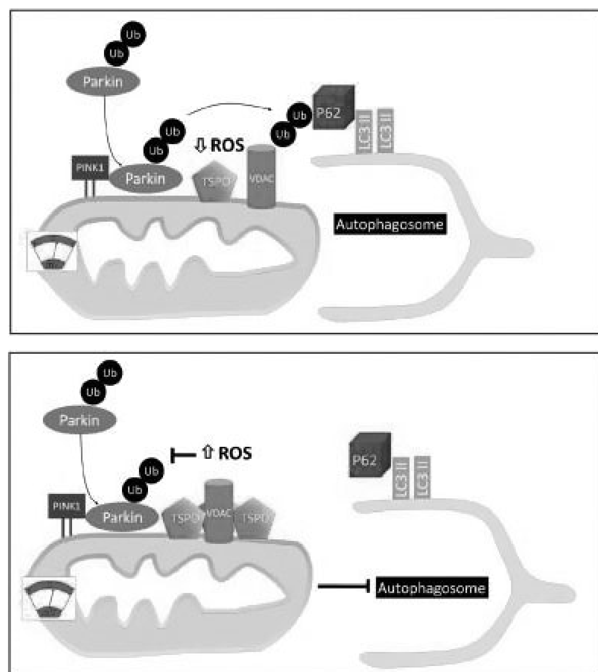

Fig. 1.

obvious molecular target to be exploited in the attempt to amend pathologies characterized by defective mitochondria.

Keywords: Mitophagy, ROS, TSPO.

MON-332
The action of citrus flavonoid – nobiletin on some aspects of mitochondrial bioenergetics

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The changes in mitochondrial biochemistry are one the most important processes accompanying pathophysiology of many types of disorders, including neurodegenerative diseases and cancer. It was revealed that some citrus flavonoids could change the activities of mitochondrial bioenergetics. Among these compounds nobiletin attracts a lot of attention for its neuroprotective and anti-inflammatory action. Nobiletin affects brain cognitive function, facilitates learning and memory formation. This polymethoxylated flavone has anti-amnesic effect and neurotrophic activity, prevents bulbectomy- and amyloid-beta protein-induced memory impairment, improves cerebral ischemia-induced memory deficits suppresses microglial activation, and produces an antidepressant-like effect. However, mechanism of action of nobiletin on the brain mitochondrial metabolism, as well as the target of nobiletin in mitochondria is not revealed. The aim of our study was to investigate the influence of nobiletin on the some aspects of the bioenergetics of isolated brain mitochondria; and determine the target molecules, involving in nobiletin-induced alterations. For this purpose the action of nobiletin on the main mitochondrial enzyme systems was investigated. We have found that action of nobiletin leads to enhancement of SDH activity and improvement of α-KGDH activity and matrix substrate-level phosphorylation in Citric Acid Cycle. Besides, the addition of nobiletin to isolated mitochondria increases NADH dehydrogenase and rotenone-insensitive NADH/CoQ reductase. Furthermore, nobiletin reduces the peroxide production, inducing by Ca^{2+} elevated concentration only in the presence of glutamate/malate; and slightly elevates mitochondrial membrane potential. Our results suggest that the possible molecular target of nobiletin is I complex of respiratory chain and the neuroprotective effects of nobiletin involve activation of α-KGDH activity coupling with acceleration of matrix substrate-level phosphorylation.

Keywords: bioenergetics, mitochondria, nobiletin.

MON-333
The differences in sensitivity to hypothyroidism in synaptic and non-synaptic mitochondria of hippocampus

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Thyroid hormones, as important regulators of growth, development, and metabolism, also exert nongenomic effects on the mitochondrial energy metabolism and several clinical complications of hypotiroidism, like fatigue, cold intolerance, weight gain, bradychardia, etc. are associated with the decrease in basic metabolic rate and oxygen consumption. It was revealed that synaptic (SM) and non-synaptic mitochondria (CM) could differently response to some pathological factors. Therefore, our purpose was study the changes of some parameters of mitochondrial bioenergetics in SM and CM fractions of hippocampus of adult rats in following groups: euthyroid (control), hypothyroid (methimazol-treated), and T4-treated hypothyroid states.

nNOS translocation to CM was observed with concomitant increase of mtNOS activity in hypothyroid rats. Furthermore, oxidation of cytochrome c oxidase and production of peroxides with substrates of complex I (glutamate + malate) were enhanced, whereas the activity of aconitase and mitochondrial membrane potential ($\Delta\phi_m$) were decreased. Additionally, the elevation of hexokinase activity in CM was also found. No differences in these parameters were observed in SM between control and hypothyroid animals. The total ATP production was significantly decreased in both synaptic and nonsynaptic mitochondria in hypothyroid conditions, but the part of ATP, produced by substrate level phosphorylation is significantly increased. The total ATP production was restored to control level in nonsynaptic mitochondria of T4-treated animals.

Our results suggest that hypothyroidism induces mild oxidative/nitrosative stress in neuronal and glial cell bodies of hippocampus that leads to acceleration of aerobic glycolysis. Such compensatory elevation in glycolytic metabolism and substrate level phosphorylation does not occur in synaptic endings and therefore, synaptic mitochondria could be more susceptible to apoptosis during hypothyroidism.

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Keywords: energy metabolism, hypothiroidism, mitochondria.

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MON-334
The effect of long term humic acid administration is on redistribution and usability of metals as cofactors rather than chelating

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The humic acids (HAs) are well documented for their antioxidant effects, but also for their chelating properties towards metals in soil. Antioxidant enzymes commonly use the oxido-reduction properties of metal cofactors for catalytic activity. The question arises of how the proposed chelating properties of HAs can affect the antioxidant status and current levels of these metals. We investigated mitochondria from major synthetic and xenobiotic-metabolizing organs and plasma from chickens, having received HAs in food (0.6%) for 42 days.

There was no demonstrated change in the activity of superoxide dismutase (SOD), but the activity of glutathione peroxidase (GPx) was significantly lower. The activity of glutathione reductase (GR) was significantly higher in the liver and kidneys. Levels of metals detected by atomic absorption spectrometry pointed to particularly significant changes in the amounts of metal present. Cu was higher in liver and plasma, but lower in the kidney. The amounts of Zn decreased in the liver and kidneys, as did Mn. The levels of Fe were uniformly significantly high. Se-containing enzymes are responsible for GPx activity but, as a non-metal, Se was not pursued here. Regarding the activity of GPx and GR enzymes and concurrent lower levels of reduced glutathione (GSH), we can only consider the oxidative stress conditions in mitochondria. Mitochondria provide cellular Fe-S clusters and GR is required to maintain oxidant-labile Fe-S enzymes such as aconitase. It is therefore logical to assume that the total Fe concentration will rise. The increased amount of Fe may contribute to oxidative stress alone, but also to increased activity of iron-dependent enzymes for synthesis of antioxidant enzymes thus directly relating to the use of Cu, Zn and Mn in the mitochondria. Conditions for the oxidation of SOD to form disulfides are essential for the activity of SOD and Cu transfer into the active site. The binding of Cu is the limiting step for the use of Zn. Mitochondrial Mn, however, is normally 1–2 orders less than Fe. Presently we cannot say whether there is metal competition for SOD binding due to an increase in Fe and thereby reduced MnSOD synthesis; however, high Mn levels in the plasma may prevent the impairment of arginase activity and nitrosative stress. Finally, GSH levels in plasma were offset. Despite this, there is an expectation that the administration of HAs does not affect the binding abilities to metals but rather competition, leading to a decline in Se use and compensatory responses. Further studies will be focused on clarifying this, as it may be an important factor for the length of safe and beneficial usage of HAs.

Keywords: chelating properties, humic acid, metal cofactor.

MON-335
The effects of repeated curcumin administration on nociception in mice

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Introduction: Curcumin is a mitochondrial modulator that protects mitochondria and modulates certain antioxidant enzymes. Latest years studies suggested that the balance between anti-oxidants and oxidants is directly involved in different types of pain so that mitochondrion as a redox modulator might by implicated in pain perception.

Aim of the Study: To evaluate the effect of repeated curcumin administration on nociception in mice.

Materials and methods: 17 BALB/c male mice were divided into two groups that received repeated gavage with either curcumin (120 mg/kg b.w., n = 5), either an equivalent dose of olive oil (n = 12). The hot-plate (HPT), tail-flick (TFT), mechanical sensitivity (MST) (*Von Frey method*) and plantar (PT) (*Hargreaves method*) tests were performed to measure nociception, mechanical allodynia and thermal hyperalgesia, before (baseline) and after two weeks of curcumin or olive oil daily administration. Percentage change from baseline was calculated and the data expressed as mean ± S.E.M. and analyzed using paired and unpaired Student's t-test.

Results: Oral administration of curcumin has a significantly effect on tail flick test ($p = 0.032$), mechanical sensitivity test ($p = 0.049$) and Hargreaves test ($p = 0.0047$) latencies expressed as changes from baseline when compared with control group. When compared with the baseline values, curcumin significantly increases latencies of TFT (3.5 ± 0.2 s versus 4.8 ± 0.1 s) and PT (6.4 ± 0.8 versus 11.8 ± 0.8 s). Even if an increase in plantar test latencies were also observed after 2 weeks of olive oil treatment, the effect of curcumin was significant ($p = 0.0047$) regarding the percentage change from baseline ($130.5 \pm 46.6\%$ in curcumin treated group versus $32.7 \pm 8.3\%$ in olive oil treated group).

Discussions: Our findings demonstrate that 14 days of curcumin administration has an antinociceptive effect on the tail flick test and no effect on the hot plate test. These results suggest that curcumin might exert its effects on the spinal level as long as tail flick is mainly spinally and HP supraspinally mediated. Curcumin in in a similar way as olive oil, increases plantar test latencies but has no effect on mechanical allodynia. However a more important percentage change from baseline ($p = 0.0047$) was observed after curcumin administration.

In *conclusion,* we demonstrate that repeated curcumin administration modulates thermal nociception and allodynia but the exact mechanisms by which this occurs need further studies.

Acknowledgements: The research was supported by the Executive Agency for Higher Education and Research Funding (UEFISCSU) Romania project PN-II-ID-PCE-2011-3-0875.
Keywords: mitochondria,, Pain, redox balance.

MON-336
The effects of single-dose intrathecal administration of methylene blue on acute and inflammatory pain in mice

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Introduction: Methylene blue (MB) acts as an electron transfer mediator, significantly increasing mitochondrial complex I–IV activity in isolated mitochondria and resistance to oxidative stress [1]. Recent studies indicate that mitochondrial modulation might influence pain perception and transmission.

Purpose: To investigate the effects of acute MB i.t. administration on the formalin orofacial pain test.

Methods: BALB/c male mice received by means of intrathecal administration single-dose of either MB (0.05 mg/kg b.w.) or saline (equivalent volume). Two hours after administration, the mice were injected with 20 μl of 5% formalin into the upper lip in order to assess the orofacial pain (FOF). The nociceptive score was determined by recording the number of seconds animals

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spent grooming the injected area and expressed as mean ± SEM, separately for the 2 phases of the formalin test: the first phase (neurogenic) respectively the second phase (inflammatory). The results were compared by means of unpaired Student t test.

Results: Intrathecal MB administration induced a statistically significant decrease in inflammatory pain behavior as assessed by the second phase. Thus, MB injected mice spent an average of 56.4 ± 14.4 s grooming the affected area, whereas control group had an average of 138.8 ± 28.1 ($p = 0.02$). MB administration had no effect on the first phase of the test (acute pain), with an average nociceptive score of 37.1 ± 9.9 s in the MB group and 49.1 ± 5.9 s in the control group.

Conclusions: Our study results indicate that i.t. MB administration exerts a significant anti-nociceptive effect. Its mechanism is linked probably to MB action on the central nervous system structures implied in the inflammatory phase of the formalin pain. Further studies are needed in order to elucidate the mechanism by which MB exerts its effects.

Acknowledgements: Research supported by UEFISCSU Romania, project PN-II-ID-PCE-2011-3-0875.

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Keywords: inflammatory pain, methylene blue, mitochondria.

MON-337
The influence of resveratrol on chemically induced mammary carcinogenesis

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Introduction: Mammary carcinogenesis was induced in female Sprague-Dawley rats by exposure to N-methyl-N-nitrosourea (NMU). Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a widely known polyphenolic agent from red wine, which has been shown to exert antioxidant, anti-inflammatory and anticarcinogenic effects. Resveratrol is a bioactive compound which can be used against cancer cells in human body but a molecular mechanism is currently not known. Resveratrol and its derivatives are resistant against metabolizing enzymes therefore were suggested for the treatment of cancer and as a pharmacologically significant modifiers of other antineoplastic drugs in use. Mitochondria is highly organized multifluorescent system of endogenous fluorescent molecules, which can be detected by fluorescence spectroscopy.

Materials and Methods: Mammary carcinogenesis was induced by N-methyl-N-nitrosourea (NMU), which was administered in two intraperitoneal doses (50 mg/kg of body weight). Chemoprevention with resveratrol (100 mg/kg of body weight) started 12 days before the first dose of NMU and the administration lasted until the end of the experiment. The tumour incidence and latency period were evaluated. Mitochondria of mammary gland tumor were isolated according to Johnson and Lardy. Protein content of isolated mitochondria was determined by the method of Bradford. The suspension of isolated mitochondria was diluted to a final concentration of 2 mg/ml using a respiration medium (pH 7.4) containing the substrate succinate. Autofluorescence of the blood serum/plasma samples was analysed by synchronous fluorescence fingerprint on Luminescence Spectrophotometer Perkin-Elmer LS 55.

Results: Resveratrol administration resulted in reduced tumour incidence (22%) and prolonged latency period ($p < 0.01$) in compare to NMU group. The significant increase of the fluorescence intensity at 280 nm (proteins) was observed in the experimental groups which received NMU ($p < 0.001$) and NMU after chemoprevention with resveratrol ($p < 0.05$) in comparison with the control group. The significant increase of the fluorescence intensity at 350 nm (reduced coenzyme NADH+H^+) was observed in the experimental groups which received NMU ($p < 0.01$) and NMU after chemoprevention with resveratrol ($p < 0.001$) in comparison with the control group.

Conclusions: Resveratrol administered at a dose 100 mg/kg showed both prooxidative and antioxidative effect with the prevalence of cytotoxic effect.

This study was supported by DIAGONKO, ITMS: 26220220153 and MediPark Košice, ITMS:26220220185, Operational Programme Research and Development (OP VaV-2012/2.2/08-RO).

Keywords: fluorescence, mitochondria, resveratrol.

MON-338
The mitochondria in testing drug-induced toxicity

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Mitochondria are now of an increasing interest in medical research due to the facts that mitochondria play a central role in various metabolic processes of the cell and that mitochondrial dysfunction is related to several important pathologies. Mitochondrial membrane potential ($\Delta\psi$) is the driving force for ATP synthesis in the cell. Maintenance of membrane potential is known to be the critical factor determining mitochondrial viability, as well as the system of mitochondrial quality control, essential for normal mitochondrial functioning. We have shown that mitochondria may be used as a biosensor for evaluation of toxic effects of potential drugs on mitochondria *in situ*. We studied effects of toxic agents – mastoparan, alamethicin and melittin (that are able to permeabilize biological membranes) in rat liver mitochondria preparations generating transmembrane potential ($\Delta\psi$). Mitochondrial $\Delta\psi$ was determined by changes in fluorescence in the same media as control oximetric measurements. Rates of succinate oxidation were determined with a Clark-type electrode. The effect of studied toxic agents was accompanied by decrease of mitochondrial $\Delta\psi$. But the magnitude of activation of State 4 respiration rate (v_4) by different peptides does not correlate with the effect on $\Delta\psi$. Melittin and mastoparan have not dissipated mitochondrial $\Delta\psi$ radically. In the presence of these peptides mitochondria retain stable $\Delta\psi$, so these peptides may be less harmful (compared with alamethicin). The comparison of $\Delta\psi$ dissipation value and value of the ratio of peptide concentration causing lysis of mitochondria/peptide concentration activating v_4 by 200%, of potential drugs may be used for comparative testing on their toxicity. The prospects of appearance of novel medicals, the problem of increasing the activity or reducing the toxicity of new drugs, the limitations of their medicinal use, problems that may enhance existing anticancer and anti-infectious therapies are in the focus of drug research now. The use of isolated mitochondria as a test system for screening potential drugs for their safety may be useful in preclinical studies of new drugs.

Keywords: mitochondria, test system.

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MON-339
The mitochondrial pyruvate carrier. Old and recent findings call for more attention to this main substrate transport system

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The first evidence of the existence of a mitochondria pyruvate carrier dates back to 1971 (Papa et al., FEBS Lett. 12, 285) followed by characterization of kinetics, substrate specificity, inhibitor sensitivity (Paradies and Papa, Abstr. Commun. International Congr. Biochem. 1973, p. 288; Papa and Paradies, Eur. J. Biochem. 1974, 49, 265) confirmed by specific inhibition by α-cyano-4-hydroxycinnamate (Halestrap and Denton, Biochem. J. 1974, 138, 313). After nearly forty years, two independent groups (Bricker at al., Science, 2012, 337, 96; Herzig et al., Science, 2012, 337, 93) identified a complex of two mitochondrial proteins, MPC1 and MPC2 and the genes of the pyruvate carrier. The carrier, since of its essential role for mitochondrial degradation to CO_2 of glycolytic pyruvate and the capacity of pyruvate/acetoacetate exchange-diffusion plays a key role in energy metabolism, ketogenesis, fatty acid synthesis, etc. Depression of the pyruvate carrier was, in fact, found in mitochondria from different experimental tumours (Paradies et al., Cancer Research, 1983, 43, 5068) and from liver of experimental diabetic rats (Kielducka et al., J. Bioenergetics and Biomembranes, 1981, 13, 123). Recently mutations in the MPC1 genes have been detected in families with children affected by lactic acidosis (Bricker, see above ref.). All this calls for more attention to the role of the pyruvate carrier in human pathophysiology (see Shell and Rutter, Cancer Metabolism, 2013, 1: 6).

Keywords: mitochondria, pyruvate carrier.

MON-340
The molecular selectivity of type II NADH: quinone oxidoreductase for quinones – a docking study

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Type II NADH: quinone oxidoreductase is a flavoprotein that catalyzes the transfer of electrons from NADH to quinones and it can, in several organisms, replace the respiratory system (complex I-V).

Recent high resolution structures show that NDH-II is a homo dimmer membrane bound protein with an amphiplilic anchor that allows the interaction with the membrane.

Different microorganisms produce different quinones: e.g. in *E. coli* the physiological quinone is ubiquinone, while *S. aureus* and *B. subtilis* synthesis menaquinone, thus NDH-II will have a different molecular interaction and selection mechanism for different organisms.

Despite the high resolution structural information available for NDH-II in complex with quinone, NAD and FAD, the mechanism behind the molecular selection and interaction with is still elusive.

To unravel the interaction mechanism of NDH-II:quinone we setup a modeling and *in silico* docking calculation based on the published high resolution structures of NDH-II. We identified the interaction hotspots of NDH-II from different species to different quinones and discuss the role in the enzymatic mechanism.

Keywords: docking, NADH: quinone oxidoreductase, quinones.

MON-341
The protective role of Sestrin2 induction against glucose deprivation via modulation of mitochondrial function

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Sestrin2, an Nrf2-dependent antioxidant enzyme, regulates diverse cellular functions in response to various stresses including metabolic and oxidative stress. In this study, we demonstrated that the role of Sestrin2 induction in glucose deprivation-induced oxidative stress. First, we observed that glucose deprivation significantly increased Sestrin2 expression in mRNA or protein levels in primary hepatocytes, AML12, Huh7 and HepG2 cells. Glucose deprivation increased intracellular reactive oxygen species (ROS) production, which contributed induction of Sestrin2. To elucidate whether induction of Sestrin2 by glucose deprivation is accompanied by Nrf2 activation, the phosphorylation of Nrf2 was examined. Glucose deprivation increased a phosphorylation of Nrf2. Moreover, Sestrin2 induction by glucose deprivation was decreased in ARE-deleted Sestrin2 reporter gene assay. To study the role of Sestrin2 induction, we used Sestrin2 overexpressed stable cells. Overexpression of Sestrin2 in cells rescued cells from glucose deprivation-induced cytotoxicity. Moreover, glucose deprivation-induced ROS production and mitochondrial impairment was decreased in Sestrin2 overexpressed stable cells. However, mitochondrial membrane potential was significantly lost by siRNA knockdown of Sestrin2. Collectively, we provide evidence for the functional importance of Sestrin2 induction in prevention of glucose deprivation-induced oxidative stress and cell death.

Keywords: Sestrin2, Metabolic stress, Mitochondrial dysfunction, Glucose deprivation, Hepatocytes.

MON-342
The role of Sestrin2 induction by resveratrol in methylglyoxal-induced toxicity

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Resveratrol, a naturally occurring polyphenolic compound, is found in grapes. Previous reports have shown that resveratrol has various beneficial effects in metabolic diseases including diabetes and obesity. This study investigated whether methylglyoxal, a glucose-derived dicarbonyl intermediate, causes ROS-mediated cytotoxicity in HepG2 cells and resveratrol protects cells from the methylglyoxal-induced cell death. Methylglyoxal significantly induced apoptosis in cells and oxidative stress was involved in its cytotoxicity. However, pretreatment of resveratrol protected cells from methylglyoxal-induced apoptosis. In addition, resveratrol recovered the level of GSH and attenuated ROS production. In addition, methylglyoxal-induced mitochondrial impairment, as evidenced by the increase of ADP/ATP ratio increase and observation of mitochondrial permeability transition was restored by resveratol. Resveratrol induced Sestrin2, as a novel antioxidant protein, which contributed to repress methylglyoxal-induced apoptosis. Collectively, our results demonstrated that resveratrol could protect cells from methylglyoxal-induced ROS production, mitochondrial impairment which is mediated with Sestrin2 induction.

Keywords: Resveratrol, Methylglyoxal, Sestrin2, Oxidative stress, Mitochondrial dysfunction.

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MON-343
The study of calcium capacity and induction of Ca^{2+}-dependent nonspecific permeability of the inner membrane in the liver mitochondria of guinea fowl (*Numida meleagris* L.)

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It is known that the mitochondria of animals not only provide cell ATP and heat, but also involved in cell death by apoptosis and necrosis. Induction of Ca^{2+}-dependent nonspecific permeability of the inner membrane (pore open) is one of the cell death pathways associated with the mitochondria. Mechanisms and regulation of the opening pores mainly studied in liver and heart mitochondria of laboratory rats, which have a relatively short lifespan. However, similar mechanisms of induction of mitochondrial nonspecific permeability in birds' vital organs, which are characterized by greater longevity, are unexplored.

In the study, we used males of 'wild' gray-speckled population of guinea fowl (*Numida meleagris* L.), and white lab male rats as control animals. Mitochondria from the liver of mature male white rats and guinea fowl were isolated by conventional differential centrifugation with subsequent separation of endogenous fatty acids with fatty acid-free BSA. Mitochondrial calcium capacity, or ability of mitochondria in the presence of inorganic phosphate and other penetrating anions capture and hold of calcium ions in matrix, was determined by a calcium-selective electrode. Induction of calcium-dependent pore evaluated by mitochondrial swelling in the sucrose incubation medium by recording the change in optical density of the mitochondria suspension at a 540 nm.

We have found that calcium capacity of the liver mitochondrial of guinea fowl in the presence of 1 mM inorganic phosphate is 1.4 mM in the absence and 2 mM to presence of 1 μM cyclosporin A (CsA). At the same time, calcium capacity of rat liver mitochondria in the absence of CsA does not exceed 100 μM. At these concentrations of calcium liver mitochondria of rats and guinea fowl intensively swell in sucrose incubation medium, that indicating on opening nonspecific pores. In experiments on rat liver mitochondria established that 20 μM hexadecanedioic acid (HDA) in the presence of 200 μM Ca^{2+} is able to induce nonspecific permeability of organelles by insensitive to CsA mechanism. Similar data were obtained on the liver mitochondria of guinea fowl. However, the intensity of the swelling of guinea fowl liver mitochondria is much less marked than in rat liver mitochondria. The mechanisms underlying the phenomena, which described above, is expected to examine in subsequent research.

This work was supported by the Ministry of Education and Science of Russian Federation (project ■ 1365) and grant of the Russian Foundation for Basic Research (■ 14-04-00688).
Keywords: calcium capacity, guinea fowl, mitochondria.

MON-344
Understanding the regulation of mitochondrial bioenergetics by the immune adaptor protein SARM1 and its role in age-related neurodegeneration

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Aging poses a risk factor for the development and progression of neurodegenerative diseases like Alzheimer's (AD) and Parkinson's disease (PD) which share common morphological features

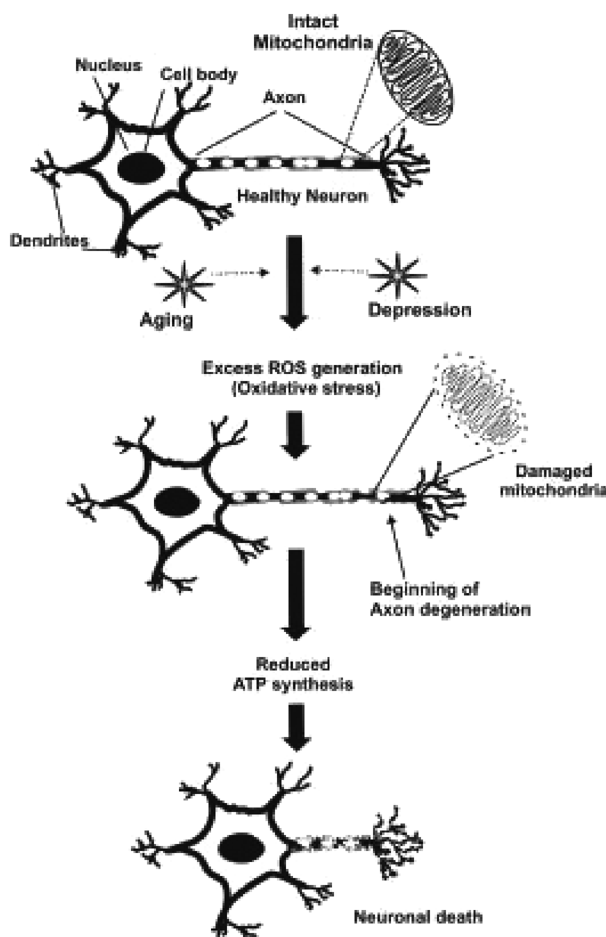

Fig. 1. Neuronal loss in aging and depression.

of mitochondrial dysfunction and axon degeneration as an early pathological mechanism. Although recent evidence suggests that normal aging and the degeneration of specific neuron populations in AD or PD may be linked by the same cellular mechanisms, this remains a topic of intense debate. Mitochondria are required to meet the high energy demands of neuronal cells and any defect in this pathway make the neurons particularly susceptible to bioenergetic failure ultimately resulting in energy crisis and neuronal loss. An exciting area of research involves understanding the various components of mitochondrial energy metabolism that may eventually lead to axon degeneration in normal aging and age-related neurodegeneration. The adaptor molecule, sterile alpha and TIR motif-containing 1 (SARM1) mediates excess reactive oxygen species (ROS) generation in the neuronal mitochondria that may lead to mitochondrial dysfunction and neuronal death. Interestingly, SARM1 plays an important role in the axonal self-destruction program through a mechanism not yet known. This study aims to analyze whether SARM1 is a direct player in the ROS generating machinery of neurons particularly axons during aging resulting in mitochondrial damage, energy crisis and axonal retraction. In summary, the regulation of an intrinsic death pathway by SARM1 may provide the missing link between the process of age-related decline in mitochondrial bioenergetics and subsequent axon loss.

Keywords: Neuron mitochondria neurodegenration SARM1 ROS.

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MON-345
Ca^{2+}-dependent fusion pore induced by α,ω-dioic acids as a possible mechanism of the mitochondrial permeability transition

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Recently we found that the α,ω-dioic acids (among them α,ω-hexadecanedioic acid (HDA) was most effective) are able to induce the opening of the 'non-classical' cyclosporin A (CsA)-insensitive pore in the liver mitochondria loaded with Ca^{2+} or Sr^{2+}. It has been suggested that such permeability is based on the formation of a pore in the membrane of organelles which has lipid nature. In this paper, in order to clarify the molecular mechanism of this process, we studied the effect of HDA on the Ca^{2+}-dependent permeability of artificial membranes using the model of large unilamellar liposomes (LUV) loaded with the fluorescent dye sulforhodamine B (SRB). It was shown that HDA, like palmitic acid (PC), is able to induce permeability of LUV for SRB in the presence of Ca^{2+}. However, the kinetics of SRB release upon the induction of Ca^{2+}-dependent permeability of LUV by HDA and PC is different. Furthermore, it was found that HDA is inferior to PC in ability to induce LUV permeability for SRB. Under the same conditions, α,ω -tetradecanedioic acid which acyl chain is 2 carbon atoms shorter than HDA is less effective. Consequently, the effectiveness of these α,ω-dioic acids as inducers of pore opening is sharply reduced with decreasing number of carbon atoms in their acyl chain, which may be associated with a decrease in their ability to dissolve in lipids. It was established that HDA/Ca^{2+}-induced SRB release from LUV is also dependent on the pH of the surrounding solution. The maximum release of SRB from LUV induced by HDA/Ca^{2+} complex formation was observed at alkaline pH (pH 9.0–10.0). Using the method of ultrasonic interferometry it's shown that HDA, unlike PC, doesn't induce a chemotropic phase transition in the lipid bilayer of membrane in the presence of Ca^{2+}, which as was shown earlier underlies the permeability of lipid membranes induced by Ca^{2+} and PC. Thus, it can be assumed that the Ca^{2+}-dependent permeability of the LUV, induced by HDA and PC occurs by different mechanisms. The method of dynamic light scattering showed that the addition of Ca^{2+} to HDA-modified LUV induces their fusion. We believe that the process of LUV fusion may be accompanied by partial SRB release from LUV due to the formation of fusion pores.

We assumed that the process of membrane fusion may underlie the previously described HDA/Ca^{2+}-induced nonspecific permeability of the inner membrane of liver mitochondria. It is possible that HDA and Ca^{2+} induce fusion of inner and outer mitochondrial membranes in the field of contact sites where the membranes are close together, with subsequent formation of fusion pore resulting in swelling of the mitochondria. This study was supported by the Ministry of Education and Science of Russia (No. 1365).

Keywords: Fusion pore, Mtochondrial permeability transition, α,ω-Dicarboxylic Acids.

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CSIII-04 – Neuronal function & imaging

MON-347

A new class of aggregation inhibitor of amyloid-β peptide based on green tea catechins

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Alzheimer's disease (AD), the major dementing disorder of the elderly which accounts for 55% of all dementia patients worldwide, is related to amyloid-beta peptide, the main component of senile plaques in Alzheimer's disease brain. Observations suggest a broad ability for green tea catechins (GTC) to disrupt early-stage and late-stage aggregation processes but the molecular mechanism has not been fully elucidated. To advance the understanding of the potential use of GTC in the prevention or treatment of AD patients, we investigated the effect of natural and synthetic compounds on APP processing. We checked the ability of gallate containing phenolic compounds, including (-)-catechin, (-)- catechin gallate, (-)-epicatechin gallate, (-)-epigallocatechin, (-)- epigallocatechin gallate, gallic acid, and trimethoxy derivatives of epicatechin-3-gallate to inhibit acetylcholinesterase (AChE) activity and AChE-promoted Aβ aggregation. Compounds were analyzed for their ability to inhibit AChE by colorimetric and radiometric assays and for determining their type of enzyme inhibition. Fluorimetric studies with thioflavin T were performed to evaluate their effect on beta amyloid aggregation. We have found that TMCG, a 3,4,5-trimethoxy derivative of catechin gallate, exhibits a non competitive inhibition of AChE, has a disaggregating effect on amyloid fibrils and decreases the secretion of both 1–40 and 1–42 amyloid peptides to the extracellular medium of SH-SY5Y APP695 cell cultures. According to stability and bioavailability, methoxy derivates of catechins are promising drug to ameliorate the Alzheimer′s pathogenesis.

Keywords: acetylcholinesterase inhibitors, Alzheimer's disease, Catechin.

MON-349

Association of Alzheimer disease with DNA topoisomerase IIβ in primary neuronal cells

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In mammalian cells, DNA topoisomerase IIβ (topo IIβ) plays an important role in the initiation of selective gene transcription, neuronal differentiation and axonogenesis. Inhibition of topo IIβ activity both in vivo and in vitro results in shorter axon length and increase of DNA damage by suppressing the transcriptional induction of differentiation- related genes. However, in which pathways of axonogenesis controlled by topo IIβ has not been clarified yet. Additionally, the observed symptoms of Alzheimer‘s disease such as axon shortening and increase in DNA damage give rise to thoughts about the abnormalities in the expression of topo IIβ or aberration of the enzyme in different levels. However, there is no outcome related to the effect of topo IIβ in the course of Alzheimer‘s Disease (AD).In our study, it was studied that whether or not the symptoms of Alzheimer‘s disease associates with the function of topo IIβ in rat primary neuronal cell culture. For this purpose, rat cerebellar granule neurons from post-natal 9 days (P9) old rats were firstly isolated and cultured. Then, amyloid beta (Aβ) 1–42 peptide fibrils were synthesized by the incubation of Aβ 1–42 peptide monomers at 370C during 24 and 48 hours, separately while shaking. These fibrils were given to primary neuronal culture on the 4th day in vitro (DIV). To confirm the success of the establishment of in vitro, immunofluorescence and Congo red staining were performed. Moreover, toxicity of Aβ fibrils given to primary neuronal culture on 4th DIV was observed through Real Time Cell Analysis (RTCA) system during a week. As a final step, in order to associate the function of topo IIβ with AD, samples collected from primary neuronal culture were applied to western blot technique upon Aβ fibrils exposure to the cells during a day. To evidence establishment of in vitro AD, amyloid beta plaques around neurons were detected both by Aβ 1–42 antibody and Congo red stain. Besides these, the choice for the concentration of Aβ 1–42 fibril as 7 μM might have been not enough to be neurotoxic to cerebellar granule cells. It would be better to prefer 14 or 28 μM and 48 hours exposure of Aβ 1–42 peptides to primary neuronal culture rather than one day exposure.To gain insight to the association of topo IIβ with Alzheimer's disease we established in vitro Alzheimer's disease model on cerebellar granule neurons isolated from post natal 9 day Wistar rats. We investigated the possible neurotoxicity of amyloid beta 1–42 aggregates on primary neuron culture affect the expression of both Alzheimer related genes (Presinilin1 and cofilin) and topo IIβ assuming its role in neuron developmnent and axonogenesis, simultenously

Keywords: Alzheimer's Disease (AD), Axonogenesis, DNA topoisomerase IIβ.

MON-350

C2 domain of CPNE1 plays a pivotal role in neuronal differentiation

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CPNE1 is known as a calcium-dependent membrane-binding proteins that regulate signal transduction and membrane trafficking. Previous study showed that CPNE1 directly induced the neuronal differentiation of hippocampal progenitor cell line, HiB5 (Mol. Cells 34, 549–554). To understand how CPNE1 induces neurite outgrowth of HiB5 cells, we investigated the effect of neuronal differentiation of its several truncated mutants depend on its known domain. Here, we showed that over-expression of CPNE1-C2-domains increased neurite outgrowth and expression of TUJ1, a neuronal marker protein. Here, calcium binding defected mutant of CPNE1 did increased interestingly neurite outgrowth and expression of TUJ1 of HiB5 cells. This may mean that C2 domain of CPNE1 related with differentiation does not depend on calcium. Furthermore, phosphorylation of AKT was increased by over-expression of CPNE1-C2-domain and(or) calcium binding defected mutant of CPNE1. These results suggest that C2 domains of CPNE1 play an important role for neuronal differentiation in HiB5 cells.

Keywords: None.

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MON-351
Determination of cresyl phosphate adduct in F-16 pilots by mass spectrometer

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The oxygen for F-16 fighter pilots is supplied by an on-board oxygen generating system (OBOGS) that uses bleed air from the engine to generate oxygen. The air passes through the jet engine before it is enriched for oxygen and breathed through an oxygen mask. While in the jet engine, the air can become contaminated with jet engine lubricating oil. An important constituent of lubricating oil is tricresyl phosphate, an anti-wear additive. The ortho isomer of tricresyl phosphate (TOCP) is a minor component of tricresyl phosphate. It is suspected that exposure to TOCP and other oil ingredients is responsible for the illness called aerotoxic syndrome. The symptoms associated with aerotoxic syndrome include nausea, blurred vision, dizziness, headache, tremor, confusion, memory loss and breathing difficulties. TOCP is metabolically converted by cytochrome P450 enzymes to cresyl saligenin phosphate (CBDP). CBDP reacts with butyrylcholinesterase (BChE) to make a covalent adduct on serine 198. The presence of phosphorylated BChE in a person's blood is an indication of exposure to TOCP. The purpose of this work was to determine whether the blood of healthy, active-duty F-16 pilots has measurable levels of the cresyl phosphate adduct.

BChE was immunopurified from 0.5 ml plasma by binding to immobilized monoclonal mAb2. BChE protein was released with acetic acid, digested with pepsin and analyzed by LC-MS/MS on the 5600 Triple TOF mass spectrometer. Positive controls for quantifying the limit of detection were plasma samples containing known amounts of cresyl saligenin phosphate treated plasma. The cresyl phosphate adduct eluted at 31.3 minutes with an observed parent ion mass of 966.4 m/z and characteristic daughter ions 778.3, 673.3, and 602.3 m/z. Control experiments demonstrated that as little as 0.1% of the 1 to 2 μg. BChE recovered from 0.5 ml plasma could be detected as the cresyl phosphate adduct on peptide $FGES_{198}AGAAS$. Mass spectrometry analysis of plasma from fifteen healthy F-16 pilots showed that none had evidence of exposure to TOCP. It was concluded that the on-board oxygen generating system, when operating properly, did not deliver tri-ortho-cresyl phosphate in the oxygen supply.

Acknowledgement: Supported by a postdoctoral research fellowship from The Scientific and Technological Research Council of Turkey (TUBITAK 2219).
Keywords: aerotoxic syndrome, cresyl saligenin phosphate, plasma butyrylcholinesterase.

MON-352
Disassembly of microtubular cytoskeleton underlays neurotoxicity of cytosolic prion protein

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Prion protein (PrP) plays a key role in the pathogenesis of transmissible spongiform encephalopathies (TSE). TSE are fatal, infectious neurodegenerative diseases that affect humans and animals. PrP is mostly extracellular glycoprotein anchored in the plasma membrane. In pathology, however, fraction of PrP mislocalized

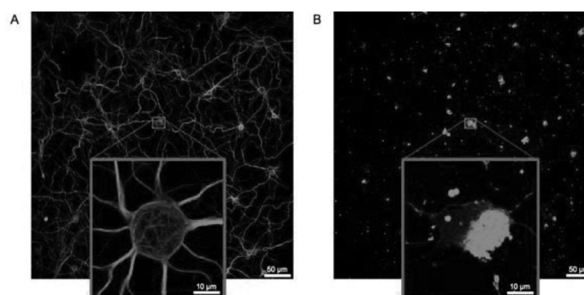

Fig. 1. PrP1-30 disintegrates microtublar cytoskeleton and causes loss of neurites. Untreated primary neuronal cells (A) and treated with PrP1-30 (B) were stained with antibodies to β-tubulin to visualize microtubules.

in the cytosol (cytoPrP) significantly raises. Our previous studies have shown that PrP interacts with tubulin. This interaction induces tubulin oligomerization/aggregation and inhibits microtubule formation. We have also demonstrated that two microtubule-associated proteins (MAPs) which regulate microtubule stability: Tau and MAP2, can prevent above-mentioned effects of PrP. Importantly, we have shown that phosphorylated Tau losses its ability to protect microtubules from deleterious effect of PrP. Noteworthy, hyperphosphorylated Tau has been frequently detected in TSE. To investigate influence of PrP on the microtubular cytoskeleton of primary neurons we have used synthetic peptide encompassing first 30 amino acid residues of PrP (PrP1-30). The peptide consists of the N-terminal signal sequence (1–22) responsible for penetration of the molecule through cell membrane and the major tubulin-binding site (23–30). By means of confocal microscopy we have demonstrated disassembly of microtubular cytoskeleton and loss of neurites of primary neurons exposed to PrP1-30. To examine the role of phosphorylation of MAPs we have modulated activity of GSK3 - main kinase responsible for the modification of Tau and MAP2. As an inhibitor of GSK3 we chose LiCl. We have examined morphology of the neurons incubated simultaneously with PrP1-30 and LiCl and have noticed that they do not differ from the control cells. We have confirmed the involvement of GSK3 in neurotoxicity of cytoPrP by using specific inhibitor of the kinase – CT98014. Cells treated with LiCl or CT98014 sustained proper morphology in contrast to the cells treated with PrP1-30 alone, which had much shorter neurites and tubulin aggregates concentrated in the cell body. To confirm the role of PrP-tubulin interaction in the toxicity of cytoPrP we conducted experiments in which neurons ware treated with taxol in order to stabilize microtubular cytoskeleton and subsequently exposed to PrP1-30. Expectedly, taxol-treated cells retained proper morphology even after incubation with PrP1-30. In cytotoxicity tests (LDH) we have confirmed protection of neurons from toxic effect of PrP1-30 by both inhibitors of GSK-3 as well as taxol. Our observations may help in understanding the molecular mechanism of neurotoxicity of cytoPrP. Since LiCl is well-known pharmaceutical used in the treatment of several mental disorders we believe that our study may be useful in design of TSE therapy.

Keywords: microtubular cytoskeleton, neurotoxicity, Prion protein.

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MON-353

Does GABA shunt contribute to schizophrenia-related GABA deficits? An in vitro study on ketamine-induced rat model of schizophrenia

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A converging body of evidence implicates GABA (γ-aminobutyric acid) in the pathogenesis of schizophrenia. In particular, GABA deficits have been linked to the symptoms of cognitive dysfunctions found in clinical studies and in animal models. *In vivo* GABA is formed by a pathway referred to as the GABA shunt. The first reaction involves the transamination of α-ketoglutarate into L-glutamic acid by GABA α-oxoglutarate transaminase (GABA-T). Then, glutamic acid decarboxylase (GAD) catalyzes the decarboxylation of glutamic acid to form GABA. GAD exists in two isoforms: GAD65 and GAD67 which differ by regulation and specific properties. It is believed that GAD65 in neurons is mainly responsible for synthesis of GABA pool for neurosecretion whereas GAD67 would produce GABA for energy metabolism. GABA is metabolized by GABA-T to form succinic semialdehyde which, in turn, can be oxidized by succinic semialdehyde dehydrogenase (SSADH) into succinate and can then reenter the Krebs cycle. To evaluate whether alterations in synthesis and/or activity of GABA shunt enzymes may affect the total available pool of GABA in schizophrenic brain, we used ketamine-induced rat model of schizophrenia. Following open-field behavioral tests, brains were dissected and cortex, striatum, hippocampus and cerebellum were isolated. Interestingly, GAD and GABA-T activity was decreased in schizophrenic cortex and striatum, an increase was detected in hippocampus but no changes occurred in cerebellum. In case of SSADH, reduced activity was noted in cerebellum and cortex, in contrast to the striatum and hippocampus in which increase or unchanged activity was demonstrated, respectively. Assessing molecular background of this phenomenon, we observed that altered activity of examined enzymes was associated with changes in their mRNAs and protein level, what we evaluated using real-time PCR and Western blot. Also, different enzymatic activities could directly affect GABA level. By using ELISA we detected lower GABA content in schizophrenic cortex and striatum and reduced GABA secretion from synaptosomes obtained from these structures. Finally, we found that changes in GABA level were accompanied by disturbed calcium homeostasis, as increased resting Ca^{2+} level was detected in all examined brain structure isolated from ketamine-treated rats. Our overall data suggest that GABA metabolism may be regulated in unique fashion in different parts of schizophrenic brain. One could assume that altered expression/activity of GABA-metabolizing enzymes may underlie disease-associated deficits in this neurotransmitter.

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Keywords: Calcium homeostasis, GABA metabolism, ketamine-induced schizophrenic symptoms.

MON-354

Effect of cannabinoids on locomotor behavior and sleep/wake regulation in Lipocalin-type prostaglandin D synthase and Adenosine A2A receptors KO mice

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Cannabinoids are known to have a number of pharmacological effects such as hypolocomotion, hypothermia, catalepsy and memory impairment in rodents. In this study, we focused on the effects of cannabinoids on sleep/wake regulation in mice. As the initial study, we examined the effects of four synthetic cannabinoids, cannabicyclohexanol (CCH), JWH-018, (-)-CP-55940, (+)-WIN-55212-2, and an endocannabinoid anandamide on locomotor activities in WT (C57BL/6) mice. Among them, CCH, JWH-018 and (-)-CP-55940 (5 mg/kg, i.p., respectively) remarkably decreased the locomotor activity. Subsequently, we compared the effects of the three synthetic cannabinoids on locomotor activities in Lipocalin-type prostaglandin D synthase (L-PGDS) KO mice and adenosine A_{2A} receptors ($A_{2A}R$) KO mice as animal models of sleep/wake disorders. The suppressive effects of CCH and JWH-018 on locomotor activities were more potent in L-PGDS KO and also $A_{2A}R$ KO mice than those of WT mice. Additionally, we tested the effects of the three synthetic cannabinoids on sleep/wake regulation evaluated by electroencephalogram (EEG) in WT mice. CCH, JWH-018 and (-)-CP-55940 significantly increased the EEG power in a frequency range of 2 – 3 Hz, which appears during non-rapid eye movement (non-REM) sleep in WT mice, for 12 h, 10 h and 10 h, respectively, as compared with the vehicle-treated control. These data suggested that the hypolocomotion caused by synthetic cannabinoids is partially regulated by prostaglandin D_2/adenosine system, and the changes in EEG by synthetic cannabinoids may relate to sleep/wake system.

Keywords: cannabinoid, locomotor activity, sleep/wake.

MON-355

Effect of YC-1 on the expression of matrix metalloproteinase 9 in single-dose pentylenetetrazol-induced epileptic seizures

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YC-1, a synthetic bezylindazol derivate (3-(50-hydroxymethyl-20-furyl)-1-benzylindazol), that has been demonstrated to have antiplatelet activity. YC-1 may be reached to central nervous system due to its lipid solubility, at the same time, it could be improved as neuroprotective agents for demonstrating protective effects directly on neurons and the blood brain barrier (BBB). Dysfunction of BBB supported epileptiform activity and showed positive correlation with seizure frequency. The BBB permeability increased in pentylenetetrazol (PTZ)-induced models of epilepsy and it leads to many functional changes. In several studies determinated that matrix metalloproteinases (MMPs) induce the formation of seizures by providing the opening of the BBB and caused epilepsy development. The aim of this study was evaluated the effect of YC-1 on the expression of MMP-9 in a single dose PTZ-induced epilepsy model. 28 male rats were divided into four experi-

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mental groups (n = 7) including: 1- received PTZ (55 mg/kg i.p.) and YC- (1 mg/kg i.p), 2-single dose PTZ –recevied rats, 3- YC-1 (1 mg/kg i.p.) as a control group and 4- % 0.1 DMSO (vehicle) treated group. YC-1 was injected 10 minutes prior to PTZ injection for animals. Electrical activity of the cortex and seizure duration and latency recorded with a PowerLab 8S System v.5 (ADI Instruments, U.K.). The expression of MMP-9 in the rat cortex was tested by Western blot. p values <0.05 accepted as statistically significant. Our preliminary findings revealed that YC-1 had no effect on PTZ seizure latencies and minor seizures, but it increased the death rate and major seizure. Additionally, the expression of MMP-9 in cortical tissue did not showed statistically significant differences between groups. As a result, we proposed that the contrabution of YC-1 in the PTZ-induced epilepsy model might be related with very different mechanisms. YC-1's effect on seizures expression should be further studied using new functional and molecular techniques.

Keywords: Matrix metalloproteinase, seizure, YC-1.

MON-356
Effects of native inorganic particles on membrane potential, proton gradient, Na+ -dependent uptake, and binding of glutamate in rat brain nerve terminals

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Recent epidemiological, observational, clinical, and experimental studies suggest that certain neurological diseases, such as Alzheimer's disease, Parkinson's disease, and stroke, may be strongly associated with ambient air pollution, one of the components of which is fine and ultrafine inorganic particles. During inhalation, nano/micro-sized particles are efficiently deposited in nasal, tracheobronchial, and alveolar regions and transported to the CNS. Glutamate is the major excitatory neurotransmitter in the CNS, which is involved in many aspects of normal brain functioning and the pathogenesis of neurological disorders. Research was focused on the analysis of the effects of fine and ultrafine inorganic particles originated from volcanic ash (JSC-1a, ORBITEC Orbital Technologies Corporation, Madison, Wisconsin) on the key characteristics of glutamatergic neurotransmission. The average size of particles (even minor fractions) before and after sonication was determined by dynamic light scattering. With the use of radiolabeled L-[^{14}C]glutamate, it was shown that there is an increase in L-[^{14}C]glutamate binding to isolated rat brain nerve terminals in low [Na^+] media and at low temperature in the presence of the particles. The initial velocity of L-[^{14}C]glutamate uptake was not changed as a result of application of the particles. Fluorimetric experiments with potential-sensitive dye rhodamine 6G and pH-sensitive dye acridine orange showed that the potential of the plasma membrane of nerve terminals and acidification of synaptic vesicles were not altered by the particles. Therefore, fine and ultrafine inorganic particles of volcanic ash JSC-1a increase glutamate binding to the nerve terminals. The effect revealed can have deleterious influence on extracellular glutamate homeostasis in the CNS which is extremely important for proper synaptic transmission.

Keywords: fine and ultrafine inorganic particles; volcanic ash; glutamate binding; glutamate uptake; the membrane potential; synaptic vesicle acidification; rat brain nerve terminals.
Keywords: glutamate uptake;, rat brain nerve terminals, synaptic vesicle acidification.

MON-358
Enhanced store-operated calcium entry in neuronal models of Huntington's disease

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by polyglutamine expansion within Huntingtin protein. The striatal neurons are most affected in HD. It has been suggested that impaired calcium signaling plays an essential role in HD pathogenesis.

Calcium influx via the store-operated calcium (SOC) channels is well established as an important and ubiquitous mechanism for calcium entry in mammalian cells. To model HD in different cell cultures we used a transfection of the full-length mutated Huntingtin (Htt138Q) or lentiviral infection of N-terminal fragment of the mutated Huntingtin (Htt138Q-1exon).

Using patch-clamp technique we demonstrated the strong enhancing of the SOC entry in models of HD in the human neuroblastoma cells (SK-N-SH), mice neuroblastoma cells (Neuro2A) and in the primary culture of striatal neurons isolated from mice (MSN). By using RNAi-mediated knockdown approach we proved a crucial role of calcium sensor STIM1 in activation of the SOC pathway in HD models. Further we demonstrated that for the SOC entry in these cells the channel-forming proteins TRPC1 and Orai1 are required.

Human induced pluripotent stem (iPS) cells hold great promise for therapy of a number of degenerative diseases such as HD, Alzheimer's disease, diabetes mellitus, etc. They also have the therapeutic potential for disease treatment not only by iPS cell-based cell replacement therapy but providing a number of cellular models of neurodegenerative disease for scientific research and drug screening.

The next step of our research was devoted to studying of the SOC entry in Huntington-specific human neurons (MSN-human) differentiated from iPS cell lines generated through somatic reprogramming of fibroblasts obtained from the HD patients. In contrast to our previous studies the expression of the mutated Huntingtin in MSN-human HD model was absolutely endogenous. It is worth mentioning that mutated Huntingtin, expressed in HD patient's brains has only 40–45 glutamine residues in its polyglutamine tract what is close to be norm. Despite this we showed a strong enhancing in the level of the SOC entry in pathological human neurons.

Further we showed that application of EVP4593 compound leads to the reduction of the increased SOC entry to the normal values in SK-N-SH, MSN and MSN-human models of HD.

Our results suggest that SOC entry constitutes a novel target for HD treatment and EVP4593 compound represents a novel potential therapeutic agent for treatment of HD.

The study was supported by the RScF, the RFBR, the Program of the RAS 'MCB', and the fellowship of the President of the RF.
Keywords: Huntington's disease, Patch-clamp, Store-operated calcium entry.

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MON-359
Enhancement of alcohol drinking in mice depends on alterations in RNA editing of serotonin 2C receptors

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Serotonin 2C receptors (5-HT_{2C}R) are G-protein-coupled receptors with various actions, including involvementin drug addiction. 5-HT_{2C}R undergoes mRNA editing, converting genomically encoded adenosine residues toinosines via adenosine deaminases acting on RNA (ADARs). Here we show that enhanced alcohol drinkingbehaviour in mice is associated with the degree of 5-HT_{2C}R mRNA editing in the nucleus accumbens and dorsalraphe nuceus, brain regions important for reward and addiction. Following chronic alcohol vapour exposure,voluntary alcohol intake increased in C57BL/6J mice, but remained unchanged in C3H/HeJ and DBA/2J mice.5-HT_{2C}R mRNA editing frequency in both regions increased significantly in C57BL/6J mice, as did expressionsof 5-HT_{2C}R, ADAR1 and ADAR2, but not in other strains. Moreover, mice that exclusively express the uneditedisoform (INI) of 5-HT_{2C}R mRNA on a C57BL/6J background did not exhibit increased alcohol intake comparedwith wild-type mice. Our results indicate that alterations in 5-HT_{2C}R mRNA editing underlie alcohol preferencein mice.

Keywords: 5-HT2C receptor, Alcohol addiction, RNA editing.

MON-360
External magnetic field-mediated movement of brain nerve terminals labeled by D-mannose-coated gamma-Fe2O3 nano-sized particles

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Movement of brain nerve terminals by application of an external magnetic field promises breakthrough in nanoneurotechnology. D-mannose-coated superparamagnetic nanoparticles were synthesized by coprecipitation of Fe(II) and Fe(III) salts followed by oxidation with sodium hypochlorite and addition of D-mannose. Effects of D-mannose-coated superparamagnetic maghemite (γ-Fe_2O_3) nanoparticles on key characteristics of glutamatergic neurotransmission were analysed. Using radiolabeled L-[^{14}C]glutamate, it was shown that D-mannose-coated γ-Fe_2O_3 nanoparticles did not affect high-affinity Na^+-dependent uptake, tonic release and the extracellular level of L-[^{14}C]glutamate in isolated rat brain nerve terminals. Fluorimetric experiments with potential-sensitive dye rhodamine 6G and pH-sensitive dye acridine orange showed that the potential of the plasma membrane of the nerve terminals and acidification of synaptic vesicles were not altered in the presence of D-mannose-coated γ-Fe_2O_3 nanoparticles. The study was focused on analysis of possibility of usage of these nanoparticles for manipulation of nerve terminals by an external magnetic field. It was shown that more than 84.3 $\pm$ 5.0% of L-[^{14}C]glutamate-loaded nerve terminals (1 mg of protein/ml) incubated for 5 min with D-mannose-coated γ-Fe_2O_3 nanoparticles (250 μg/ml) moved to area, where the magnet (250 mT, gradient 5.5 T/m) was applied versus 33.5 $\pm$ 3.0% of label in control and 48.6 $\pm$ 3.0% of label after the application of uncoated nanoparticles. Therefore, it was demonstrated the possibility to manipulate brain nerve terminals by an external magnetic field using D-mannose-coated γ-Fe_2O_3 nanoparticles, which did not affect key characteristics of glutamatergic neurotransmission, that is, D-mannose-coated γ-Fe_2O_3-labeled nerve terminals with normal functioning were obtained.

Keywords: brain nerve terminals;, glutamate transport;, magnetic nanoparticles.

MON-361
From IDP to Alzheimer's disease - structural features of beta-amyloid prefibrillar aggregates by sedimented solute NMR

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Beta amyloid protein (Aβ) is one of the intrinsically disordered proteins associated with neurodegenerative diseases like Parkinson's, prion disease and Alzheimer's disease (AD) in particular. Although the direct involvement of Aβ peptides in AD is well documented and their aggregative ability is closely related to their neurotoxicity, the precise mechanism of the neurotoxic effects of Aβ peptides remains unclear. Moreover, there is still a significant gap between the site-specific structural information and the complex structural diversity of Aβ amyloids. Most of the published data also reported that the neurotoxicity of Aβ peptides might be ascribed to the oligomeric species, not to the fibrils. Detailed structural and dynamical properties of the various prefibrillar intermediates are crucial for the understanding the role of amyloid formation in AD pathogenesis. NMR spectroscopy is a useful tool to obtain atomic resolution information on IDPs. In this work we report new atomic-level structural features of Aβ assemblies over 60 kDa by a new technique referred to as sedimented solute NMR (SedNMR). We have obtained a qualitative picture of the structure of prefibrillar Aβ40 aggregates prepared from standard ultracentrifugation and some clues toward structural evolution from prefibrillar aggregates to the final fibrillar materials. We prove that *in situ* SedNMR directly inside SSNMR rotors by magic angle spinning (MAS) can be used to address the kinetics of formation of soluble Aβ assemblies by monitoring the disappearance of monomer and the appearance of the oligomers at the same time. *Ex situ* SedNMR via an ultracentrifuge device provides a way to select certain species and reveals atomic – level structural features of soluble Aβ assemblies. These high resolution studies contribute to understand structural polymorphisms of Aβ amyloids and provide valuable information of the molecular basis of AD onset-progress.

Keywords: Alzheimer's Disease (AD), Beta-amyloid, sedimented solute NMR.

MON-362
Fullerenes promote neurite outgrowth of nerve growth factor-treated PC12 h cells by activation of phospholipase C signaling pathway

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Fullerenes (C_{60}) have unique structural, electrical, and mechanical properties with many potential applications in medicine and electronics. Although low concentrations of fullerenes promoted

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the neurite outgrowth of nerve growth factor (NGF)-treated PC12 h cells, the precise cellular and molecular mechanisms underlying neurite outgrowth by fullerene addition are unclear. We have already found that low concentrations of amino group-modified carbon nanotubes promoted the neurite outgrowth of NGF-treated neurons by stimulating extracellular signal-regulated kinase (ERK) through the activation of the phospholipase C (PLC) signaling pathway. Therefore, we investigated whether PLC may play a role in the mechanisms underlying the fullerene-induced potentiation of NGF-treated PC12 h cells.

We investigated whether fullerenes or pyrrolidine-modified fullerenes may stimulate the neurite outgrowth of NGF-treated PC12 h cells by the activation of ERK through the PLC signaling pathway independent of the Ras/Raf/MEK cascade. Fullerenes and pyrrolidine-modified fullerenes stimulated the neurite outgrowth of NGF-treated PC12 h cells at a range of concentrations of 0.11–0.85 μg/ml. On the other hand, they did not influence the proliferation of PC12 h cells at a range of concentrations of 0.11–0.85 μg/ml. When the concentrations of fullerenes were increased to a range of 1.7–17 μg/ml, the numbers of both NGF-treated PC12 h cells with neurite outgrowth and proliferated PC12 h cells decreased. Fullerenes and pyrrolidine-modified fullerenes enhanced the phosphorylation of PLC-γ1 and ERK in NGF-treated PC12 h cells. The activation of PLC-γ1 is known to induce the release of Ca^{++} from intracellular Ca^{++} stores. These fullerenes induced an increase in intracellular Ca^{++} levels of NGF-treated PC12 h cells.

Fullerenes and pyrrolidine-modified fullerenes promote the neurite outgrowth of NGF-treated PC12 h cells by the activation of ERK independent of the Ras/Raf/MEK cascade through the PLC signaling pathway.

Keywords: Fullerene, PC12 h cells, PLC signaling pathway.

MON-363
Gaining insight into the molecular basis of brain damage in the PKU mouse model

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Phenylketonuria (PKU) is the most common inherited disorder of amino acid metabolism. The primary cause of PKU is lack of phenylalanine hydroxylase (PAH), the enzyme that in liver metabolizes L-phenylalanine (L-Phe) to L-tyrosine (L-Tyr) using 6R-L-erythro-5,6,7,8-tetrahydrobiopterin (BH4) as essential co-factor. In untreated PKU patients, Phe concentrations in blood and tissues are high. Hyperphenylalaninemia (HPA) leads to a variety of symptoms, but the most prominent symptom occurs in the brain. If not detected and treated in newborns, HPA causes severe neurologic dysfunction with severe and irreversible mental retardation, microcephaly, and cognitive and behavioral deficiencies. At present, prevention of the major symptoms of the disease, including mental retardation, consists of a low Phe diet throughout life. Despite the biochemical characterization of PKU, the molecular mechanisms underlying PKU-associated brain dysfunction remain poorly understood.

In this study, we analyzed the protein expression profiles in brain tissue of homozygous PKU mice versus the normal heterozygous counterpart using two-dimensional differential gel electrophoresis (2D DIGE) technology. Our differential proteomic approach, combined to mass spectrometry, revealed 21 proteins whose expression is affected by HPA: four were overexpressed and 17 underexpressed. The bioinformatic analysis of the identified protein data set showed that about 60% of species are related to nervous system development and functions (neuron differentiation and dendritic growth/branching) and to neurological disorders (progressive motor neuropathy and movement disorders). Moreover, functional annotation analyses showed that some identified proteins were involved in oxidative metabolism. To investigate further the proteins involved in the neurological damage, we validated by western blot two of the proteins that were most strikingly underexpressed, namely, Syn2 and Dpys12, which are involved in synaptic function and neurotransmission. Furthermore, we found that Glu2/3 and NR1 receptor subunits were overexpressed in PKU mouse brain. In conclusion, these findings confirm that alteration in synaptic function, in transmission and in energy metabolism underlie brain damage provoked by PKU.

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Keywords: differential proteomics, neuronal dysfunction, phenylketonuria.

MON-364
Global analysis of S-nitrosylation sites of the post synaptic density proteins in mouse models of Alzheimer's disease

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Alzheimer's disease (AD) is the most frequent neurodegenerative disorder, characterized by the progressive deterioration of cognitive functions, learning and memory. AD is characterized by synaptic loss, nerve cell loss, extracellular deposition of beta amyloid protein (forming senile plaques) and tau protein (neurofibrillary tangles). The pathogenesis of this disorder is linked to a condition of oxidative and nitrosative stress occurs due to an imbalance in the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). One of the consequences of interaction reactive nitrogen species with proteins is S–nitrosylation. S–nitrosylation is the selective covalent modification of cysteine thiols in proteins by nitric oxide to form nitrosocysteine. This modification is emerging as an important redox signaling mechanism which can regulate board range of biologic physiologic and cellular function.

Our research focused on systemic analysis of endogenous S-nitrosylation of proteins from postsynaptic densities (PSDs)–multiprotein structures responsible for the proper functioning of excitatory synapses. PSDs were isolated from transgenic mice with neuronal expression of human mutant APP gene (which are an accepted model of Alzheimer's disease) and control FVB mice. In the project we used methods enabling us to selectively enrich the PSD fraction in S–nitrosylated proteins and mass spectrometry for their identification.

We have worked out a protocol for mapping of the S-nitrosylated peptides coupling liquid chromatography (LC) and mass spectrometry (MS) with SNOSID and biotin switch method. As a result we obtained two dimensional maps (molecular mass versus HPLC retention time) of peptides present in the sample and peptide sequences. We used new bioinformatics tools for datasets analysis which were developed in our laboratory.

This type of S–nitrosome analysis help to defining the quantitative and qualitative differences in S-nitrosylation of proteins among different biological states. The progress in the understand-

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ing of the molecular alterations underlying Alzheimer's disease will be useful in developing successful preventive and therapeutic strategies able to block the neurodegenerative process.

Keywords: Alzheimer's disease, S-nitrosylation, synapse.

MON-365
Glycogen synthase kinase-3 regulates synaptic Matrix Metaloproteinase-9

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Glycogen synthase kinase-3 (GSK3) activity is crucial for hippocampal function where it plays an important role in the regulation of synaptic functional and structural plasticity. Control of functional synaptic plasticity by GSK3 may be through the regulation of Long Term Depression (LTD). Structurally, GSK3 regulates hippocampal size, dendritic tree morphology and spine density. However, molecular pathways, by which GSK-3 exerts its synaptic functions are not fully known. Matrix Metaloproteinase-9 (MMP-9) has been implicated in regulation of the synaptic plasticity and dendritic spine moprhology. Hence, we decided to investigate whether there is a link between MMP-9 and GSK3. To understand how these two molecules are linked, we used isolated synaptoneurosomes. LTD was induced by the application of DHPG that we found to induce a rapid cleavage of MMP-9 substrate β-dystroglycan. Furthermore, this effect was inhibited by application of Chiron, selective GSK3 inhibitor. Interestingly, application of broad spectrum MMP-9 inhibitor I potentiated DHPG-induced decrease of GSK3 phosphorylation suggesting a possible reciprocal effect.

Next, we analyzed MMP-9 activity, by gel zymography, in mice overexpressing GSK3β (GSK3β[S9A]) and lacking GSK3β (GSK3β(n-/-)), in response to PTZ-induced seizures. Overexpression of GSK3β[S9A] potentiated MMP-9 activity in mice that were treated to PTZ. In contrast, in GSK3β(n-/-) MMP-9 activity was diminished upon PTZ injection as compared to WT mice.

Finally, we confirmed the effect of GSK3β on spine morphology by analyzing spine's shape in brain sections derived from mice overexpressing or lacking GSK3β. The effect of GSK3-MMP-9 relationship on spine morphology is being investigated.

Taken together, these data indicate that GSK3β regulates MMP-9 at the excitatory synapse, and the relationship may be crucial for the structural plasticity regulation.

Keywords: dendritic spines, GSK3, MMP-9.

MON-367
Identification of PP2A as a novel interacting partner of LRRK2

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Mutations in the leucine-rich repeat kinase 2 (LRRK2) are the most common cause of genetically inherited Parkinson s disease (PD). Two of the common found mutations namely R1441C and G2019S, mediate their toxic effects through their increased kinase activity. Furthermore, the kinase activity of LRRK2 is regulated through some essential serine and threonine autophosphorylation residues, that are located in the LRR, ROC and kinase domain. In this study we identified Protein Phosphatase 2A (PP2A), as an interacting partner of LRRK2. We were able to show that the alpha subunit of PP2A (PP2Aa) is interacting with LRRK2 in the perinuclear region of Hela cells. Finally we investigated the physiological role of PP2A in SH-SY5Y cells transiently expressing R1441C-LRRK2, by silencing the catalytic subunit of PP2A (PP2Ac). As a result, cell death which was induced by R1441C-LRRK2 was significantly aggravated. All these data suggest that PP2A is a new interacting partner of LRRK2 and reveal the importance of PP2A as a potential therapeutic target in PD.

Keywords: LRRK2, Parkinson's disease, PP2A.

MON-368
Limiting in vivo conditions of reversed glutamate transport in platelets

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Platelets express high-affinity glutamate transporters EAAT1-3 in plasma membrane and vesicular glutamate transporters VGLUT1-2 in secretory granules. The mechanisms of glutamate release from platelets are uncertain though glutamate is considered to be an important signalling molecule for the platelets, the other blood cells and endothelium. In current research we assess the availability of non-stimulated, transporter-mediated and exocytotic release of glutamate in platelets and compare glutamate transport in presynapse and in peripheral (non-neuronal) type of the cells. We use the radiolabelled assay (L-[^{14}C]glutamate) and glutamatedehydrogenase assay to monitor the endogenous glutamate release from platelets and isolated brain nerve terminals (synaptosomes).

The absence of non-stimulated and depolarization-induced (high K^+) release of endogenous glutamate from platelets was shown even after the inhibition of glutamine synthetase and glutamate uptake by methionine sulfoximine or DL-threo-*b*-benzyloxyaspartate, respectively. Transportable inhibitor of glutamate transporters DL-threo-*b*-hydroxyaspartate (DL-THA) failed to induce glutamate efflux from the cytosol of platelets by means of heteroexchange.

The protonophore cyanide-p-trifluoromethoxyphenyl-hydrazon (FCCP) and inhibitor of H^+-ATPase V-type bafilomycin A1 caused dissipation of the proton gradient on both platelets and synaptosomes but did not stimulate glutamate release from platelets. It was shown that platelet degranulation (stimulated by ADP or thrombin) was accompanied by massive endogenous glutamate release. High K^+, DL-THA, FCCP and bafilomycin A1 did not prevent this exocytotic release of glutamate.

The depolarization of the plasma membrane of synaptosomes at Ca^{2+}-free conditions caused transporter-mediated release of endogenous glutamate from the cytosol into the extracellular space (glutamate transporter reversal). In contrast to nerve terminals, the heteroexchange, transporter-mediated and non-stimulated release of glutamate are not inherent to platelets. We suggested that the absence of the transporter-mediated release of glutamate was associated with the minority of the cytosolic pool of glutamate and a lack of depolarization-induced Na^+ influx in platelets, i.e., the factors, which determined glutamate transporter reversal. Thus, the glutamate is released from platelets by means of exocytosis only.

Keywords: exocytosis, glutamate transporters, platelets.

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MON-370
MMP-3, MMP-9 and TIMP-1 in serum during thrombolytic treatment of acute ischaemic stroke

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Introduction: The recommended treatment of acute phase of ischaemic stroke (IS) in humans is limited to recombinant tissue plasminogen activator (rtPA). Previous studies have indicated that intracranial bleeding during rtPA administration can be caused by high activity of matrix metalloproteinase (MMP)-9. MMP-9 is capable to digest type IV collagen, the component of basal membranes. During thrombolysis the enzymatic cascade that follows through plasmin and MMP-3 is triggered by rtPA. Finally, MMP-3 activates MMP-9, theoretically leading to the destruction of basal membranes of brain vessels and intracranial bleeding thereafter. Our objective was to find the relationship between rtPA treatment versus MMP-9 activity, MMP-3, and TIMP-1 (tissue inhibitor of MMP) serum levels related to patients' neurological status during acute ischaemic stroke.
Material and Methods: Thirty five ischaemic stroke patients were enrolled. Fourteen of them underwent thrombolytic therapy with rtPA (rtPA group). The serum samples were obtained at 3 time-points in rtPA group (time-point 0: 1st – 4th hour of stroke; time-point 1: immediately after rtPA administration; time-point 3: on day 5–7 from stroke onset). In remaining patients venous blood was obtained at two time-points: time-point 1: 5th – 10th hour of stroke and time-point 2: on day 5–7 of stroke. MMP-9 was analyzed with gelatin zymography, MMP-3 and TIMP-1 serum levels were analyzed using an ELISA method. Neurological status was assessed by National Institutes of Health Stroke Scale (NIHSS) performed on the admission and discharge of patients. NIHSS improvement ratio (IR) was calculated as a difference between NIHSS score at the admission and discharge of patient.
Results: The active form of MMP-9 (86 kDa) was not observed in any analyzed samples. MMP-9 activity was estimated as the sum of activities of each detected form 92 kDa, 130 kDa and 200 kDa (corresponding to pro-MMP-9, heterodimer MMP-9/lipokalin and homodimer MMP-9/MMP-9, respectively). MMP-9 was significantly elevated and MMP-3 was decreased in serum at time-point 1 in rtPA group in comparison to non-rtPA group. MMP-3 was negatively correlated with IR values for all stroke patients but the results did not reach full statistical significance ($p = 0.06$).
Conclusion: Thrombolysis applied for the treatment of ischaemic stroke increases MMP-9 activity in serum, however rtPA seems not to facilitate the conversion of pro-MMP-9 into the active form. In addition, our results can suggest the involvement of MMP-3 to the biochemical processes occurring during acute phase of IS.
Keywords: ischaemic stroke, thrombolysis, MMP-9.

MON-371
Modulation of AMPA receptors by Type II-TARPs

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AMPA receptors (AMPARs) are involved in fast synaptic transmission in the CNS. They are transmembrane proteins that assemble as homo- or heterotretameric combinations of four distinct subunits: GluA1 to GluA4. Phenomena such as alternative splicing and RNA editing contribute to the high diversity of receptor subunit combinations, which ultimately result in an increased multiplicity of channel architectures and properties.

A group of accessory proteins has been found to further increase this broad diversity of channel characteristics. Transmembrane AMPA receptor regulatory proteins (TARPs), are classified into two groups. Besides γ3, γ4, and γ8, type I-TARPs include γ2, also called stargazin, the first TARP discovered. All of these proteins are known to efficiently regulate all AMPA receptor combinations, modulating channel gating and assuring proper receptor expression and trafficking to the membrane. Type II-TARPs comprise γ5 and γ7. Together with γ6, type II-TARPs were initially regarded as 'non-TARPs', because they did not seem to modulate any of the biophysical channel properties. A view that has been recently changed.

Therefore, the role of type II-TARPs in the regulation of AMPARs needs to be further clarified. It is necessary to investigate whether type II-TARPs interact with other glutamate receptor subfamilies, such as kainate and NMDA receptors. It is still not clear which molecular mechanisms and which TARP-interacting domains are involved in type II-TARP modulation of AMPARs. In addition, very little is known about the trafficking pathways and stoichiometry of AMPAR-type II-TARP complexes.

Here, using two-electrode voltage-clamp (TEVC), we investigate the roles that type II-TARPs play in modulating channel properties. Our results show that, in *Xenopus laevis* oocytes, type II-TARPs play a clear role in differentially modulating steady state currents of AMPA receptors, depending on the subunit, splice variant, and the editing form involved. Furthermore, in accordance with the literature, γ6 does not seem to have any significant effect on channel functionality, confirming the 'non-TARP' status that has been attributed to this subunit. Our results also show that kainate receptors are not modulated by type II-TARPs, suggesting an AMPAR-selective modulation.
Keywords: None.

MON-372
Modulation of intracellular proteolytic pathways in rational neuroprotection

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Neurodegeneration both pathology- or age-related is accompanied to the disturbance in protein turnover in nervous tissue and imbalance in intracellular proteolytic pathways. Thus (i) proteasome-dependent protein quality control machinery is inefficient against protein aggregates, (ii) the autophagic elimination of abnormal proteins, organelles or cells is suppressed so that lysosomal compartment is overloaded, and (iii) on the contrary the proteases promoting cell death by apoptotic or necrotic pathways (e.g. caspases and calpains) are excessively activated. Calpain/calpastatine system overactivation in damaged brain depends on multiple factors such as calcium excess in cytosol, calpain expression upregulation, and calpastatine (calpain inhibitor) degradation and spatial separation with controlled protease. Hitherto the resultant calpain hyperactivation effect is considered as the critical event (or 'point-of-no-return') in neurodegeneration. In order to solve the pending issue on rational neuroprotection and neurodegeneration manifestation delay the effectiveness of some selective calpain regulators and exogenous agents of pleiotropic activity aiming to stimulate endogenous neuroprotective mechanisms was estimated. The work was supported by RFBR grant 12-04-01597 and the program 'Leader scientific schools' project 1410.2014.4.
Keywords: Neurodegeneration, neuroprotection, protease.

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MON-373
Molecular mechanism of the interaction between gating modifier toxin VSTx1 and voltage-sensing domain of potassium KvAP channel revealed by NMR spectroscopy

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Voltage-gated K+ channels (Kv) play essential role in the cellular excitability and propagation of nerve signals. The spatial organization of Kv channels is modular and involves a pore domain surrounded by a 'ring' of four loosely associated voltage-sensing domains (VSDs). The VSD is composed of four consecutive transmembrane helices (S1-S4). The S4 helix, sometimes called 'voltage sensor', accommodates several positively charged residues (usually four Arg), which are directly involved in the channel gating. Kv channels are targeted by plethora of toxins produced by venomous animals. The spider toxin VSTx1 (tarantula *Grammostola spatulata*) inhibits activation of the prototypical K+ channel KvAP (archea *Aeropirum pernix*) by blocking the VSD in the depolarized conformation and delaying the domain return to the hyperpolarized conformation (Schmidt et al, JMB, 2009).

In the present work the interaction of VSTx1 with isolated VSD of KvAP channel having depolarized conformation was studied by NMR spectroscopy. The membrane mimicking environment of zwitterionic DPC/LDAO (2:1) micelles and nanodiscs containing partially anionic POPC/DOPG (3:1) membranes were used. VSTx1 demonstrates moderate membrane activity and binds to the micelle surface by large hydrophobic patch surrounded by the ring of the positively charged residues (Lys4, Lys8, Arg24 и Lys26). Changes in the line-width and chemical shift of NMR signals observed upon mutual VSTx1 and VSD titrations permit to map binding interfaces in the toxin/domain complex. The usage of spin-labeled VSD variants permits to determine relative orientation of VSTx1 and VSD molecules. The Phe5-Lys8 loop of the toxin is contacted with the S1-S2 loop of the VSD, and C-terminal VSTx1 fragment (Ser22-Phe34) is directed toward S3b-S4 helical hairpin. NMR data obtained in micellar and nanodisc environment also revealed the presence of additional low-affinity nonspecific interactions between VSTx1 and VSD molecules.

Summarizing the obtained results, we could propose that VSTx1 binds to the interface region of the lipid bilayer and from this state forms the complex with the VSD S1-S2 loop. The bound toxin molecule blocks the S3b helix, thus hinder the movement of the covalently linked voltage-sensor (S4 helix). The presented study for the first time provides direct structural information about interaction of spider gating modifier toxins with the VSD of voltage-gated channels.

This work was supported by the Russian Foundation for Basic Research (14-04-01270).
Keywords: None.

MON-374
Molecular pharmacology of synthetic neurosteroidal agonists of Nerve Growth Factor (NGF) receptors: differential neurotrophin receptors signaling

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Neurotrophins control neuronal cell fate and function during development and adulthood. They act through Trk and $p75^{NTR}$ receptors, exerting potent neuroprotective effects. However, neurotrophins' therapeutic usefulness is compromised by their polypeptidic nature and limited penetrance to the blood-brain barrier (BBB). We have synthesized analogs of neurosteroid dehydroepiandrosterone with anti-apoptotic properties, deprived of androgenic/estrogenic actions. In the present study we provide evidence that derivative BNN27, binds to NGF receptors, TrkA and $p75^{NTR}$, at nanomolar concentrations (Kd: 1.86 ± 0.4 nM and 3.9 ± 1.2 nM respectively). Mutagenesis assays have shown that binding of BNN27 to TrkA receptors does not require its extracellular, NGF binding domain, in contrast to mutated $p75^{NTR}\Delta$ECD receptor where no binding is observed. BNN27 induced TrkA tyrosine phosphorylation in all three tyrosine residues (490, 675 and 785), affecting downstream signaling of Akt and MAPKs in primary sympathetic neurons. However, BNN27 differentially regulated TrkA internalization: it induced internalization and fast return of the receptor into the membrane through activation of rab5 protein and docking of the receptor in the early endosomes. Using cholesterol depletion agents and separating lipid raft microdomains, we showed that BNN –in contrast to NGF- caused TrkA to effectively segregate into lipid raft fractions, presenting differential membrane localization of the receptor. BNN27 significantly reversed apoptosis of NGF-dependent embryonic sensory neurons of NGF null mice and of sympathetic neurons in primary culture. Moreover, BNN27 was effective in promoting the interaction of $p75^{NTR}$ receptors with its interactors RhoGDI, RIP2 and TRAF6. Interestingly, BNN27 was ineffective by itself in inducing differentiation and neurite outgrowth of PC12 cells. However, it enhanced the effects of NGF at low concentrations in both phenomena. NMR studies with BNN27 and recombinant NGF receptors suggest that it facilitates binding of NGF to its receptors. In conclusion, BNN27 exerts strong anti-apoptotic, neuroprotective actions via NGF receptors, differentially activating prosurvival signaling, and thus it may serve as a lead molecule to develop BBB permeable, neurotrophin-like small molecules (microneurotrophins) with potential applications in the treatment of neurodegenerative diseases.

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Keywords: Molecular neuropharmacology, Neurodegeneration, Neurotrophins.

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MON-375
NeuroD2 transcription factor regulates expression of Id2 gene during neuronal differentiation

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NeuroD2 is a neuron-specific basic helix-loop-helix (bHLH) transcription factor with important roles in neuronal differentiation and development. NeuroD2 forms heterodimers with ubiquitous E-proteins to form a transcriptionally active complex which induces transcription of neurogenic genes by binding to E-box elements in their promoters. In order to identify target genes of NeuroD2 during development of the cortex we have compared gene expression profiles between wild-type and *neuroD2* knockout cortex tissue by microarray analysis. We have identified *id2* gene as a top candidate gene and have demonstrated that expression of *id2* is significantly reduced in *neuroD2* knockout mice by using northern blot and *in situ* hybridization. Id2 protein is a member of Id proteins that inhibit E-box mediated gene expression by interfering with binding of neurogenic bHLH proteins to their target promoters. NeuroD2 heterodimerizes with Id (inhibitor of differentiation) proteins which results in being sequestered away from DNA and hence repression of transcriptional activity. Additionally, our results suggest that NeuroD2 and Id2 protein localization are dynamically co-regulated during neuronal differentiation. In young cortical neurons both NeuroD2 and Id2 are predominantly localized to the nucleus, however as neurons mature localization of both proteins shift to the cytoplasm. We continue to investigate the mechanisms of by which NeuroD2 regulates *id2* expression and how these two proteins interact during neuronal differentiation.

Keywords: NeuroD2, Id2, differentiation.

MON-376
Oxidative stress and antioxidants in diabetic neuropathy

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Oxidative stres occurs in a cellular system when the production of free radical moieties exceeds the antioxidant capacity of that system. If cellular antioxidants do not remove free radicals, radicals attack and Damage biomolecules such as carbohydrates, proteins, lipids, and nucleic acids. The oxidized and nitrosylated products of free radical attack have decreased biological activity, leading to loss of energy metabolism, cell signaling, transport, and other major functions. On the other hand, diabetes is characterized by chronic hyperglycemia that results dysregulation of cellular metabolism. Oxidative stres and free radicals play important key roles and significant mediators in the development of diabetic neuropathy. In our study, we have examined the contribution and roles of oxidative stres parameters and antioxidants in the pathogenesis of diabetic neuropathy. Blood samples were obtained from both Patients with diabetic neuropathy and healthy subjects. Superoxide dismutase and catalase activities as well as glutathione and glutathione-related antioxidant enzymes were examined in erythrocytes while lipid peroxidation and protein oxidation parameters were measured in plasma. Our results suggest a role for oxidant/antioxidant balance in diabetic neuropathy pathogenesis which may be applied to treatment protocols in the future.

Keywords: Antioxidants, Diabetic Neuropathy, Oxidative Stress.

MON-377
PDGFRB mutations associated with idiopathic basal ganglia calcification impair the receptor activity and signaling

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Platelet-derived growth factor receptors (PDGFRα and PDGFRβ) are tyrosine kinase receptors that stimulate cell proliferation and motility and that are encoded by two different genes, *PDGFRA* and *PDGFRB* respectively. Recently, heterozygous *PDGFRB* mutations have been described in patients diagnosed with idiopathic basal ganglia calcification (IBGC) but were not characterized. Moreover, it was shown that these mutations were segregating with the disease. IBGC, also called Fahr disease, is a rare inherited neurological disease characterized by symmetric and bilateral calcifications which are mostly located in the basal ganglia, the dentate nucleus of the cerebellum and the cerebral cortex. The goal of the present study was to determine whether these mutations had a positive or negative impact on the PDGFRβ activity. Therefore, we stably expressed two different PDGFRβ mutants associated with IBGC, L658P and R987W, in HT-1080 cells. Their expression was confirmed by flow cytometry. Then, we analyzed the signaling pathways that are known to be activated by PDGFRβ. We observed that the L658P mutant did not activate any of these pathways in response to PDGF whereas the R987W mutant activated phospholipase Cγ (PLCγ) and Akt but not signal transducer and activator of transcription 3 (STAT3) after PDGF stimulation. These results were confirmed by luciferase assays in MCF7 and HT-1080 cells. *In vitro* kinase assays demonstrated that the L658P mutant had no kinase activity whereas the R987W mutant had a weaker activity than the wild-type receptor. To conclude, our results indicate that PDGFRβ mutations associated with IBGC impair the receptor activity and signaling. This is consistent with the recent description of loss-of-function mutations in the gene encoding the PDGF-B ligand. Our data suggest that a defect in STAT3 activation by PDGF may play a critical role in this disease.

Keywords: Fahr disease, PDGF receptor, STAT3.

MON-378
Protein disulfide isomerase gene variants are associated with amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease affecting motoneurons in the brain and spinal cord leading to paralysis and death. Disruption of protein homeostasis at the endoplasmic reticulum (ER) and abnormal protein aggregation is a salient feature of ALS. Here we report several novel missense variants in two genes encoding key ER foldases (PDIA1 and PDIA3/ERp57) of the protein disulfide

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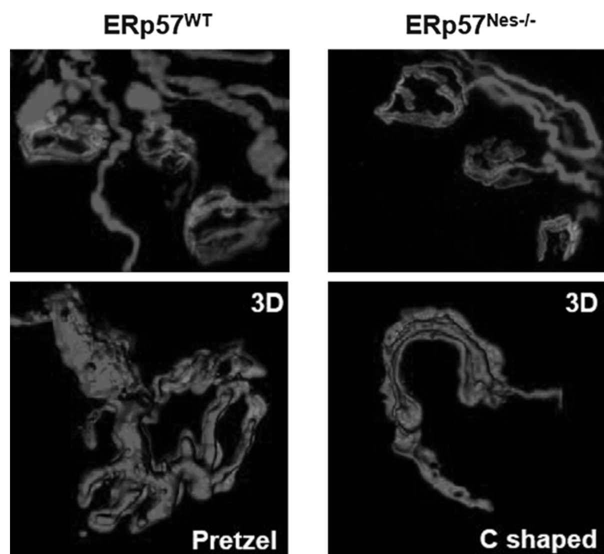

Fig. 1.

isomerase family in ALS patients. Initial phenotypic screening in zebrafish revealed that mutations in these genes can induce motor defects associated with a disruption of motoneuron connectivity and functionality. Consistent with this, expression of mutant PDIs impaired dendritic outgrowth in three different motoneuron cell culture models, including in human motoneurons derived from a human embryonic stem cell line. Cellular and biochemical studies identified distinct molecular defects that underlie the pathogenicity of these ALS-linked PDI mutants. Finally, specific deletion of ERp57 in the nervous system of mice led to a severe motor dysfunction associated with a loss of neuromuscular synapses. We speculate that persistent changes in cellular proteostasis, may result in perpetual decline of neuromuscular connectivity caused by decreased efficiency in the folding of synaptic proteins. Our findings argue strongly that the ER proteostasis is affected in ALS and that ER-protein misfolding mediated by the PDIA1 and ERp57 variants leads to ALS pathogenesis. This study identifies ER proteostasis imbalance as a risk factor to develop ALS.

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Keywords: Amyotrophic Lateral Sclerosis, Protein folding.

MON-379
Quercetin increases bioavailability of nitric oxide in jejunum in normoglycemic and diabetic rats and induces neuronal plasticity in the myenteric plexus

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The aim of the present study was to investigate the effect of quercetin on nitric oxide (NO) levels, lipid peroxidation, and total radical-trapping antioxidant potential (TRAP) using chemiluminescence techniques and assess different subpopulations of myenteric neurons: double-staining for neuronal nitric oxide synthase (nNOS) and the pan-neuronal marker anti-HuC/D and determining vasoactive intestinal peptide (VIP) immunoreactivity in the muscular layer of the jejunum from diabetic rat. Thirty-two male Wistar rats were divided into four groups: normoglycemic (C), normoglycemic supplemented with quercetin (Q), diabetic (D), and diabetic supplemented with quercetin (DQ). Supplementation with quercetin in normoglycemic and diabetic rats significantly increased NO tissue detection compared with the control group (Q group, $p < 0.05$; DQ group, $p < 0.001$). Lipid peroxidation was unaltered in the D group, but the DQ group exhibited a significant reduction ($p < 0.05$) of lipid peroxidation compared with the C group, which was followed by a significant decrease ($p < 0.01$) in the TRAP of the intestinal tissue. The assessment of the different subpopulations of myenteric neurons showed that supplementation with quercetin in diabetic animals prevented the diabetes-induced increase in the cell body area (6.44%) of nNOS-immunoreactive neurons and diabetes-induced increase in the area of VIP-immunoreactive varicosities (31.6%) and promoted an increase in the cell body area (16.11%) of HuC/D-immunoreactive neurons but did not prevent the diabetes-induced loss of HuC/D- and nNOS-immunoreactive myenteric neurons. Supplementation with quercetin increased the bioavailability of NO in the jejunum in normoglycemic and diabetic rats and induced neuronal neuroplasticity in the myenteric plexus in these animals.

Keywords: diabetes mellitus, neuroplasticity, oxidative stress.

MON-380
Quercetin increases bioavailability of nitric oxide in jejunum in normoglycemic and diabetic rats and induces neuronal plasticity in the myenteric plexus

J. N. Zanoni[1], J. V. Colombo Martins Perles[2], S. R. Garcia de Souza[2], F. C. Vieira Frez[3], C. Melo[4], F. Guarnier[5]
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Keywords: diabetes mellitus, neuroplasticity, stress oxidative.

MON-381
Real time molecular dynamics of extracellular matrix components

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It has been established that extracellular matrix (ECM) components are actors in neuronal signaling. There is evidence of highly localized niches of extracellular matrix which suggests specific and fine functional control. During development, the ECM protein and carbohydrate composition changes with time. However, it is commonly believed that it is a relatively fixed structure topologically. Using confocal time-lapse microscopy (0.5–1 Hz) and single particle tracking (SPT) with quantum dots (QD), we demonstrate, in primary neuronal cell cultures, that extracellular matrix components are dynamic and that the diffusion rates of chondroitin sulfate and hyaluronan and associated molecular components change with maturation. At the synapse, ECM components display confined movements and the diffusion rate increases with maturation between DIV 10 (1×10^{-3} μm^2/sec) and DIV 15 (1×10^{-2} μm^2/sec). In extra-synaptic locations, the ECM components exhibit Brownian motion with relatively fast diffusion rates. The rates of diffusion of chondroitin sulfate components are influenced by pharmacological agents. On average, they slow down when neuronal activity is blocked (treatment with TTX) and accelerate when neuronal activity is increased (treatment with bicuculline or 4AP). These data show that the neuronal extracellular matrix is not a static structure and that its molecular movement is regulated by activity. Moreover, single particle tracking has revealed subpopulation of ECM. In DIV 30 cultured cells, chondroitin 6-sulfate is more frequently confined to synapses with a monomodal distribution of diffusion coefficients. Whereas, chondroitin sulfate monitored by the frequently used WFA lectin is extrasynaptic with a multimodal distribution. Thus, the heterogeneity of ECM and ECM binding protein microdomains is accessible using single particle tracking. This may in turn play a key role in activity dependent signaling and tuning the balance of excitation and inhibition.

Keywords: extracellular matrix, Neurons, Single particle tracking.

MON-382
Role of spinal melanocortins/melanocortin receptor MC3 system in the development of diabetes-induced neuropathy

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Aims: One of the recent breakthroughs in pain research has been recognition that non-neuronal cells might play an important role in initiating and modulating activity in primary afferent nociceptors. This study was aimed at defining diabetes-related changes in the activity of glial cells in dorsal horns of the lumbar spinal cords. As the possible role of melanocortins (MC) in nociception has recently attracted attention in the field, we focused on an attempt to better define whether spinal MC/melanocortin receptor MC3 (MC3R) system contributes to neuropathic pain by evaluating the therapeutic efficacy of a specific MC3R agonist Dtrp-g-MSH.

Methods: After 4 weeks of streptozotocin-induced diabetes male C57Bl6/J mice were maintained with or without acute treatment with Dtrp-g-MSH (1 μM in 3 μl, intrathecal infusions). Neuropathy variables included motor and sensory nerve conduction velocities and tactile response (by flexible von Frey filament test). Glial cells markers (Iba-1 and GFAP), phosphorylated p38 MAP kinase and MC3R expressions were measured by spinal cord tissue immunostaining.

Results: Diabetic mice developed nerve conduction velocity deficits and tactile allodynia. Quantification of glial cell revealed prominent elevation of microglia in the dorsal horns, whereas a slight reduction in the number of astrocytes was established in diabetes. These alterations were accompanied by p38 MAP kinase activation in the spinal cord in diseased animals as it is evident from a substantial increase in the spinal levels of phosphorylated p38. It is noteworthy that p38 was activated in cells labelled with microglial marker Iba-1 antibodies but not in GFAP-expressing astrocytes. Acute intrathecal infusion of Dtrp-g-MSH to diabetic mice reversed neuropathic pain behaviour as early as the first hour after each of two administrations and its effect was maintained for 4 h. Dtrp-g-MSH administration was shown to decrease the number and activity of microglial cells. Agonist action was unlikely to be mediated via alterations in the expression of the MC3 receptors as no detectable changes in MC3R immunoreactivity in the dorsal horn were observed compared with vehicle-treated groups.

Conclusion: Taken together, current data suggest that diabetic neuropathy is associated with microglia activation in the dorsal horns and melanocortins/MC3R system might be involved in enhanced nociception. Our findings support the potential use of MC3R agonists to treat diabetes-induced neuropathy.

Keywords: None.

MON-383
S-acylation in neurodegeneration

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Neuronal Ceroid Lipofuscinoses (NCLs) are a group of hereditary, progressive neurodegenerative disorders characterized by the accumulation of intracellular autofluorescent lipopigment (ceroid lipofuscin) in neurons and other cells of the body. This aggregation causes the loss of nerve cells in the brain, leading to neurodegeneration. Mutations in the DNAJC5 gene encoding cysteine-string protein (CSP) have been recently identified as the cause of autosomal-dominant adult-onset NCL (ANCL) (Hachiya et al. 2006; Nosková et al. 2011; Anderson et al. 2013). These mutations (L115R and ΔL116) are situated within the cysteine-string domain, a region of CSP that is extensively S-acylated. S-acylation is a post-translational modification consisting of the attachment of fatty acids to cysteine residues through a thioester bond.

We found that ANCL mutations cause CSP to form SDS-resistant aggregates, which are induced and maintained by S-acylation (Greaves et al. 2012). This observation is interesting as protein aggregation is a hallmark feature of different neurodegenerative conditions. The present study aims to extend upon these observations by assessing: (i) how ANCL mutations affect the intracellular localisation of CSP; (ii) the turnover and degradation of wild-type CSP and mutant CSP aggregates; (iii) how different fatty acids affect the aggregation of CSP mutants; and (iv) the potential of specific S-acyltransferase enzymes to serve as drug targets to prevent CSP aggregation in ANCL. Collectively, these analyses will

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shed light on potential mechanisms whereby CSP aggregates lead to ANCL and potential therapeutic mechanisms to treat this disorder.

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Keywords: Cysteine-String Protein (CSP), Neuronal Ceroid Lipofuscinoses (NCL), S-acylation.

MON-384
Sensory dysfunction in oral mucosa of patients with fibromyalgia

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Studies have described oral problems associated with fibromyalgia syndrome (FM), including sicca, oral ulcerations, and orofacial pain. Our preliminary observations on a small group of FM patients indicated a change in the non-nociceptive, tactile sensory function in oral mucosa and provided evidence for the hypothesis of a neuropathic etiology in orofacial pain.

In the present clinical study on a group of 5 FM patients were studied. The sensory nerve endings of the oral mucosa, were studied using electron microscopy analysis. The specimens were fixed and embedded for study. The nerve fibers in the oral mucosa were examined by electronic microscopical analysis for localization. These fibers run very close to the basal lamina of the epithelium and extend into the filliform and fungiform papillae. Electron microscopic observations showed clearly nerve fibers, which are located very close to the basal lamina of epithelial cells. Some electron-dense granules may be observed in the axoplasms.. The terminal axons revealed numerous mitochondria, neurofilaments, microtubules and clear vesicles in the base of axoplasmic protrusions. All the structures observed are involved in the transmission of pain and mechanoreceptors stimulus of these oral mucosae.

The present findings with strong evidence for neuropathic background in FM will hopefully provide insights for new therapeutic strategies.

Keywords: fibromyalgia, oral mucosa, sensory nerves.

MON-385
Single molecule analysis reveals coexistence of stable serotonin transporter monomers and oligomers in the live cell plasma membrane

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The human serotonin transporter (hSERT) is responsible for the termination of synaptic serotonergic signaling. The formation of dimers of SERT in the plasma membrane has already been indicated by various approaches, including optical methods such as Förster resonance energy transfer (FRET) and classical biochemical ensemble analysis. However, neither application is suitable to yield quantitative interpretation and decipher the exact configuration of the oligomerization states; additionally, biochemical methods do not take the influence of the membrane environment into account. Here we used single molecule fluorescence microscopy to obtain the oligomerization state of SERT via brightness analysis of single diffraction limited fluorescent spots. The techniques applied in this study allow for identification and quantitative evaluation of subpopulations of SERT complexes exhibiting different degrees of oligomerization in a living cell. Heterologously expressed SERT was labeled either with the fluorescent cocaine derivate JHC 1-64, or via fusion to mGFP. We found a variety of oligomerization states of membrane-associated transporters, revealing molecular associations at least up to pentamers and demonstrating the coexistence of different degrees of oligomerization in a single cell; the data is in agreement with a linear aggregation model. The oligomerization was found to be independent of SERT surface density, indicating a stable interaction of the subunits. Therefore, we developed a special bleaching protocol to decipher the interaction kinetics of SERT oligomers; the complexes remained stable over several minutes in the live cell plasma membrane. Together, the results indicate kinetic trapping of preformed SERT oligomers at the plasma membrane. Indeed, by evaluating the oligomerization of SERT in the endoplasmic reticulum we show that the oligomerization process is chemically equilibrated at ER membranes; after trafficking to the plasma membrane, the SERT stoichiometry remains fixed.

Keywords: Serotonin Transporter, Single Molecule Analysis, Stoichiometry.

MON-386
STIM sensors as a molecular targets for memory loss treatment connected with calcium homeostasis impairments in Familial Alzheimer's Disease

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Familial Alzheimer's disease (FAD) which leads to memory impairments is caused by mutations in presenilin-1 (PS1) gene in approximately 50% of cases. PS1 is well known as a component of the gamma-secretase enzyme which cleaves APP to A-beta. To become a catalytic part of enzyme PS1 holoprotein undergoes an endoproteolysis. It was shown that FAD connected mutations in PS1 gene disrupt endoproteolysis increasing holoprotein level. In our study we found effects of FAD PS1 mutants (PS1DE9, PS1 D247A) on ER calcium storage and activity of store-operated and voltage-gated calcium channels in neurons and Neuro2a cell line. The PS1 endoproteolysis levels were estimated with SDS PAGE and western-blot of total cell lysates which demonstrated increased PS1 holoproteins levels. Disrupted channels activities were detected with direct single-cell electrophysiological measurements. Intracellular calcium concentrations were measured in calcium imaging experiments with fura2-AM. The effects were connected with calcium sensors STIM impaired signal transduction from ER to calcium channels in plasmatic membrane (PM) under control of ER calcium levels, which was demonstrated by experiments with sensors knock-downs. The impaired intracellular signal transduction by STIM sensors was revealed in live confocal imaging experiments. Moreover a feeding of Drosophila

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melanogaster transgenes expressing human mutated PS1 in cholinergic nervous system with pharmacological inhibitor of STIM sensor signal transduction led to rescue of the memory loss detected by courtship based assay.

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Keywords: Alzheimer's disease, calcium channels, Drosophila.

MON-387
Structural NMR study of the isolated voltage-sensing domain from the second pseudosubunit of human sodium channel Nav1.4

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Voltage-gated Na+ channels (Nav) are essential for signal transduction in nervous, cardiac, and muscle systems of multicellular organisms. These membrane proteins represent promising targets for treatment of epilepsy, psychotic syndromes, ataxia, migraine and chronic pain. In spite of the large significance for basic science and pharmacology applications little is known about Nav structure at atomic level. Polypeptide chain of eukaryotic Nav channel (α-subunit) encloses 24 transmembrane helices. This makes expression, purification and structural studies of full-sized Nav channels extremely difficult. Each α-subunit includes four pseudosubunits which form four voltage-sensing and one pore domain. This multidomain organization make possible to use 'divide and conquer' approach for studies of Nav channels.

Here we present the first structural study of isolated voltage-sensing domain from the second pseudosubunit of human Nav1.4 channel (VSD). VSD samples were produced by cell-free expression. To find optimal conditions for NMR structural study, the different types of membrane mimicking media (micelles of zwitterionic and anionic detergent, lipid/detergent bicelles) and sample conditions (pH, temperature, ionic strength) as well as several variants of VSD amino acid sequence were tested. It was found that the lyso-palmitoylphosphatidylglycerol (LPPG) micelles at pH 6.0 provided sufficient NMR spectra quality and sample stability. At this conditions, using ^{13}C,^{15}N-labelled VSD sample the nearly complete backbone and partial side-chain resonance assignments were obtained. The VSD secondary structure determined from the chemical shift values was in general agreement with the expected VSD topology and involved four conservative helical regions (S1-S4). However, two additional short helices were observed at the N-terminus of VSD. Moreover, unlike the situation observed in the VSDs of K+ channels, the S3 segment of VSD-Nav1.4 represents one continuous helix without pronounced hinges and kinks, and S4 helix contains the break at the level of R2 residue. The Mn^{2+} titration revealed that S1, S2 and S4 helices adopt transmembrane orientation within the micelle, but S3 is water accessible. These data revealed the formation of water-filled cavities in the spatial structure of micelle embedded VSD. Obtained ^{15}N relaxation data pointed to the enhanced mobility of the VSD loops in the ps-ns timescale. The overall reorientation of VSD/micelle complex in solution takes place with characteristic time ~15 ns (45 °C).

Keywords: membrane proteins, NMR Spectroscopy, voltage-gated sodium channels.

MON-388
Structure and function of a sodium channel gating modifier from the crab spider *Heriaeus melloteei*

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Voltage-gated sodium channels are critical for propagating action potentials in the nervous system. Their malfunction may lead to severe diseases, such as epilepsy, myotonia, long QT syndrome etc. Sodium channels are therefore considered as prospective drug targets. The known variety of sodium channel ligands is classified based on the interaction site and the produced effect. For instance, tetrodotoxin from the puffer fish is a classic pore blocker affecting site 1. Scorpion α-toxins bind to so-called site 3 and inhibit inactivation, whereas β-toxins bind to site 4 and promote activation of the channels. Here we present structural and functional studies of a sodium channel activation inhibitor from crab spider venom.

Hm-3 is a peptide toxin isolated from the spider *Heriaeus melloteei*, which consists of 35 residues. It was produced recombinantly in *Escherichia coli* with a yield of ~0.4 mg per 1 L of bacterial culture. Hm-3 solution structure was determined by NMR spectroscopy. It was found to adopt the so-called 'inhibitory cystine knot' fold stabilized by three disulfide bonds. Electrophysiological studies showed that at concentration of ~100 nM the toxin effectively inhibited both mammalian and insect sodium channels. What was more interesting, the toxin shifted the dependence of channel activation to more positive voltages. This effect is shared by a few other spider toxins, such as JZTX-III from *Chilobrachys jingzhao*, HWTX-IV from *Haplopelma schmidti*, and ProTx-I and II from *Thrixopelma pruriens*. This type of toxin is likely to bind to receptor site 4. To distinguish between site 1 and site 4 activity, competitive binding with tetrodotoxin was performed demonstrating a lack of pore blocking activity for Hm-3. Moreover, the toxin was shown to bind to both neutral and negatively charged lipid vesicles testifying in favor of a 'membrane-access' mode of Hm-3 action.

This work was supported by the Russian Foundation for Basic Research (grant number 12-04-01712), the Program of Molecular and Cell Biology of the Russian Academy of Sciences, and by the stipend of the President of the Russian Federation.
Keywords: gating modifier, spider toxin, voltage-gated sodium channels.

MON-389
Studies of a glial-specific G-protein-coupled receptor GPR37L1

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Glial cells are the most abundant cells in the brain. There is growing interest in the normal functions of glia and their potential roles in neurological diseases. It has long been known that oligodendrocytes (OLs, the myelin-forming cells) and their precursors (OPs, also known as NG2 glia) are critically involved in demyelinating diseases such as multiple sclerosis. More recently, astrocytes have been recognized as having essential physiological functions in the normal central nervous system

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(CNS) and have also been implicated in stroke, brain trauma, Alzheimer's disease, Parkinson's disease, motor neuron disease and Rett syndrome. Our understanding of glial heterogeneity is limited. For example, astrocytes have been classified according to their morphology (e.g. fibrous versus protoplasmic astrocytes in the white and grey matter respectively) but little is known regarding their molecular and regional diversities. The molecular characterization of different subsets of astrocytes could shed light on their functions in various regions of the brain.

The superfamily of G-protein-coupled receptors (GPCR) is a large family of seven-pass transmembrane proteins. The majority of these bind specific extracellular ligands, which trigger an intracellular response. GPCRs are major drug targets; it has been estimated that more than half of clinically useful drugs interact with these receptors. We conducted a visual screen of expression patterns in the Allen Brain Atlas for gene products that seemed likely to mark glial cells and analysed these further by *in situ* hybridization and immunocytochemistry. Among several genes picked in this way, we found one GPCR (GPR37L1) that is expressed exclusively in glia. GPR37L1 is first expressed at early postnatal stages (P7-P14), at the onset of synaptogenesis, and maintains its expression into adulthood. It is expressed throughout the adult brain and spinal cord in subsets of astrocytes and OPs. To explore the functional properties of GPR37L1 and its potential role in neuromodulation or myelination, we generated several transgenic lines: GPR37L1-null and over-expressing mice, mice that express the Diptheria toxin A chain (DTA) in GPR37L1-positive cells and lines that conditionally express green fluorescent protein (GFP) and/or tandem-duplicated Tomato (tdTom) in GPR37L1 cells. Preliminary examination of GPR37L1 knockout mice suggests that this receptor might be involved in synaptic function in the hippocampus.

Keywords: central nervous system, glial cells, G-protein-coupled receptor.

MON-390
Study the specificity of mutants forms of extracellular domain of α7 neuronal nicotinic acetylcholine receptor

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Neuronal nicotinic acetylcholine receptors (nAChR) are widely represented in the central nervous system (CNS). Cholinergic innervation in the CNS, acting through nAChR, regulates the processes of mediator release, neuronal excitability, which affects a number of physiological and cognitive functions. α7 nAChR – one of the most widely presented neuronal nAChR subtype in in CNS, which dysfunction associated with diseases such as schizophrenia, Alzheimer's, Parkinson's, nicotine addiction. α7 nAChR is a target for many drugs, therefore, binding of ligands mechanisms and spatial structure of the receptor of interest for investigation.

Chimeric receptor α7/GlyR is an appropriate model for the study of the extracellular domain of α7 receptor [1, 2]. Using α7/GlyR facilitates some stages of the experiment. Based on bioinformatics and computer modeling data it had been hypothesized that the replacement of Tyr168 and Glu189 in extracellular domain to alanine will affect the specificity of the interaction α7/GlyR with agonists. The methods of site-directed mutagenesis in the coding sequence of α7/GlyR chimera were introduced appropriate replacements. Expression of of α7/GlyR and Tyr168Ala, Glu189Ala mutant forms was carried out in oocytes *Xenopus laevis*.

The results of two-electrode voltage clamp measurements revealed that the value of EC_{50} mutants Tyr168Ala and Glu189Ala for acetylcholine decreased by 38% and 49%, respectively, and for — nicotine decreased by 49% and 43%, respectively, compared with α7/GlyR.

The results obtained that introduction of an alanine residue at position 168 or 189 of the extracellular domain increases the α7/GlyR affinity for acetylcholine and nicotine. Thus, it is experimentally confirmed that Tyr168 and Glu189 do not contact to agonist but in the same time contribute to low affinity of α7/GlyR.

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Keywords: alpha 7 nAChR, Alzheimer's Disease (AD), LGIC.

MON-391
Synthesis and screening of hMAO inhibitory activities of some new 2-pyrazoline and hydrazone derivatives

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The monoamine oxidase (MAO), a mitochondrial outer membrane enzyme, catalyzes the oxidative deamination of biogenic amines. MAO exists in two isoforms as MAO-A and -B (1). Selective MAO-A inhibitors are widely used in the treatment of depression and anxiety while selective MAO-B inhibitors are used in the treatment of Alzheimer's and Parkinson's diseases (2,3). Most of the prodrug-based MAO inhibitors having hydrazine, hydrazide and amine moiety such as isocarboxazid, phenelzine and moclobemide show prominent antidepressant activity (4). Earlier studies demonstrated MAO inhibitory activities of 2-pyrazolines (4-6). Hydrazone compounds also have antidepressant activities (4). Based on the above literature and in pursuing our previous research concerning to the synthesis of novel selective MAO inhibitors, we report here the synthesis, docking studies and hMAO inhibitory activity of some new 2-pyrazoline and hydrazone derivatives. Chemical structure of the compounds have been elucidated by their IR, ^{1}H NMR, Mass, and elementary analysis data. All compounds inhibited hMAO isoforms competitively and reversible. Compound **VIr,** which carries 2-hydroxy-4-methoxyphenyl group at the 3rd position and 2-methoxyphenyl group at the 5th position of the 2-pyrazoline ring, showed the highest hMAO-A inhibitory activity with a Ki value at nM concentration. Newly synthesized compounds were docked computationally to the active site of the hMAO-A and -B forms, and the data indicated a significant correlation between the docking results and experimental ones. Further derivatives should be prepared to explore the possibility of getting a potent and selective hMAO-A inhibitor of this kind.

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Keywords

2-Pyrazoline and Hydrazone Synthesis, Docking, Human Monoamine Oxidase Inhibition.

MON-392
Systematic study on alpha-Synuclein internalization and accumulation

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Background: Parkinson's disease (PD), a progressive neurological disorder of unknown etiology, is characterized by two processes, massive death of neurons of the subtantia nigra pars compacta and accumulation of insoluble inclusions enriched in unfolded alpha-Synuclein (aSyn) called Lewy Bodies (LB). Recent works have shown that uptake of extracellular aSyn can enormously contribute to LB formation, however, the molecular species responsible for aSyn internalization and accumulation have not been systematically characterized. In this study, we studied the ability of structurally different aSyn species to be internalized and accumulate in neuronal cells.

Observations: In order to study internalized aSyn accumulation, SH-SY5Y neuronal cells were treated with 13 structurally different aSyn species, including monomers, oligomers and fibrils. Internalized aSyn was quantified by Selected Reaction Monitoring mass spectrometry (SRM) and confocal microscopy. We found that insoluble aSyn fibrils are efficiently internalized and accumulate in neuronal cells. aSyn uptake was strongly dependent on the insolubility of the fibrils, which are internalized in a dose and time-dependent manner. In contrast to insoluble higher-order structures, we did not observe accumulation of aSyn monomers, oligomers, or dissociable fibrils. Importantly, accumulation of insoluble fibrils leads to a significant reduction of cell viability. We then assayed the ability of cell-produced aSyn to be internalized, and established a cellular model system to produce fibrillar aSyn in neuronal cells. These cells were used to set up a cell-to-cell propagation assay. We observed transmissibility of high-molecular weight aSyn, indicating that donor cells secrete aSyn fibrils, which are internalized by acceptor cells. Finally, and in order to characterize the cellular responses to aSyn accumulation, we quantified the proteomes of cells treated with monomeric, oligomeric and fibrillar aSyn using a mass spectrometry-based approach. We found pronounced proteomic changes in cells treated with insoluble aSyn fibrils, indicating that aSyn accumulation leads to a substantial cellular response.

Conclusions: By establishing a novel cellular model system that recapitulate some aspects of the cell-to-cell propagation of pathological aSyn and Parkinson's Disease pathology, we found that insoluble aSyn fibrils are efficiently internalized and accumulate in neuronal cells, leading to impaired cell viability and substatial proteomic changes.

Keywords: Alpha-synuclein, neurodegenerative diseases, Parkinson's disease.

MON-393
Systemic approaches to identifying mobile homeodomain transcription factors

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Owing to the inherent function as transcription regulators, the homeodomain transcription factors (HM-TFs) have been believed to function in the nuclei of cells that expressing them. However, several HM-TFs have been identified as having unexpected non-cell autonomous functions in developing nervous system. The HM-TFs, including Engrailed-2, Pax6, Otx2, and Vax1, can be secreted out from the cells express their transcripts and move into neighboring cells that don't express the transcripts. The secretion and internalization of HM-TFs were shown to be mediated by secretion (Sec) and penetration (Pen) motifs in homeodomains. Although the Sec and Pen sequences are highly conserved in most HM-TFs, which are over 200 in human genome, it is still unclear all HM-TFs possess these unconventional biophysical properties. Here, we evaluated secretion of 168 human HM-TFs in culture cell-lines by semi-quantitative dot blot method. Our screening results indicate that differential secretion properties of each sub-homeodomain class; importance of extra-homeodomain motifs for HM-TF secretion.

Keywords: homeodomain transcription factor.

MON-394
Systemic oxidative stress might be in the path from normal cognitive function to dementia: data from cross-sectional and longitudinal study

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Growing evidence from *in vitro* and animal model experiments suggest a role of oxidative stress (OxS) in the pathogenesis of late Alzheimer's disease (LOAD) and vascular dementia (VAD). However, the definitive appreciation of the involvement of OxS in these two most common forms of dementia-related diseases need a confirmation in a population-based study on a large sample. The aim of our study was to investigate the possible relationship between OxS and the onset and clinical progression of LOAD and VAD. To achieve this purpose a sample of 431 older individuals: 101 LOAD, 43 VAD, 188 mild cognitive impairment (MCI, pre-dementia condition) and 99 healthy controls were enrolled and assessed for serum levels of hydroperoxides (HY; i.e., by products of lipid peroxidation), total amount of non-enzymatic antioxidants (RAP), uric acid (UA), thiols (TH) and advanced oxidation protein products (AOPP). In addition, a subgroup ($n = 126$) of MCI patients were followed-up for averagely 2 years to evaluate if baseline levels of OxS markers were either related or not to the conversion to LOAD or VAD. Multivariate analysis (covariates: age, gender, smoking and comorbidities) of our cross-sectional data showed a significant ($p < 0.01$ for all) decrease of RAP levels compared to controls in MCI (−24.5%), LOAD (−24.2%) and VAD (−29%) patients. On the other hand, HY emerged as significantly higher ($p < 0.05$) in LOAD patients

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(+30.6%), (but not in MCI and VAD) with respect to healthy. Multivariate logistic regression highlighted the presence of a similar unfavourable oxidative balance (high HY and low RAP) in MCI (O.R.: 4.87; 95%CI: 1.02–23.55) and LOAD (O.R.: 6.99; 95%CI, 1.87–25.21). Noteworthy, no differences were found in OxS markers between MCI patients who converted to LOAD (n = 29) or to VAD (n = 15) and those that remained stable (n = 82) after follow-up time. Overall, our results suggest that systemic redox imbalance might be a significant feature of early stage of dementia. Moreover, the similar (deranged) oxidative profile in MCI and LOAD, could account for the observed inability of OxS markers to predict the conversion from prodromal phase to dementia.

Keywords: Alzheimer, Oxidative stress, Vascular dementia.

MON-395
Tetrahydropalmatine to rats ameliorates development of anxiety and depression related symptoms induced by singles prolonged stress

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Abnormal adaptation of the stress-response system following traumatic stress can lead to an altered hypothalamic-pituitary-adrenal (HPA) axis that may contribute to post-traumatic stress disorder (PTSD) development. The present study was designed to investigate the anxiolytic-like and antidepressant activity of tetrahydropalmatine (THP) in an experimental rat model of anxiety and depression induced by single prolonged stress (SPS) animal model of PTSD by several behavioral tests. For 8 consecutive days, male rats were treated intraperitoneally (i.p.) with vehicle or varied doses of THP, 30 min prior to SPS. Daily THP (50 mg/kg) administration significantly increased open arms visits in the elevated plus maze (EPM) test, spent longer time in open arms, had lower anxiety index, higher risk assessment, and more head dips over borders in open arms performed after SPS. They also exhibited spent longer time in the center of the open filed, reduced grooming behavior in the elevated plus maze (EPM) test, and reduction of immobility time in the forced swimming test (FST). It also blocked the increase in neuropeptide Y (NPY) and decrease in corticotrophin-releasing factor (CRF) expression in the hypothalamus. This is the first study revealing pronounced anxiolytic-like and antidepressant effects of THP on development of behavioral and biochemical symptoms co-morbid with PTSD, indicating its prophylactic potential. Thus, THP reversed several behavioral impairments triggered by the traumatic stress of SPS and has potential for non-invasive PTSD therapeutic intervention.

Keywords: None.

MON-396
The M146V mutation in presenilin-1 gene cause disruption of calcium homeostasis

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Mutations in presenilin-1 (PS1) gene are found in approximately 40% cases of Familial Alzheimer's disease (FAD). The M146V mutation in PS1 gene causes disruption of calcium homeostasis. We aimed to detect channels types involved in the calcium homeostasis impairments. Calcium imaging experiments were performed with Fura2-AM with human neuroblastoma SK-N-SH cell line, transfected with PS1-M146V mutant or PS1-WT as a control. To measure calcium concentration in endoplasmic reticulum (ER) we used ionophore ionomycin. The PS1-M146V expression elevated the calcium concentration in ER comparing to control value. There are two calcium sensors in ER which activate store-operated calcium channels in case of decrease in free calcium concentration in ER. Sensor STIM1 has higher affinity to calcium than sensor STIM2 and works in 'all-or-nothing' way as it needs strong signal for activation. On the other hand spontaneous activity of STIM2 is known to be important for refilling ER calcium stores. Knockdown of STIM2 as well as SOC channel subunit TRPC1 with shRNA recovered the calcium concentration in cells with PS1-M146V mutant to control values. We measured SOC entry induced by thapsigargin (inhibitor of calcium ATPase of ER). In 9 hours after transfection SOC entry was higher in cells expressing mutant than in control cells, but after 24 hours of mutant expression SOC entry was suppressed comparing to control. Therefore cells expressing PS1 M146V might had spontaneous SOC entry through TRPC1-formed channels driven by STIM2 that preceded detected total SOC entry reduction due to inactivity of STIM1 sensor. Experiments on primary cultures of mouse hippocampal neurons revealed the increase in ionomycin sensitive pool and reduction of SOC entry in neurons expressing PS1 M146V mutant comparing to control as well. Active STIM1 is known to be an inhibitor of L-type voltage-gated calcium channels. Depletion of internal calcium stores in free-calcium solution following by depolarization with 140 mM KCl in 2 mM calcium solution revealed that voltage operated calcium entry in neurons expressing PS1 mutant was higher than in control. Thereby we concluded FAD PS1-M146V mutant ceases a misbalance in STIM sensors activity that leads to overload of internal calcium stores and interfere with store-operated channels activation and voltage-gated calcium channels inhibition.

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Keywords: Alzheimer, Calcium homeostasis, Store-operated calcium entry.

MON-397
The molecular aspects of peptide regulation of Glutamate and GABA- ergic receptor systems functional activity

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The processes of normal functioning of nerve cells, ensuring intercellular signaling, synchronization of neural activity, etc. will largely depend on the chemical environment of cells, the presence of neurotransmitters and readiness of receptor cell systems to interact with signaling molecules. The main endogenous neurotransmitters of cellular excitation and inhibition processes are glutamate and GABA. Medical action of many drugs is based on the modulation of receptors binding functional activity. In the work presented, we have studied the molecular base of action of short synthetic neuropeptides with double psychotropic effects (nootropic and anti-anxiety) - Selank (used in clinical practice) and its C-terminal fragment (RPGP). Were also researched molecular effects of potential neuroleptics - biologically active analogues of neurotensin: WPYF and APYF. By using the radioligand-receptor method of analysis of specific intermolecular interactions we found that neuropeptides above able to modulate [^{3}H]GABA and [^{3}H]Glu specific binding to rat brain cells plasmatic membranes. The quantitative assessment of regulatory peptides impact on specific binding of [^{3}H]GABA

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shown the existence of two areas of peptides concentration, which is a sharp (almost two-fold) increase in the specific binding. So, for Selank it makes from 5 nM to 20 nM and from 5 mkM up to 50 mkM. In addition, it was shown that in the presence of ultralow concentrations of peptides (from 1 pM to 10 pM), exist the increase (in 1.5 times) in the number of binding places of tritium labeled GABA. At the next stage of research we tested the joint introduction of synthetic peptides and different GABA(A) receptors allosteric modulators (some of them use in clinical practice, like Diazepam). It is shown that in the presence of one of the neuropeptides (Selank and other) and one of known allosteric modulators of the GABA(A) receptor there is a formation of cooperative effects of specific [^{3}H]GABA binding. Neuropeptides above shown a good influence on [^{3}H]Glu specific binding as allosteric modulators in wide range of peptide concentrations. Also we study the 'ex vivo' effects of delayed in time after intranasal delivery of the neuropeptides influence on specific [^{3}H]GABA binding. In two hours after administration of neuropeptides some changes in the number of binding sites and in the affinity of specific [^{3}H]GABA binding were observed. Thus, we can conclude that neuropeptides with anxiolytic effects (Selank and other) able to make modulatory changing in specific GABA and Glu binding, and also can cause changes in functional activity of receptors by starting cell biochemical mechanisms (when peptide molecules presence is need no more).

Keywords: Glu and GABA -ergic systems, neuropeptides, specific binding.

MON-398
The protective effects of tideglusib against NMDA receptor activation in neural stem cells

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N-methyl-D-aspartate (NMDA) receptor is a subtype of the ionotropic glutamate receptors. It plays important roles in brain function, brain development, learning, memory and neurotoxicity. These receptors are major pharmacological targets to prevent or reduce the progression of neurodegenerative diseases where NMDA receptor overactivation occurs. Successful therapy with NMDA receptor antagonists in humans has been limited by the severe side effects of complete receptor blockade. The aim of the present study was to investigate the possible protective effects of tideglusib, a glycogen synthase kinase-3 inhibitor, against NMDA receptor overactivation induced by NMDA and D-serine in neural stem cells. We measured the alteration in membrane integrity, free radical generation, intracellular Ca^{2+} accumulation and mitochondrial membrane potential following treatments. We found that NMDA treatment, alone or with the combination of D-serine, significantly increased LDH leakage and triggered cell death in neural stem cells. Reactive oxygen species (ROS) formation and intracellular Ca^{2+} levels were increased following NMDA receptor overactivation as well. Furthermore, the significant reduction in mitochondrial membrane potential was found in NMDA/D-serine-treated cells. On the other hand, treatment with tideglusib significantly decreased ROS production, membrane degradation, but did not change intracellular Ca^{2+} levels following NMDA receptor activation. Both in the presence or in the absence of NMDA/D-serine, tideglusib had no effect on mitochondrial membrane potential. As a conclusion, tideglusib may be considered as a neuroprotective agent against NMDA receptor-mediated excitotoxicity in the brain, but data obtained by Ca^{2+} measurements showed that the protection by tideglusib was far from NMDA receptor inactivation. Our preliminary results suggest that the mechanisms involved in ROS generation or mitochondrial dysfunction may be crucial targets for tideglusib against neurodegeneration triggered by NMDA receptor overactivation.

Keywords: excitotoxicity, neuroprotection, tideglusib.

MON-399
The role of peripheral anionic site in binding of Toluidine blue O to human butyrylcholinesterase

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Toluidine blue O (TBO) is a cationic phenothiazine dye which is widely employed in the staining applications. Previously, TBO has been reported as an antifungal and antibacterial drug for the inactivation of yeast and some gram-positive/negative bacteria. Our recent kinetic studies have showed that TBO is a potent inhibitor of human plasma butyrylcholinesterase (BChE) (K_i = 7 nM) as well as human erythrocyte acetylcholinesterase (AChE) (K_i = 46 nM). Considering that TBO may be a potential drug candidate for Alzheimer's disease, in the present study, we investigated the role of peripheral anionic site (PAS) in inhibition of human butyrylcholinesterase by TBO. Site-directed mutants of BChE with PAS modifications were generated by PCR-mediated site-directed mutagenesis. Recombinant wild-type and mutant BChEs were expressed in human embryonic kidney cells in serum free medium and then purified by DEAE-Trisacryl anion exchange and procainamide-Sepharose affinity chromatography. The inhibitory effects of TBO on wild type and mutant BChEs were studied spectrophotometrically at 25 °C, in 50 mM MOPS buffer (pH 8), containing 0.025–0.4 mM butyrylthiocholine as substrate, and
0–8 μM TBO. TBO acted as linear mixed type inhibitor of A277W, Y332A and Y332A/A277W. Based on the rapid equilibrium inhibitory model, K_i values for A277W, Y332A and Y332A/A277W were 0.11 ± 0.01 μM, 0.42 ± 0.06 μM and 0.30 ± 0.014 μM, respectively. On the other hand, TBO caused complex nonlineer inhibition of wild type BChE and T120F. K_i values determined by nonlinear regression analysis were 0.007 μM (for wild type) and 0.297 μM (for T120F). Inhibition studies showed that the mutations in peripheral anionic site of human BChE cause a 15–60-fold increase in K_i value, compared to recombinant wild-type. These results suggest that peripheral anionic site of human BChE may contribute in binding of TBO to enzyme.

Acknowledgement: Supported by a grant (SBAG-113S256) from the Scientific and Technical Research Council of Turkey.
Keywords: butyrylcholinesterase, site-directed mutagenesis, Toluidine blue O.

MON-400
Thrombolysis affects leucine serum level during ischaemic stroke

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Introduction: Several compounds specific to neurons or glial cells are releasing through damaged blood-brain barrier into the circulation during ischaemic stroke (IS). Branch chain amino acids (BCAAs) were considered as a potential biomarker of numerous diseases including cerebrovascular diseases. The

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decrease of BCAAs during acute phase of IS has been noticed previously. However, there is no information about the influence of intravenous thrombolytic treatment with recombinant tissue plasminogen activator (rtPA) on BCAAs serum level. In addition, the relationship between patients' neurological status and BCAAs level during acute phase of IS is also unknown.

Material and Methods: Seventeen ischaemic stroke patients and ten healthy controls were enrolled into the study. Seven of them underwent intravenous thrombolytic therapy with rtPA (rtPA group). The serum samples were obtained at 3 time-points in rtPA group (time-point 0: 1st – 4th hour of stroke; time-point 1: immediately after rtPA administration; time-point 3: on day 5–7 from stroke onset). Remaining stroke patients (without thrombolysis, n = 10) had venous blood collection at two time-points: time-point 1: 5th – 10th hour of stroke and time-point 2: on day 5–7 of stroke. Control serums were obtained once. Before the analysis samples were deproteinised with 6% sulphosalicylic acid in lithium–citrates buffer (pH 2.8) and centrifuged. BCAAs were determined by the automated ion-exchange chromatography with five lithium-citrate buffers using Amino Acids Analyser (AAA 400) by INGOS Corp., Praha, Czech Republic. Amino acids were separated using analytic column OSTION LG FA. The amino acids were identified in comparison to the standards provided by INGOS Corp. The original software MIKRO version 1.8.0 (INGOS) was used for BCAAs evaluation. The amino acids serum level was expressed in microM/ml.

Results: The leucine and valine serum level was gradually increased at time-point 1 and 2 in comparison to the time-point 0 in rtPA group. In addition the leucine level was significantly higher at time-point 1 of rtPA group compared to corresponding time-point at group non treated with rtPA or control patients (median value: 0.134 versus 0.089 or 0.099 microM/ml in rtPA versus non-rtPA or control groups respectively, $p < 0.05$). Neither the significant fluctuation of isoleucine serum level nor the relationship between BCAAs serum level and the degree of neurological deficits was observed.

Conclusion: We suggest that the increase of leucine serum level in patients treated with rtPA could be the indicator of quickened recanalization resulting in the washing out of leucine from the ischaemic focus.

Keywords: ischaemic stroke, thrombolysis, branched chain amino acids.

MON-401
Toluidine Blue O is a potent inhibitor of human cholinesterases

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by synaptic loss, impairment of cholinergic function and abnormal protein depositions in brain. The symptomatic treatment of AD is based on the enhancement of cholinergic function by cholinesterase inhibition. Human brain contains two major forms of cholinesterases: acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). In healthy human brain, AChE activity is higher than BChE activity. In AD, AChE activity decreases while BChE activity increases with disease progression.

In this study, we tested inhibitory effects of three cationic phenothiazine dyes [(toluidine blue O (TBO), thionine (TH) and methylene violet (MV)] and a structurally related phenazine dye, neutral red (NR) on human plasma BChE. Human plasma BChE was purified from outdated human plasma by DEAE-Trisacryl anion exchange chromatography and procainamide- Sepharose affinity chromatography. Inhibition studies were performed at 25 °C in the assay mixture containing 50 mM MOPS buffer pH 8, 0.125 mM 5,5′-dithiobis (2-nitrobenzoic acid), 0.025–0.4 mM butyrylthiocholine and 0–80 μM dye. Kinetic analyses yielded following information: MV acted as a linear mixed inhibitor of human BChE with K_i value of 1.42 μM. TBO, TH and NR caused nonlinear inhibition of human BChE pointing to cooperative binding at two sites. K_i values for TBO, NR and TH estimated by nonlinear regression analysis were 0.007 μM, 0.93 μM and 2.0 μM, respectively. The inhibitory effect of TBO was also tested on human erytrocyte AChE using acetylthiocholine as substrate. TBO acted as linear mixed type inhibitor of human erytrocyte AChE with K_i value of 0.046 μM. Our kinetic results indicate that TBO is a potent inhibitor of human cholinesterases with K_i in nM range. We suggest that it may be a potential drug candidate for treatment of Alzheimer's disease.

Acknowledgement: Supported by a grant (SBAG-113S256) from the Scientific and Technical Research Council of Turkey.
Keywords: Alzheimer's disease, cholinesterase, Toluidine blue O.

MON-402
Variability of potassium channel blockers in *Mesobuthus eupeus* scorpion

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The lesser Asian scorpion *Mesobuthus eupeus* (Buthidae) is one of the most widely spread species of the genus *Mesobuthus*, and its venom is thoroughly investigated. Currently, two main strategies are used in animal toxin research. One is direct isolation of toxins by chromatographic methods and the other is based on cDNA cloning and analysis. Nevertheless, considerable amount of active compounds remain elusive due to the high complexity of *M. eupeus* venom.

Here we present a comprehensive study on the variability of potassium channel blockers in *M. eupeus*. We combine methods from both strategies, including multi-dimensional chromatography, mass spectrometry, venom gland transcriptome sequencing and computational analysis. As a result, we report in total 59 candidate potassium channel toxins assigned to two types of fold. Nine toxins were found to effectively interact with some potassium channel isoforms, and six of them represent novel Kv1.1 and Kv1.3 ligands that may find future application in drug development. We rationalize that our integrated approach is a powerful method to characterize potassium channel toxins from scorpions.

This work was supported by the program for fundamental research of the Presidium of Russian Academy of Sciences no. 24 and by the stipend of the President of the Russian Federation.
Keywords: potassium channel blockers, scorpion, toxin.

MON-403
α-synuclein-mediated perturbation of ionic gradient in neurons

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Recent works suggest that α-synuclein assemblies propagate in a prion-like manner, thus contributing to Parkinson's disease pathology. The interaction of exogenous α-synuclein assemblies

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with neuronal plasma membrane and proteins is poorly documented. Using single particle tracking and immunofluorescence studies, we observed that α-synuclein assemblies form aggregates on the neuronal plasma membrane. A fraction of these aggregates are localized at synapses both *in vitro* and *in vivo*. The presence of α-synuclein aggregates impaired stimulus-evoked Ca^{2+} and Na^{+}-flux in neurons as measured by Ca^{2+}- and Na^{+}-imaging respectively. Lastly, using proteomics approach, we have identified several α-synuclein interacting membrane proteins. Our results demonstrate that the interaction of extracellular α-synuclein initiates a signaling cascade leading to an impaired ion-gradient in neurons.

Keywords: Parkinson's Disease, Single particle tracking, α-synuclein.

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CSIII-05 – Non-coding RNAs

MON-405
Artificial analogues of small nucleolar RNAs modulate the gene expression in human cells

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In order to analyze the regulatory potential of artificial small nucleolar RNAs (snoRNAs), the series of box C/D snoRNA analogues were designed and synthesized. Synthetic RNAs hold canonical elements of human snoRNAs, such as U24 and U25, and novel guide sequences, so these RNAs were targeted at modifying nucleotides of different types of cellular RNAs. We have found that such RNA-constructions directed to pre-mRNA nucleotides affected splicing of target RNA and suppressed the mature mRNA level [1, 2]. Our results have shown possibility to use artificial box C/D snoRNAs as specific gene regulators.

Moreover the transfection with box C/D RNAs analogues was found to decrease human adenocarcinoma cell viability. To reveal the characteristic features of the influence of snoRNAs on gene expression we analyzed whole transcriptome of human adenocarcinoma cells MCF-7 with Illumina HT microarrays. It was found that genes relating to innate immune response and apoptotic cascades were activated in cells transfected by artificial snoRNAs compared with control cells. The data suggest that box C/D RNAs are able to act as mediators of cellular stress and modulate cell viability and proliferation via stress signal cascades. Interestingly, the list of RNAs with increased level included several miRNAs. Further bioinformatic analysis allowed us to establish that the box-C/D-RNA-dependent activation of these miRNAs induced downregulation an extensive mRNA group. The last fact sheds light on the relationship regulatory pathways controlled by different types of small non-coding RNAs.

Acknowledgments: The work was supported by Russian Foundation for Basic Research grants 14-04-31468 and 13-04-01058.

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Keywords: None declared.

MON-406
Biochemical characterization of two Cas5d ribonucleases in CRISPR-mediated microbial immunity

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Clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR-associated (Cas) proteins constitute an RNA-guided microbial immune system against invading foreign genetic materials. Cas5 proteins belong to one of the most prevalent Cas protein families in CRISPR-Cas systems and are predicted to have RNA recognition motif (RRM) domains. Cas5d is a subtype I-C-specific Cas5 protein that can be divided into two distinct subgroups, one of which has a long C-terminal tail while the other contains an insertion in the middle of its N-terminal RRM domain. Here, we report crystal structures of two Cas5d proteins from *Streptococcus pyogenes* and *Xanthomonas oryzae*, which respectively represent the two Cas5d subgroups. Despite a common domain architecture consisting of an N-terminal RRM domain and a C-terminal β-sheet domain, the structural differences between the two Cas5d proteins are highlighted by the presence of an extended helical region protruding from the N-terminal RRM domain of *X. oryzae* Cas5d. Cas5d proteins contain not only specific endoribonuclease activity for CRISPR RNAs but also non-specific double-stranded DNA binding affinity. These findings suggest that Cas5d may play multiple roles in CRISPR-mediated immunity. Furthermore, the specific RNA processing was also observed between *S. pyogenes* Cas5d protein and *X. oryzae* CRISPR RNA and vice versa. This cross-species activity of Cas5d provides a special opportunity for elucidating conserved features of the CRISPR RNA processing event.

Keywords: Cas, CRISPR.

MON-407
Characterization of stress-inducible ncRNAs in *L. monocytogenes*

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Listeria monocytogenes is a Gram-positive, intracellular foodborne pathogen. It is the causative agent of listeriosis, a severe infectious disease affecting elderly, immune-compromised and pregnant individuals. Regulation exerted by small non-coding RNAs (ncRNAs) is a key event in all organisms. In *L. monocytogenes* studies have shown that many ncRNAs are specifically expressed in response to various stress conditions or in environments encountered by the bacteria during infection such as blood and the intestinal lumen. This suggests that ncRNA-mediated regulation is important for the stress tolerance and pathogenesis of *L. monocytogenes*. At present, more than 150 ncRNAs have been identified in *L. monocytogenes*. However, only a subset of these has been studied in detail.

This project aims to characterize seven stress-inducible ncRNAs recently identified in *L. monocytogenes*. The ncRNAs will be investigated in regards to 1) expression profile, 2) target genes, and 3) regulatory mode of action. Studies from our laboratory indicate that these ncRNAs are expressed in response to various stress conditions, including osmotic stress and exposure to antibiotics affecting the integrity of the bacterial cell envelope. Furthermore, our results suggest that the expression of the seven ncRNAs is regulated by the two-component system LisRK or the alternative sigma factor σ^B, which are known to be key regulators of the cell envelope- and general stress response of *L. monocytogenes*. Future studies aim to further characterize these ncRNAs. Through transcriptomic analyses it will be possible to identify genes affected by one or several of the seven ncRNAs. Putative target genes will be verified by quantitative PCR, and the effect of the ncRNAs on target gene expression on both the transcriptional and translational level will be investigated through implementation of reporter gene assays. Moreover, direct targets

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and the molecular mechanism by which the ncRNAs exert their regulation will be studied by means of *in vitro* binding assays. Lastly, *ex vivo* as well as *in vivo* infection assays may help to determine the role of these ncRNAs in bacterial virulence.

The results of this project may ultimately lead to a better understanding of the function of ncRNAs in *L. monocytogenes* and how they contribute to bacterial stress tolerance and pathogenesis.

Keywords: Listeria monocytogenes, non-coding RNA, stress regulation.

MON-408
Characterization of the heart miRNome of the Nile tilapia (*Oreochromis niloticus*)

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Heart is the first organ to form in the embryo and all subsequent events in the life of an organism depend on its function. In this sense, miRNAs had been described to operate as key regulators on diverse biological processes, including the cardiac biology. In fact, several miRNAs highly or specifically expressed in cardiac muscle have been identified through experiments carried out in a restrict number of model organisms. The Nile tilapia *O. niloticus* can be considered an excellent biological model for investigating miRNAs due to its economic importance and genome fully sequenced. Hence, the main aim of the present study was to characterize the heart miRNome and evaluate evolutionary relationships throughout vertebrates by comparative analysis. For this purpose, Illumina next-generation sequencing was used to identify and quantify the heart miRNAs of tilapia. As result, a total of 891 594 mapplable reads were generated, of which 891 476 referred to 113 known conserved Chordata miRNAs expressed, which potentially represents novel orthologs. A suit of 118 reads referred to three novel putative miRNAs expressed in cardiac tissue. Preliminary comparative analysis revealed that miR-26, -30, -126 and let-7 present a highly conserved expression in vertebrate's heart (from fish to mammals), indicating they might be involved in conserved pathways despite of large divergence times isolating these organisms. Intriguingly, there is a feasible exchange of function between miR-499 and miR-208 once we detected a higher expression of miR-499 and no expression of miR-208 in fish and birds with an inverse expression pattern in mammals. Indeed, miRNA target analysis revealed miR-208 and -499 shared >95% of the total number of predicted targets. Furthermore, the Nile tilapia heart miRNome represents a valuable resource for future teleost functional and comparative genomic studies, as well as for relationships between human and fish cardiac evo-devo. Further functional experiments are under development to determine interactions between selected miRNAs and their targets in cardiac development and homeostasis.

Keywords: cardiac biology, evolution, non-coding RNA.

MON-409
Conservative subtelomeric tandem PO41 repeat is transcribed both in chicken and Japanese quail somatic cells

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Transcriptional silencing was for a long time considered to be a fundamental property of satellite DNA arrays located in (peri) centromeric, subtelomeric and telomeric regions of chromosomes. Occasional reports about satellite DNA transcription remained unnoticed until recently. Nowadays, active transcription of satellite DNA both in somatic and germ cells is an evident fact. Here we aimed to analyze transcriptional activity of subtelomeric tandem repeat in two representatives of Galliform species: domestic chicken (*Gallus gallus domesticus*) and Japanese quail (*Coturnix coturnix japonica*). Subtelomeric tandem PO41 ('pattern of 41 bp') repeat was chosen due to its high primary DNA sequence conservation and similar chromosomal distribution in Galliform species, which indicate that its transcripts could have important functions.

Using RNA FISH we demonstrated transcription of both strands of PO41 repeat in chicken and Japanese quail tissues: cerebellum, neoncephalon, muscles, oviduct, small and large intestine. To confirm the results of RNA FISH we adapted a method of RT-PCR on RNA samples from indicated tissues. Comparative analysis of the pattern of transcription in normal somatic and malignized cells demonstrated the similar distribution of transcripts in interphase nuclei. One, two or dispersed foci of PO41 transcripts were detected in euchromatin, close to clusters of heterochromatin. RNases treatments indicate that transcripts are predominantly single-stranded and partly able to form double-stranded structures such as hairpin or supercoiled RNA. Using immuno-FISH we revealed that RNA Pol II participates in transcription of PO41 repeat. Our results also indicate that PO41 RNA does not co-localize with known nuclear bodies like Cajal bodies, splicing speckles and nuclear stress bodies. A scheme representing possible structure of PO41 repeat transcripts is suggested. Presumably, nuclear foci with high concentration of PO41 RNA correspond to transcription factories and consist of nascent PO41 repeat transcripts associated with RNA polymerases.

In prophase PO41 transcripts were detected as bright foci in nuclei, while in metaphase they were located between condensed chromosomes. As can be seen on 3D-reconstrunctions, in early anaphase transcripts of tandem PO41 repeat are oriented on spindle equator. In early telophase PO41 RNA divide equally between daughter cells. It late telophase PO41 RNA is associated with chromosomes indicating its potential regulatory role in heterochromatin maintenance.

The work was partially performed using experimental equipment of the Research Resource Centers 'Chromas' and 'Molecular and cell technologies' of St Petersburg State University.
Keywords: cell cycle, non-coding RNA, tandemly repeated DNA.

MON-410
Development of DNA origami based nanocarriers for cancer therapy

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Biotechnological developments created new possibilities for the treatment of diseases. New drugs and drug carrier systems have been trying to develop and these show opportunities for efficient therapy applications. Biomacromolecules can be used for drug delivery applications as effective drug carrier systems or targeting agents. Especially DNA based systems present biocompatibility, biodegradability, and non-toxic features and these properties make them good candidates for biotechnological applications. The aim of this study is to prepare DNA origami structures for drug targeting, and gene silencing, applications.

In this study, the DNA origami structures were prepared and the use of these structures as a delivery vehicle was investigated in vitro conditions. The DNA origami structures were synthe-

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sized as single and multi-unite structures, which can be used for drug or gene delivery, targeting, and releasing. Doxorubicin, a common therapeutic agent for several cancer types, was intercalated and covalently bound to origami. The effects of the system were determined on the MDA-MB-231 and A549 cell lines via toxicity trials. DNA origami structures were modified with different targeting agents such as lactose, folic acid, and RGD peptide for targeting cancer cells.

The synthesis of single and multi-unit origami structures were carried out. Therapeutic agent, doxorubicin, was incorporated into the origami structure with intercalation or covalent binding. The intra-cellular uptake of the doxorubicin-DNA origami adduct was determined using cancer cells with comparing free doxorubicin. The cellular uptake of doxorubicin loaded origami structures was shown using confocal microscopy and fluorescence spectroscopy. The fluorescence spectroscopy results confirmed that our carrier system deliver the drug more efficiently than free doxorubicin.

Using programmable DNA hybridization and its structural features, many of functionalized materials and structures with desired shape and size can be prepared. These structures will contribute to the development of delivery systems, which is based on biomacromolecules. *In vivo* studies of these delivery systems will contribute to the development of effective biomolecular carrier systems.

Acknowledgement: This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) (112T208).
Keywords: drug delivery, naonomedicine.

MON-411
Development of DNA origami based nanocarriers for cancer therapy

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Acknowledgement: This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) (112T208).
Keywords: drug delivery, naonomedicine, proteomics.

MON-412
Discovery of natural antisense transcripts in *Plasmodium vivax* clinical isolates

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Plasmodium vivax is the most geographically widespread human malaria parasite causing approximately 130–435 million infections annually. It is an economic burden in many parts of the world and poses a public health challenge along with the other *Plasmodium sp*. Despite this the biology of this parasite is less studied and poorly understood. Emerging evidence of severe complications due to infections by this parasite provides an impetus to focus research on the same. Gene expression studies of this parasite directly obtained from the patients has provided evidence of gene regulation resulting in varying amount of transcript levels in the different blood stages. However, the mechanisms regulating gene expression in malaria parasites are not well understood. Discovery of natural antisense transcripts (NATs) in *P. falciparum* has suggested that these might play an important role in regulating gene expression.

Studies have not been initiated to document genome-wide distribution of NATs in *P. vivax* isolates until a study published by our lab. To identify *in vivo* prevalence of antisense transcripts in *P. vivax* clinical isolates, we performed whole-genome expression profiling using custom designed microarray having strand-specific probes. We identified a total of 1348 NATs against annotated gene loci. Our data shows condition specific expression patterns of varying sense (S) and antisense (AS) transcript levels. Few genes with AS transcripts have been validated using strand-specific reverse transcriptase polymerase chain reaction (RT-PCR) and ratios of AS to S transcripts were confirmed using strand-specific quantitative real-time PCR (qPCR). We have also linked experimental and *in silico* studies to understand possible mechanisms of AS transcript production in this parasite.

The results of this study was recently published and is the first study to reveal the presence of NATs in *P. vivax* clinical isolates (Boopathi et al., 2013). In conclusion, our data suggests differential regulation of gene expression in diverse clinical conditions, as shown by differing sense/antisense ratios and thus would lead to detailed investigation of gene regulaion in future.

Keywords: Natural antisense transcripts, *Plasmodium vivax*, Strand specific microarray.

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MON-413
Genome wide identification of uridylated RNAs in humans

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The 3′-end RNA modification by uridylation has been generally linked to RNA degradation. The uridylated pre-miRNAs in mammals and uridylated mRNAs in fission yeast are targeted by the DIS3L2 3′ to 5′ exoribonuclease. DIS3L2 has been associated with Perlman syndrome development and Wilms tumor progression. However, no functional link has been made between uridylation and the involvement of DIS3L2 in these diseases.

Here we report on the RNA binding studies of human DIS3L2 that were performed by Crosslinking in vivo and immunoprecipitation (CLIP) method. Our study uncovers a broad spectrum of DIS3L2 RNA substrates in vivo. A specific bioinformatics approach identifies that many of these targets contain untemplated oligo(U) tail in average length of 8–10 nucleotides. The spectrum of such modified RNAs includes snRNAs, snoRNAs, tRNAs, pre-miRNAs, rRNAs and mRNAs. This points to the essentiality of oligo(U) modification in the cell metabolism.

Keywords: dis3 l2, uridylation.

MON-414
Heat-shock can strongly increase the lifetime of a small RNA with rapid decay

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4.5SH RNA is a 94 nt small nuclear RNA with an unknown function. Hundreds of its genes are present in the genomes of four rodent families including Muridae [1]. 4.5SH RNA genes contain an internal RNA-polymerase III promoter consisting of A and B boxes. It was reported that 4.5SH RNA turns over rapidly, but its lifetime remained unknown [2]. We estimated 4.5SH RNA half-life in Krebs ascites carcinoma (KAC) cells, in different rodent cell lines (L929, 3T3NIH, and Rat1), and in HeLa cells after their transfection with 4.5SH RNA gene. It was found that 4.5SH RNA was indeed short-lived with $t_{1/2}$ about 20 min in various cell types. Then we studied how cell stress could influence 4.5SH RNA in cell. 4.5SH RNA level strongly increased under heat-shock in KAC (10 times), L929 (5 times), 3T3NIH (3 times), Rat1 (5 times), and in transfected HeLa cells (3 times). Additionally, 4.5SH RNA level increased in KAC cells during infection with encephalomyocarditis virus (5 times). We determined the RNA half-life on the peak of heat-shock reaction or viral infection. It turned out to be much longer than in control cells: 90 min in KAC cells, 90 min in L929, 80 min in Rat1, 40 min in transfected HeLa cells after heat-shock, and 30 min in KAC cells after viral infection. Thus, 4.5SH RNA accumulation under heat-shock or viral infection was due to its lifetime increase. It may be a specific stress reaction of this RNA. Alternatively, it can be suggested that heat-shock and viral infection affect the RNA degradation machinery.

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Keywords: cell heat-shock, RNA lifetime, small non-conding RNA.

MON-415
Identification of flax microRNAs involved in nutrient stress response.

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Nutrients are needed for plant growth and development. Expression level alterations of microRNAs (miRNAs) in plants were shown under different nutrient concentrations in soil or nutrient solutions. We sequenced three flax (*Linum usitatissimum* L.) small RNA libraries and obtained 7.2M (inorganic phosphate – Pi - deficiency), 11.6M (normal nutrition), and 7.6M (excessive fertilizer) raw reads. We identified 76 conserved miRNA homologues which belong to 20 distinct miRNA families. Pi deficiency and excess fertilizer responsive miRNAs were identified in flax for the first time. The most significant down-regulation was shown for miR395 (fold change = –2.3, p-value = $4.4*10^{-6}$) and miR169 (fold change = –1.9, p-value = $3.4*10^{-4}$) in flax grown in redundancy of macro- and micronutrients. Under Pi deficiency up-regulation of miR398 (2.0-fold change; p-value $1.5*10^{-11}$), miR399 (2.2-fold change; p-value 0.02) and miR408 (2.3-fold change; p-value $6.6*10^{-7}$) was observed.

We identified 156 potential novel flax miRNAs with predicted hairpins secondary structures. Predicted target genes for new miRNAs are involved in a wide range of processes occurring in the plant, including cell differentiation, immune responses, phosphate homeostasis regulation, plant growth and development. We identified potential novel flax miRNA lus-miR-N1 which was down-regulated under Pi deficiency.

Quantitative real-time PCR (qPCR) on the extended sampling was performed to validate the results obtained from the high-throughput sequencing for miR169, miR395 and lus-miR-N1. However, qPCR confirmed differential expression only for miR395 and lus-miR-N1, but not for miR169. Target genes of miR395 are involved in sulfate uptake and assimilation. We speculate that alterations of the expression level of miR395 could be associated not only with excess sulfur application but also with redundancy of other macro- and micronutrients especially phosphorus and nitrogen which are necessary for protein synthesis. Target gene of lus-miR-N1 encodes ubiquitin-activating enzyme E1. This enzyme catalyzes the first step in the ubiquitination reaction, and in this way this miRNA could regulate phosphate starvation response in flax.

Revealed alterations of flax miRNA expression under excess fertilizer and Pi deficiency clarify molecular mechanisms of nutrition regulation in plants and provide a wide area for further investigations.

The work was financially supported by grants MK-6205.2012.4, RFBR 12-04-01469 and RFBR 13-04-01770. Part of this work was performed at the EIMB RAS 'Genome' center.
Keywords: flax, high-throughput sequencing, microRNA.

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MON-416
Identification of transcribed Alu elements in the human genome

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Non-long terminal repeat retrotransposons, including Long and Short Interspersed Elements (LINEs and SINEs), are the most successful and abundant mobile elements in the human genome. The primate-specific *Alu* elements (*Alus*), belonging to the SINEs class, are over one million copies in the human genome and continuously retrotranspose contributing to disease through insertional mutagenesis. Although most human *Alu* loci are inactive or silenced, *Alu* RNA overexpression may occur under various cell perturbation conditions and it also takes place under pathological conditions including cancer. *Alus* derepression might also impact on human transcriptome to increase genomic instability through both transcriptional and post-transcriptional roles played by *Alu* RNAs. The body of modern *Alus* is about 300 bases in length and it is composed of two divergent dimers separated by a short A-rich region. The left arm contains the internal *cis*-regulatory promoter elements, A box and B box, recognized by the RNA polymerase III (Pol III) transcription machinery, while the right arm is followed by a poly A stretch. The *Alu* transcription unit further includes the genomic sequence extending to the transcription termination site, mainly consisting of four thymines (T4) in the flanking genomic region. Most *Alu*s in the human genome are unique in sequence, due to accumulated mutations in the *Alu* body and to the presence of the 3′ trailer (the sequence between the poly A stretch and the T4) of variable length and sequence. Thus, most *Alu* RNAs, originating from transcription of specific *Alu* loci, can be unambiguously identified, and they can be distinguished between *Alu* RNAs derived from individual *Alu*s directly transcribed by Pol III, and *Alu* RNAs embedded within protein-coding or other RNA polymerase II-transcribed genes whose expression can occur from host gene promoters. A computational screening of RNA-seq and ChIP-seq datasets let us identify dozens of *Alu* RNAs expressed from individual, transcription-prone *Alu* loci in different human cell types. Some of these loci are intergenic, others are within introns of protein-coding genes in antisense orientation, suggesting the possibility of regulatory effects based on sense-antisense RNA pairing. The parallel exploration of ChIP-seq datasets revealed the association of the Pol III machinery to some of the transcript-producing *Alu*s. We identified the internal Pol III promoter elements in a number of active *Alus,* and we demonstrated they are actively transcribed *in vitro* with a key role played by the B box as demonstrated by mutational analysis. Our data also show evidence of Pol III terminator *read through* and recognition of non-canonical termination sites, as well as of post-transcriptional processing of primary *Alu* transcripts.

Keywords: non-coding RNA, RNA polymerase III, Transcription.

MON-417
Identification, functional analysis of non-coding Y RNAs from Chinese hamster cells and binding analysis with kin17 protein

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Non-coding Y RNAs are essential for chromosomal DNA replication in vertebrate cells. They are functionally required for the reconstitution of the initiation of chromosomal DNA replication in late G1 phase template nuclei in a human cell-free system. In vertebrates, genes coding for Y RNAs are evolutionarily conserved. These genes form a small family of up to four short RNA polymerase III transcripts that are located in close proximity to each other in vertebrate genomes, including human, mouse and *Xenopus*. So far, no information is available about Y RNAs in Chinese hamster cells. Here, we report the identification and functional characterization of Y RNAs from Chinese hamster (chY RNAs) as factors for chromosomal DNA replication, and we perform an analysis of interaction among chY RNAs and kin17 protein. We have found DNA sequences in the Chinese hamster genome of four Y RNAs (chY1, chY3, chY4 and chY5), with upstream promoter sequences, which are homologous to the four main types of vertebrate Y RNAs. The chY1, chY3 and chY5 genes were most conserved, whilst the chY4 gene showed the highest degree of diversification from the other vertebrate Y4 genes. We analysed the expression of all chY RNAs in the Chinese hamster cells line GMA32 using quantitative RT-PCR. Despite the presence of genes for all four Y RNAs in hamster genome, we only found that the chY1 and chY3 RNA are expressed. This pattern of expression is consistent with that of other rodents like mouse and rat. After that, we tested if chY RNAs support DNA replication in a cell-free system. We synthesised all four chY RNAs by in vitro transcription from synthetic templates and purified them by ion exchange chromatography. All four chY RNAs were able to initiate chromosomal DNA replication in human late G1 phase nuclei in the additional presence of human initiation proteins. Finally, we carried out the interaction analysis of chY1 RNA with kin17 protein. We chemically coupled purified chY1 RNA covalently to agarose beads and pull-down proteins from GMA32 cell extracts that interact with chY1 RNA. Western blot made with pulled-down proteins revealed the presence of kin17 protein, that confirming its binding to chY1 RNA. These data therefore establish that non-coding chY RNAs can substitute for human Y RNAs in a reconstituted cell free DNA replication initiation system. These experiments suggest that Y RNAs are evolutionarily and functionally conserved in Chinese hamster cells.

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Keywords: Chinese hamster Y RNAs, DNA replication, non-coding RNA.

MON-419
Knockdown of antisense noncoding mitochondrial RNAs targets survivin and Bcl-2 for selective death of tumor cells

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The Noncoding Mitochondrial RNAs (ncmtRNAs) are a family of long noncoding transcripts derived from the mitochondrial 16S ribosomal RNA gene. These RNAs contain inverted repeats (IR) at their 5′ ends, forming stem-loop structures. The family comprises Sense transcripts (SncmtRNAs), composed mainly of the sense 16S transcript, and Antisense counterparts (ASncmtRNAs), containing mainly the antisense 16S transcript. Knockdown of the

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ASncmtRNAs by transfection of antisense oligonucleotides (ASO) causes selective death of several tumor cell lines without affecting viability of normal cells. Cell death induced in this manner displays hallmark features of apoptosis such as nuclear and chromatin fragmentation, loss of mitochondrial membrane potential and plasma membrane asymmetry, activation of caspases and release of cytochrome c from the mitochondria. In this work we show that apoptosis is brought on by relocation of the anti-apoptotic protein Bcl-2 to the nucleus, where it has been reported to exert a pro-apoptotic function. The change in subcellular localization of Bcl-2 is caused by a reduction in FKBP38, a chaperone that carries Bcl-2 to the cytoplasm. Notably, knockdown of ASncmtRNAs also induces a strong downregulation of survivin, a member of the inhibitor of apoptosis protein (IAP) family, which is over-expressed in virtually every known cancer type. This reduction in protein is not reflected in mRNA levels of survivin, which remain unaffected by the treatment, suggesting a post-transcriptional mechanism which affects only translation of survivin mRNA. To explore this possibility, we conducted a luciferase reporter assay, in which the 3′UTR sequence of survivin mRNA was cloned downstream of the firefly luciferase ORF. Luminiscence was reduced in cells transfected with the ASO directed to the ASncmtRNAs, suggesting the induction of microRNAs (miRNAs; miRs) that bind to the 3′UTR of survivin thus inhibiting its translation. We hypothesized that these putative miRs could be derived from the double-stranded region of the ASncmtRNAs. In agreement with this notion, we found that these transcripts co-immunoprecipitate with a specific antibody against Dicer, suggesting that the ASncmtRNAs are substrates of this enzyme involved in miRNA generation. Taken together, our results suggest a role for the ncmtRNAs in gene expression control through the miRNA pathway. [FONDECYT #1110835 and #1140345; INNOVA-Corfo 12IEAT-16317]

Keywords: mitochondria, non-coding RNA, survivin.

MON-420
MicroRNA-499 distinctively regulates target genes sox6 and rod1 to resolve skeletal muscle phenotype in Nile tilapia fish

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Small non-coding RNAs (microRNAs or miRNAs) have been shown essential for the regulation of specific cell pathways, including the skeletal muscle development, maintenance and homeostasis in vertebrates. However, the relative contribution of miRNAs for determining red and white cells phenotype is far to be fully comprehended. Aiming to better characterize miRNA roles into skeletal muscle cells biology, we investigated muscle-specific miRNAs (myomiRs) signatures in Nile tilapia fish. Quantitative (RT-qPCR) and spatial (FISH) expression analyses revealed a highly differential expression (forty-four fold base on qPCR) of miR-499 in red skeletal muscle in comparison to white cells, whereas remaining myomiRs were equitably low expressed in both muscle cell types. Deep examination on miR-499 targets through bioinformatics led us to sox6 and rod1 genes, which were found to be down-regulated at red muscle cells according to RT-qPCR, FISH, and immunofluorescence profiling experiments. Interestingly we verified that miR-499 overexpression distinctively affects target genes expression by predominantly promoting sox6 mRNA destabilization and ROD1 protein translational decay. We also demonstrate, through a genome-wide comparative analysis of SOX6 and ROD1 protein domains and through an *in silico* gene regulatory network, that both proteins are essentially similar in vertebrate genomes, suggesting their gene regulatory network may be also widely conserved. Overall, our data shed light on the mechanisms governing miR-499 target regulation associated to slow-twitch muscle fiber type phenotype, as well as brings up novel valuable insights into the evolutionary dynamics of miRNA and target genes enrolled in a molecular pathway potentially constrained in the skeletal muscle cells of vertebrates.

Keywords: gene expression, myomiRs, non-coding RNA.

MON-421
Mitochondrial non-coding RNAs as therapeutic target in cervical cancer

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Human cells express a family of noncoding mitochondrial RNAs (ncmtRNAs). Normal proliferating cells express both sense and antisense transcripts (SncmtRNA and ASncmtRNAs). However, tumor cells in culture or human biopsies express only the SncmtRNA and down regulate the ASncmtRNAs. This expression pattern is independent of tumor origin and distinguish tumor cell from normal cell. The goal of this work was to establish primary cultures of human biopsies of advance cervical cancer. We obtained five successful cultures that were characterized by western blot and HPV genotyping. In vitro 'therapy' using antisense oligonucleotides (ASO) against the AS-ncmtRNA show a massive cell death mediated by apoptosis and strong reduction in tumorigenic properties as showed by sphere formation assay.

The established primary cultures were used to evaluate the efficacy of the ASO treatment in xenografts models. We established the temporal tumor growth curve for each culture and 2 out of five were successful. Once the tumor reach 100 mm^3 we initiated the ASO treatment and found that after ten ASO injections a strong reduction in growth tumor was observed. We evaluated the effect over growth tumor comparing the ASO administration pathway (I.P v/s E.V). We will discuss the effect of ASO treatment *in vivo* over surviving and dose regimen.

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Keywords: cervical cancer, mitochondria, non coding RNA.

MON-422
Molecular mechanisms of piRNA amplification

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Germline-specific Piwi-interacting RNAs (piRNAs) protect the genome against selfish genetic elements and are essential for fertility in animals. piRNAs targeting active transposons are amplified by a feed-forward loop known as the Ping-pong cycle, which links endonucleolytic slicing of target RNAs by Piwi proteins to biogenesis of new piRNAs. However, the biochemical framework for this pathway remains elusive. Here, we describe the identification of a transient Amplifier complex mediating the biogenesis of secondary piRNAs in insect cells. This complex is nucleated by the DEAD box RNA helicase Vasa and contains the two Piwi proteins participating in the Ping-pong loop, the Tudor protein Qin/Kumo and antisense piRNA guides. These components assemble on the surface of Vasa's helicase domain, which functions as an RNA clamp to anchor Amplifier onto transposon transcripts. We show that ATP-dependent RNP remodelling by

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Vasa facilitates the transfer of 5′-sliced piRNA precursors between the Ping-pong partners, and failure to achieve this results in Drosophila female sterility. Taken together, our results reveal the molecular basis for amplification of small RNAs that confer adaptive immunity against transposons.

Germline-specific Piwi-interacting RNAs (piRNAs) protect the genome against selfish genetic elements and are essential for fertility in animals. piRNAs targeting active transposons are amplified by a feed-forward loop known as the Ping-pong cycle, which links endonucleolytic slicing of target RNAs by Piwi proteins to biogenesis of new piRNAs. However, the biochemical framework for this pathway remains elusive. Here, we describe the identification of a transient Amplifier complex mediating the biogenesis of secondary piRNAs in insect cells. This complex is nucleated by the DEAD box RNA helicase Vasa and contains the two Piwi proteins participating in the Ping-pong loop, the Tudor protein Qin/Kumo and antisense piRNA guides. These components assemble on the surface of Vasa's helicase domain, which functions as an RNA clamp to anchor Amplifier onto transposon transcripts. We show that ATP-dependent RNP remodelling by Vasa facilitates the transfer of 5′-sliced piRNA precursors between the Ping-pong partners, and failure to achieve this results in Drosophila female sterility. Taken together, our results reveal the molecular basis for amplification of small RNAs that confer adaptive immunity against transposons.

Keywords: piRNA, transposon, Vasa.

MON-423
Ms1, a novel sRNA interacting with the RNA polymerase core in mycobacteria

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Gene expression and its regulation are vital for all organisms. An important class of molecules that regulate gene expression are non-coding small RNAs (sRNA). In mycobacteria we found a new sRNA, called Ms1 in *Mycobacterium smegmatis*. Ms1 is a ~300 nt long sRNA that is highly abundant in stationary phase of growth. By several approaches, we demonstrate that Ms1 binds to the RNA polymerase (RNAP) core. This is in contrast with most other species where the RNAP-binding sRNA (6S RNA) binds the holoenzyme containing the primary sigma factor (σ^A). This difference can be explained by the composition of the transcription machinery in mycobacteria that in stationary phase of growth contain a relatively low level of σ^A and the RNAP-σ^A holoenzyme. Thus, Ms1 represents a new class of sRNAs interacting with RNAP.

This work is supported by grant No.P305/12/G034, from the Czech Science Foundation.

Keywords: Ms1, Mycobacteria, RNA polymerase.

MON-424
Noncoding 7SK human snRNA and HIV1 Tat interaction

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Human immunodeficiency virus (HIV) exploits host's cellular proteins during its replicative cycle and latent infection. The positive transcription elongation factor b (P-TEFb) is a key cellular transcription factor critical for these viral processes.

7SK is an abundant, 331-nucleotide small nuclear RNA (snRNA) that functions as a transcriptional regulator during the elongation phase. 7SK sequesters P-TEFb as 7SK/HEXIM1/P-TEFb ribonucleoprotein complex. 7SK RNA binds to HEXIM1 regulatory domain and promotes the binding of the HEXIM C-terminal domain to cyclin T1/T2 of P-TEFb. P-TEFb shows little CTD kinase activity which indicates that 7SK snRNA in collaboration with HEXIM1 function as an inhibitory factor of P-TEFb.

During viral replication, P-TEFb is recruited via interactions of its cyclin T1 subunit with the HIV Tat (transactivator of transcription) protein and TAR (transactivation response) element.

Currently 7SK snRNA structure is understood and Tat contains an arginine-rich motif (ARM) in which a single arginine residue has been shown to confer specific binding to the TAR bulge region.

In the presentation we will be presenting the strong interaction of 7SK snRNA and Tat through 2D NMR spectroscopic studies and ITC (isothermal calorimetric analysis). In our study we have evidence for preformed arginine binding motifs in 7SK snRNA with pseudo triple platform which is responsible for its strong interaction with Tat peptide. ITC data and NMR data also supports this strong binding interaction with preliminary data indicates a high binding affinity of TAT peptide to 7SK-SL1 (Kd = ~50 nM, n = ~1.36).

Keywords: None.

MON-425
On-enzyme refolding permits small RNA and tRNA surveillance by the CCA-adding enzyme

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Eukaryotic transcription yields a wide variety of long non-coding RNAs (lncRNAs) with important, yet largely mysterious functions. Two of these lncRNAs, MALAT1 and Menβ, give rise to a tRNA-like small RNA in addition to the mature lncRNA. The stability of these tRNA-like small RNAs, as well as of *bona fide* tRNAs, is assessed by the CCA-adding enzyme which initiates degradation of unstable RNAs through tandem addition of CCA. Here, we are able to characterize this second CCA-addition cycle by combining nine co-crystal structures and biochemical experiments. We find that a unique RNA bulge enables the second catalytic cycle. Nucleotide binding to the active site triggers a clockwise screw motion, which initiates RNA refolding with the RNA's 3′-end refolding 'on-enzyme' while its 5′-end stays put. Intriguingly, unstable RNAs are not specifically detected by the CCA-adding enzyme. Instead, the versatility of RNA folding allows the enzyme to proofread unstable RNAs without altering its mode of substrate recognition.

Keywords: non-coding RNA, protein-RNA complex, RNA folding.

MON-426
Polyadenylation of transcripts synthesized by RNA polymerase III: requirements for RNA structure

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Until recently it was believed that only transcripts synthesized by RNA polymerase II (e.g., mRNA) were subject to AAUAAA-

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dependent polyadenylation. We previously showed that RNA transcribed by RNA polymerase III (RNAPIII) from B2 elements of the mouse genome III could be polyadenylated in AAUAAA-dependent manner [1]. B2 is referred to mobile genetic elements called SINEs (Short Interspersed Elements). SINEs are capable of RNAPIII transcription due to the presence of the internal promoter which consists of two boxes (A and B) spaced by 30–35 bp. Many species of SINEs end with the RNAPIII transcriptional terminator (TTTTT) and contain AATAAA hexamers in their A-rich tail. Such SINEs were united into Class T+, whereas SINEs lacking TTTTT and AATAAA sequences were classified as T- [2]. Here we studied requirements for polyadenylation of RNAPIII transcripts of SINEs. Seven and six SINEs families from Class T+ and T-, respectively, were analyzed. Derivatives of these SINEs with nucleotide substitutions and insertions were constructed. The resulting constructs were transiently transfected in HeLa cells and isolated RNA was analyzed by Northern blot hybridization. It was found that replacement of AATAAA with AACAAA in T+ SINEs abolished the RNA polyadenylation. In this work, we determined the areas of T+ class SINEs that are important for polyadenylation of their RNAPIII- transcripts. Three species of SINEs were studied: B2 (mouse), DIP (jerboa) and VES (bat) [3]. Derivatives of these SINEs with nucleotide deletions, insertions, and substitutions, were constructed. The resulting constructs were transiently transfected in HeLa cells and isolated RNA was analyzed by Northern blot hybridization. It has been shown that the presence of the polyadenylation signal (AATAAA) as well as two other regions was important for polyadenylation of transcripts of these three SINEs. The first (beta) resides immediately downstream the promoter box B and the second (tau) is located upstream the A-rich tail. In the case of SINEs DIP and VES (but not B2), the tau region is a polypyrimidine motif. Such a motif is also located immediately upstream the A-rich tail in most other SINEs of class T+ [2]. Apparently polyadenylation mechanisms for RNAPIII-transcripts and mRNA greatly differ.

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Keywords: RNA polyadenilation, RNA polymerase III, SINE.

MON-427
Prediction of RNA-RNA interactions in *H. salinarum* by using self-orgazning map (SOM)

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Ribonucleoprotein (RNP) interactions engage in critical roles within a broad range of cellular processes, ranging from transcriptional and post-transactional regulation of gene expression to host protection in opposition to pathogens. High throughput experiments to recognize RNA–protein interactions produce details about the complexity of interaction networks, but require time and considerable efforts. Therefore, there is need to have for trustworthy computational approaches for predicting ribonucleoprotein interactions. In this paper, we predict the RNA-RNA interactions as ncRNA by Self Organizing Map (SOM). SOM is an unsupervised learning algorithm proposed by Kohonen. The principal goal of the SOM algorithm is to map an incoming pattern in a higher dimensional space into a lower usually one or two dimensional space, and perform this transformation adaptively in a topological ordered fashion.

Keywords: None.

MON-428
Quantitative measurement of iron in bacterial cells using a new fluorescent probe

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During a bacterial infection, iron becomes rapidly limiting. Thus, bacteria must cope with this deficiency. Stress response mechanisms involve a series of regulatory RNAs called small non-coding RNAs (sRNAs). Among them, RyhB sRNA regulates the expression of about fifty proteins involved in iron homeostasis. When the intracellular concentration becomes too low, RyhB transcription activation occurs, resulting in an inhibition of the expression of non-essential protein using iron to redirect resources to essential proteins. The sRNA exerts its action by pairing with target mRNAs in order to modulate their translation efficiency and stability. Nevertheless, cellular factors that could influence the action of RyhB are ill-understood. Furthermore, methods to correlate iron concentration to cellular stresses are still lacking. In this context, we initiated the development of a new molecular tool to probe iron and measure its concentration *in vivo*. Our progress toward studying the effect of RyhB-based regulation on iron concentration will be presented during this meeting.

Keywords: fluorescence, iron metabolism, non-coding RNA.

MON-429
Regulation of the colon cancer methylome by long intergenic non-coding RNAs

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We have previously identified a novel class of non-coding RNAs in humans termed long intergenic non-coding RNAs (lincRNAs). The human genome encodes over 8000 lincRNAs, and numerous lincRNAs are associated with epigenetic-modifying complexes and affect gene expression *in trans*. In a preliminary study, we identified ~148 lincRNAs that associate with the DNA methyltransferase DNMT1 in a colon cancer cell line HCT116. Since DNMT1 is a key enzyme that regulates DNA methylation patterns, we hypothesized that the deregulation of DNMT1-associated lincRNAs may disrupt DNMT1 normal function; and thus, contribute to cancer-associated abnormal DNA methylation. First, we examined the expression of DNMT1-associated lincRNAs in normal colon and patient-derived colon cancer cell lines, and found DNMT1-associated lincRNAs to be highly deregulated in colon cancer. For example, the expression levels of a lincRNA, designated lincSMAD, are significantly down-regulated in colon cancer cell lines in comparison to normal colon samples. Transfecting lincSMAD back into colon cancer cell lines significantly surprised their ability to form colonies *in vitro*. Moreover, cells into which we transfected lincSMAD showed significantly altered DNA methylation patterns at multiple loci distributed

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throughout the genome, as assayed by Illumina 450K methylation arrays. These results suggest that loss of lincSMAD in colon cancer may be a novel mechanism involved in cancer associated aberrant DNA methylation, and that deregulation of lincRNAs may contribute to colon cancer etiology.

Keywords: Colon Cancer, Epigenetics, lincRNAs, DNA methylation, DNMT1.

MON-430
Resveratrol promotes apoptosis, autophagy and suppressed cell division via upregulated miR-181 family and target genes in human K562 cells

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Chronic myeloid leukemia (CML) is a malignant disorder of the haematopoietic stem cell characterized by philadelphia chromosome. Resveratrol is a natural phytoalexin that induces apoptosis and autophagy. MicroRNAs (miRNAs) are noncoding, single stranded RNAs which hold regulation of gene expressions in different cancers.

In this study we aimed to evaluate the cytotoxic, apoptotic and autophagic effects of resveratrol in CML cells by questioning miRNA expression levels and gene expression of miRNA targets which are associated with CML progression.

K562 cells were treated with RES time and dose dependent manner and cytotoxicity was evaluated by using WST-1 assay. The RT-qPCR is used for miRNA and gene expression analysis. miRNAs and genes expression levels were evaluated by using miScript miRNA PCR Array and RT2 Profiler PCR Array, respectively.

Significant increase in miR-181 family was observed in K562 treated with resveratrol. Resveratrol upregulated miR-181a, miR-181b, miR-181c and miR-181d expressions 8.96, 6.42, 10.15, 17.19 fold according to the control cells, respectively. Target gene expression levels of miR-181 family such as ATG5, CASP3 and p27KIP1 were increased respectively 10.41, 8.63 and 36 fold via up-regulated miR-181 family.

Our findings showed that Resveratrol induced apoptosis and autophagy in K562. Resveratrol upregulated tumor suppressor miR-181 family and the target genes, leaded CASP3 activation and modulation of Atgs in autophagic pathway in K562. Resveratrol regulates autophagosome formation via upregulated-ATG5 expression. p27KIP1 upregulation induced prevented progression from G1 to S-phase and suppressed cell division. These results provide that Resveratrol may be a therapeutic candidate for CML treatment.

Keywords: Chronic Myeloid Leukemia, miRNAs, Resveratrol.

MON-431
Ribonucleic acids of circulating complexes from human blood plasma

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Circulating RNAs were shown to play an important role in normal physiological processes, such as immune response, cell differentiation as well as in pathological processes, including tumorigenesis and neurodegeneration. It is well-known that ribonucleic acids are present within extra-cellular membrane covered vesicles, and as part of circulating ribonucleoprotein complexes not associated with cells or vesicles. Circulating RNAs were found within exosomes, microvesicles, apoptotic bodies, ribonucleoprotein complexes and high-density lipoproteins. The object of the present work was RNA of exosomes, microvesicles, apoptotic bodies, ribonucleoprotein complexes from blood of healthy donors and of non-small cell lung cancer patients.

To analyze circulating RNA structures we used an approach which allowed to consider all types of RNA fragments from biological samples. Pooled non-hemolyzed blood samples were separated into 5 fractions: blood cells, plasma, microvesicles, exosomes and membrane-free ribonucleoprotein complexes. For each blood fraction RNA the cDNA-libraries were obtained and analyzed by SOLiD high-throughput massively parallel sequencing technology. To analyze SOLiD sequencing data we applied the unique bioinformatic approach utilizing Bowtie and Cufflinks software. It was found that circulating membrane vesicles as well as membrane-free ribonucleoprotein complexes contain: fragments of rRNAs, tRNAs, mtRNAs, mRNAs, lncRNAs, snRNAs, snoRNAs and mature microRNAs. Distribution of particular RNA species in different blood plasma fractions was documented. We also detected diagnostically significant differences between RNA distributions in particular blood fractions of healthy donors and lung cancer patients.

The work is supported by the Russian Foundation of Basic Research grants: No 13-04-01058, 14-04-31468.
Keywords: circulating RNAs, exosomes, microvesicles.

MON-432
Sensing viral RNA in *Drosophila melanogaster*

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RNA interference plays a central role in antiviral innate immunity in flies. Indeed, flies mutant for the three key components of the small interfering (si)RNA pathway, namely Dicer-2, R2D2 and Argonaute2 (AGO2) are highly sensitive to a wide range of viruses (1). Drosophila Dicer-2 is a RNaseIII able to cleave long dsRNA (dsRNA) into 21 nt long small interfering RNAs (siRNAs). Dicer-2 contains a DExD/H-box helicase domain at its N-terminus sharing important phylogenetic similarity with DExD/H-box helicase of known mammalian viral sensors, namely the RIG-I-Like-Receptors (RLRs). The RLRs (e.g. RIG-I, MDA-5) sense conserved viral molecular patterns, like 5′ triphosphates or dsRNA duplexes, and activate, through their caspase recruitment domains (CARD), a signaling cascade leading to interferon production. The structural similarity between the DExD/H-box helicase domains of mammalian RLRs and Dicer-2, added to their common functional role in sensing viral RNAs, point to some similarities between antiviral immunity in mammals and flies.

Although *in vitro* and *in vivo* experiments clearly indicate that Dicer-2 can process long double stranded RNA, the exact nature of the viral RNAs sensed *in vivo* in infected cells remains mysterious. We are interested in understanding how Dicer-2 senses viral RNAs, with a particular focus on the contribution of the N-terminal DExD/H helicase domain, which is conserved in mammalian RIG-I like receptors. Indeed, *in vitro* experiments have revealed a critical role of this domain in both processivity of the enzyme and discrimination of the extremities of the template

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RNA[1,2,3]. To address this question, we take advantage of a combination of approaches including *Drosophila* genetics, next-generation sequencing technologies and bioinformatics analysis.

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2. Cenik *et al*., Mol Cell. 2011.
3. Welker *et al*., Mol Cell. 2011.

Keywords: Antiviral immunity, Dicer-2, RNAi.

MON-433
Specific labeling of miRNAs and siRNAs by HEN1 methyltransferase

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Small non-coding RNAs such as microRNAs (miRNAs) and small-interfering RNAs (siRNAs) are among the most studied biological objects of the last decade. Present in the widest range of eukaryotes, including humans, these small molecules with its numerous functions have high importance in almost all main biological processes. It is not surprising that differences in small RNA levels were determined as potential biomarkers in cancer, neurological and many other diseases. However detection and extraction of these molecules are still challenging to scientists. Here we present novel fast and easy one-step labeling of small RNAs that allows extraction of these molecules and more labile two-step labeling that provides wide selection of different available reporter groups, such as biotin and fluorophores. Both methods use synthetic analogues of S-adenosyl-L-methionine and exploit high specificity of methyltransferase HEN1 to double-stranded 21 to 24 nt long RNA molecules, namely miRNA/miRNA* and siRNA/siRNA*, minimizing non-specific detection and/or extraction of DNA or irrelevant types of RNA molecules, such as tRNAs or fragments of long RNAs.

Keywords: labeling, miRNAs, siRNAs.

MON-434
Structural analogs of tRNA form a complex with Cytochrome C

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One of the most fundamental RNA molecules in every living cell is transfer RNA (tRNA). It plays an essential role in the protein biosynthesis process, where it is responsible for amino acids delivery to the ribosome machinery and their arrangement based on the codon-anticodon complementarity with mRNA sequence. Recently, it has been shown that specific tRNAs interact with cytochrome C stronger then other RNA types [1]. Cytochrome C is a relatively small protein involved in electron transfer process in the cellular respiratory system [3]. Additionally, cytochrome C plays a critical role in the internal pathway of programmed cell death process (apoptosis). Triggered by the internal signals such as DNA damage or excessive reactive oxygen species (ROS) formation, cytochrome C is released from mitochondrial membrane, forms an apoptosome complex and activates the caspase cycle, finally leading to cell death [4].

Recently it has been postulated that the specific tRNA molecules interact with cytochrome C and this complex may influence the apoptosis process [1,2]. To test this hypothesis, we synthetized the series of specific tRNA analogs (RNA transcripts) and used them to study the complex formation with cytochrome C *in-vitro*. Our results suggest, that synthetic tRNA molecules (without nucleobase modifications) interact with cytochrome C in the similar fashion as natural tRNAs. Gel mobility assay and spectroscopic analysis indicate the ratio of RNA to protein is in the range 2–5. However, slight differences can be found in the circular dichroic and fluorescents spectra, suggesting, the structure of RNA component is important for the nature of the complex. For instance analogs of $tRNA^{Ala}$ and $tRNA^{Phe}$ apparently undergo larger structural change upon binding to cytochrome C, compared to $tRNA^{His}$. In summary, this study shed new light on the meaning of the tRNA/cytochrome C interactions.

This research was supported by the National Science Centre agency in Poland (2011/01/B/NZ3/02090).

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Keywords: Cytochrome c, tRNA.

MON-435
Suppression of STAT5A and STAT5B chronic myeloid leukemia cells via siRNA and antisense-oligonucleotide applications with the induction of apoptosis

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Signal transducers and activators of transcription (STAT) proteins function in the JAK/STAT signaling pathway and are activated by phosphorylation. As a result of this signaling event, they affect many cellular processes including cell growth, proliferation, differentiation, and survival. Increases in the expressions of STAT5A and STAT5B play a remarkable role in the development of leukemia in which leukemic cells gain uncontrolled proliferation and angiogenesis ability. At the same time, these cells acquire ability to escape from apoptosis and host immune system. In this study, we aimed to suppress STAT-5A and -5B genes in K562 CML cells by siRNA transfection and antisense oligonucleotides (ODN) targeting and then to evaluate apoptosis rate. Finally, we compared the transfection efficiencies of these approaches. Quantitative RT-PCR and Western blot results indicated that STAT expressions were downregulated at both mRNA and protein levels following siRNA transfection. However, electroporation mediated ODN transfection could only provide limited suppression rates at mRNA and protein levels. Moreover, it was displayed that apoptosis were significantly induced in siRNA treated leukemic cells as compared to ODN treated cells. As a conclusion, siRNA applications were found to be more effective in terms of gene silencing when compared to ODN treatment

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based on the higher apoptosis and mRNA suppression rates. siRNA application could be a new and alternative curative method as a supporting therapy in CML patients.

Keywords: Chronic myeloid leukemia, K562, STAT5, siRNA knockdown, antisense oligonucleotides, apoptosis.

MON-436
The molecular function of the long noncoding RNA SPRY4-IT1 in human melanocytes

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Expression of the long noncoding RNA (lncRNA) *SPRY4-IT1* is low in normal human melanocytes but high in melanoma cells. siRNA knockdown of *SPRY4-IT1* blocks melanoma cell invasion and proliferation, and increases apoptosis. To investigate its function further, we affinity purified *SPRY4-IT1* from melanoma cells and used mass spectrometry to identify the protein lipin 2, an enzyme that converts phosphatidate to diacylglycerol (DAG), as a major binding partner. *SPRY4-IT1* knockdown increases the accumulation of lipin2 protein and upregulate the expression of diacylglycerol O-acyltransferase 2 (DGAT2) an enzyme involved in the conversion of DAG to triacylglycerol (TAG). When *SPRY4-IT1* knockdown and control melanoma cells were subjected to shotgun lipidomics, an MS-based assay that permits the quantification of changes in the cellular lipid profile, we found that *SPRY4-IT1* knockdown induced significant changes in a number of lipid species, including increased acyl carnitine, fatty acyl chains, and triacylglycerol (TAG). Together, these results suggest the possibility that *SPRY4-IT1* knockdown may induce apoptosis via lipin 2-mediated alterations in lipid metabolism leading to cellular lipotoxicity.

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Keywords: long noncoding RNA, melanocytes.

MON-437
The non-coding tandem repeat LL2R on chicken chromosome 2 is transcribed in growing oocytes at the lampbrush stage

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The role of non-coding RNA in a cell remains largely unknown. Massive transcription of tandemly repetitive DNA is typical for certain period of diplotene in avian and amphibian oocytes. Here we characterize the transcriptional activity of the novel non-coding tandem 'lumpy loop 2 repeat' (LL2R) that was revealed on the q arm of the chicken chromosome 2 by means of bioinformatic analysis. Using different approaches, such as DNA/RNA FISH, confocal imaging, atomic force microscopy and scanning electron microscopy, we have previously shown that synthesized during oogenesis non-coding transcripts of LL2R repeat highly enrich RNP-matrix of special transcription units (so-called 'lumpy loops') on lampbrush chromosome 2. Here we demonstrate data on transcriptional activity of the non-coding LL2R cluster by the reverse transcription (RT)-PCR on RNA isolated from chicken ovary. The RT-PCR products generated from transcripts of one of the LL2R repeat strands differed slightly in size and band intensity from the products from another DNA strand transcripts. These data indicate transcription of both strands of LL2R repeat and elucidate the tandem organization of the non-coding LL2R transcript, demonstrating that it may contain not only direct, but also inverted repeats. RT-PCR products of about 380 base pairs were cloned in AT-vector and sequenced. The alignment of cDNA sequences isolated from several clones with the *Gallus gallus* genome confirmed that the RT-PCR products consist of LL2R repeat unit. RNA FISH on chicken lampbrush chromosome 2 with strand-specific LNA probes to LL2R repeat demonstrated transcriptional activity of only one strand of this genomic locus in chicken growing oocytes. The observed difference between FISH with LNA probes data and RT-PCR results may indicate differential transcriptional activity of LL2R strands in growing oocytes and somatic cells of the ovary. Immunostaining technique has demonstrated that elongating form of RNA polymerase II associates with LL2R repeat transcription unit on chicken lampbrush chromosome 2. Loops formed by LL2R-repeat transcription unit do not contain markers of repressed chromatin but demonstrate low rate of transcription as shown by BrUTP injection experiments. We also confirmed that non-coding LL2R RNA co-transcriptionally associates with splicing factors including U6 snRNA and SR-protein SC35. An emerging model of the LL2R repeat transcription and co-transcriptional association of LL2R transcripts with splicing factors will be presented.

The work was partially performed using experimental equipment of the Research Resource Centers 'Chromas' and 'Molecular and cell technologies' of St Petersburg State University.

Keywords: lampbrush chromosome, non-coding RNA, tandem repeat.

MON-438
The peculiarities of complex formation of porphyrins with transfer RNA

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The interaction of meso-tetra-(4N-oxyethylpyridyl)porphyrin (TOEPyP4) and its Zn(II), Cu(II), -metallocomplexes with tRNA at low ionic strength we have investigated earlier [1]. In this work we have studied the binding of the same porphyrins with tRNA which has 2 forms: hairpin structure and spatial reversed 'L' structure. The interaction of tRNA from E.Coli with porphyrins is studied by UV/Vis Spectrophotometry and Circular Dichroism methods.

The measurements were performed in 0.1 BPSE and 1BPSE buffers (1 BPSE = 6 mM Na_2HPO_4+ 2 mM NaH_2PO_4 +185 mM NaCl + 1 mM Na_2EDTA), correspondingly $\mu = 0.02M$ and $\mu = 0.2M$, pH 6.57. (tRNA has hairpin form at $\mu = 0.02M$ and reversed 'L' structure, when $\mu = 0.2M$.)

From the spectrophotometric titration datas the Scatchard binding isotherms for porphyrin-tRNA complexes are built and binding parameters are calculated (N – the number of binding sites per molecule of tRNA, and K – the binding constant).

For all complexes of tRNA-porphyrin there are also found induced CD spectra, which are crucially different from ICD spectra of DNA-porphyrin complexes. In case of tRNA's hairpin structure for the values of induced CD spectra (at 400–470 nm) for complexes tRNA with TOEPyP4 and CuTOEPyP4 there is an optimum concentration of porphyrins at which the anisotropy of system is maximal. For complexes of ZnTOEPyP4 with tRNA the induced CD spectra are essentially different. The induced CD spectra of complex change a sign and continue to grow (remain-

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ing negative) starting from a certain relative concentration. This unusual ICD spectra profile is found for all three porphyrin-tRNA complexes in case of tRNA's reversed 'L' structure. It is possible that at high relative concentration of porphyrins the liquid crystal form may exist in the solution.

The binding constants with tRNA in case of hairpin form are an order of magnitude greater than in case of reversed 'L' structure. It means that this porphyrin interacts stronger with tRNA when it has hairpin form.

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Keywords: tRNA, porphyrins, complexes.

MON-439
The spatiotemporal expression patterns of microRNA in response to high temperature stress in rice

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Global warming coupled with climate change is one of the important limiting factors that affect crop yields throughout the world. MicroRNAs (miRNAs) are a novel class of endogenous, non-coding small RNAs that have been established as ubiquitous regulators of post-transcriptional gene regulation via degradation or translational repression of the cognate mRNA targets. Thus, understanding the miRNAs mediated regulatory schemas for heat stress tolerance is necessary to raise novel crop varieties that can withstand or avoid stresses imposed by changing environment. In the present study, Analysis of NGS datasets (IIlumina, GA) of control and heat stressed c-DNA libraries generated from Flag leaf and Spikelet's of heat tolerant and sensitive indica rice cultivars identified several known and novel miRNAs that displayed a spectrum of response ranging from stable to very variable in nature between tissues and across cultivars. In depth profiling of miRNA expression patterns across tissues under various heat stress treatments also identified specific miRNAs that are highly de-regulated by high-temperatures. To understand the functional implications of miRNAs,their targets were identified. The predicted targets include transcription factors, protein kinases, ATP-binding proteins, HSPs, HSFs, Growth-regulating Factors, oxidoreductases, antioxidants etc. that are linked with various metabolic & cellular processes thereby regulating the stress responses. Future studies of these miRNAs may provide better understanding of the molecular links behind these regulatory networks and may deliver fresh application routes for rice improvement during heat stress.

Keywords: Functional genomics of plant abiotic stresses, Heat Stress, microRNAs.

MON-440
Twister ribozymes: from synthetic biology to in vivo function investigations

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The recent description by Breaker and coworkers (2014) of a new class of small endonucleolytic ribozymes termed Twister opened new avenues into the field of the alternative regulatory functions of RNA and in particular into the field of catalytic RNAs.

One of the most exciting applications of ribozymes is their use in synthetic biology and in particular in the construction of artificial genetic circuits. In particular ribozymes can be used in association with aptamers and riboswitches (generating the so-called aptazymes) to create ligand-controlled genetic circuits.

Here we present a method to design and select new Twister based ligand-dependent riboswitches, to control gene expression *in vivo*, not only in bacteria, but also in eukaryotic model systems. In bacteria, our approach is based on the use of artificial constructs in which the ribozyme moiety acts as a molecular scaffold for the sequestration of the ribosome-binding site (RBS). Aptamer domains are attached to the ribozyme as exchangeable ligand-sensing domains. Addition of ligands to the bacterial growth medium changes the activity of the ligand-dependent self-cleaving ribozyme which in turn switches gene expression on or off. Alternative designs and apatameric moieties binding different ligands were successfully used paving the way to the development of Boolean logic operators at the post-transcriptional level. Besides the important implications for synthetic biology, our data represent the first evidence for Twister ribozyme activity in bacteria *in vivo*.

Moreover, we investigate the possibility of employing our Twister-based aptazymes as ligand-dependent gene expression switches in eukaryotic model systems such as *Saccharomyces cerevisiae*, *Caenorhabditis elagans* and mammalian cell cultures. In these organisms the cleavage of mRNA in either the 5′-end or 3′-end regions is sufficient to cause the degradation of mRNA.

These new findings provide new tools for the study of the *in vivo* function of Twister ribozymes.

Keywords: Bacteria, Riboswitch, Twister ribozyme.

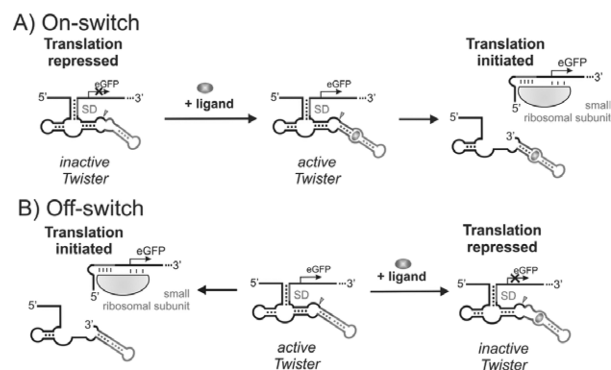

Fig. 1.

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CSIII-06 – Synthetic biology

MON-442
A new type of peptide nucleic acids derived from L-Glu

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Methods for obtaining a new modification of nucleic acids - negatively charged peptide nucleic acids (PNA) – are described. Introduction of charged groups into PNA backbone ensures good solubility of PNA oligomers in aqueous solutions and enables their complexation with cationic transfectants, such as gene-carrying peptides or lipid-like amphiphilic compounds. The synthetic part of our work was aimed at consecutive development of methodology for obtaining all structural components of PNA – pseudopeptides, monomers and some oligomers of alpha- and gamma-series. Hybridization properties of the newly synthesized negatively charged PNA oligomers were studied using UV-spectroscopy (thermal dissociations profiles) and circular dichroism (CD) spectroscopy (Figure 1). Homothymidine g-PNA decamers were shown to form duplexes of increased stability with complementary DNA, while alpha-PNA oligomers of various sequences demonstrated significantly weaker (if any) binding affinity towards DNA. The results of our work suggest that gamma-derivatives are appear generally more promising for constructing DNA mimics with a predetermined structure than alpha-isomers.

This work was supported by RSF [14-25-00013].
Keywords: hybridization, negatively charged pseudopeptides, PNA.

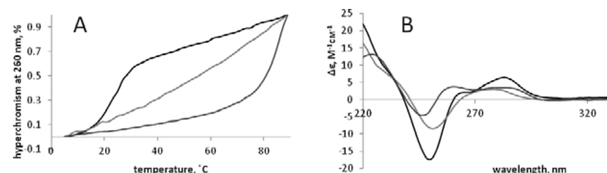

Fig. 1. Physico-chemical properties of homo-T/A duplexes. Black lines refer to DNA/DNA duplexes, green - α-PNA/DNA, red - γ-PNA/DNA. Concentration of each duplex was 5 mkM. Buffer conditions: 10 mM Na_2HPO_4, 140 mM KCl, 5 mM $MgCl_2$, pH 7.8. A: Thermal dissociation profiles. B: CD-spectra. Molar ellipticity is given per one base pair.

MON-443
Allele-specific genome editing and correction of disease-associated phenotypes in rats using the CRISPR/Cas platform

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The bacterial CRISPR/Cas system has proven to be an efficient gene-targeting tool in various organisms. Here, we employed CRISPR/Cas for accurate and efficient genome editing in rats. The synthetic chimeric guide RNAs (gRNAs) discriminated a single nucleotide polymorphism (SNP) difference in rat embryonic fibroblasts, allowing allele-specific genome editing of the dominant phenotype in (F344 × DA)F1 hybrid embryos. Interestingly, the targeted allele, initially assessed by the allele-specific gRNA, was repaired by an interallelic gene conversion between homologous chromosomes. Using single-stranded oligodeoxynucleotides, we recovered three recessive phenotypes: the *albino* phenotype by a SNP exchange; the *non-agouti* phenotype by integration of a 19-bp DNA fragment; and the *hooded* phenotype by eliminating a 7098-bp insertional DNA fragment, evolutionary-derived from an endogenous retrovirus. Successful *in vivo* application of the CRISPR/Cas system confirms its importance as a genetic engineering tool for creating animal models of human diseases and its potential use in gene therapy.

Keywords: None.

MON-444
Antigenic properties of VP1 protein of human polyomaviruses

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The aim of this study was to identify immunodominant epitopes of yeast-expressed major capsid proteins VP1 of different polyomaviruses. Sequence alignment of VP1 proteins of human JC and BK (JCPyV, BKPyV), primate (SV40), hamster (HaPyV) and mouse (MPyV) polyomaviruses revealed homologous sequences within these proteins. Recombinant yeast-expressed VP1 proteins of polyomaviruses have a capacity to self-assemble to virus-like particles (VLPs). Therefore, it is expected that recombinant VLPs may share antigenic properties with native polyomavirus VP1 proteins.

VP1 proteins of JCPyV, BKPyV, SV40, HaPyV and MPyV were expressed in yeast *S.cerevisiae* and purified by density gradient centrifucgation. The antigenic structure of recombinant VP1 proteins was investigated according to their reactivity with 4 monoclonal antibodies (MAbs) raised against recombinant JCPyV VP1 protein. Yeast-expressed JCPyV-VP1 protein was reactive with serum antibodies induced by JCPyV infection. These results demonstrate that the immunodominant epitopes recognized by serum antibodies are exposed in recombinant VLPs thus confirming the antigenic similarity between yeast-expressed and virus-derived JCVPyV-VP1 protein. The reactivities of MAbs raised against yeast-expressed JCPyV-VP1 with recombinant VLPs of JCPyV, BKPyV, SV40, HaPyV and MPyV were investigated by ELISA and Western blot assay. It was determined that two MAbs (clones #8E8, #11C8) are cross-reactive with all tested VP1 proteins except MPyV-VP1 protein. One MAb (clone #8G8) was cross-reactive with all tested VP1 proteins except SV40-VP1 protein. The MAb #2E4 demonstrated a broad cross-reactivity with all tested VP1 proteins. For epitope mapping, deletion mutants of VP1 protein were employed. The MAb epitopes were localized at the C–terminus (aa 295–354) of VP1 protein. In conclusion, the current study enhances knowledge on the antigenic structure of polyomavirus VP1 protein and its immunodominant epitopes.

This work was founded by the European Social Fund under national Integrated Programme Biotechnology and Biopharmacy, grant VP1-3.1-SMM-08-K01-005.
Keywords: polyomavirus, monoclonal antibody, capsid protein.

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MON-445
Application of novel non-viral gene delivery carrier to mammalian cells

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Gene delivery is a promising method to cure various diseases, such as cancer, diabetes, and genetic and autoimmune diseases. The progress of gene delivery is limiting by its inefficiency due to the presence of biological barriers for gene transfer, as well as high toxicity and immunogenicity. Non-viral gene delivery vectors, especially cationic polymers, have attracted much attention in gene therapy.

Novel comb-like carriers, named BG-2, based on dimethyl aminoethyl methacrylate were synthesized at Lviv National Polytechnic University, Ukraine via controlled radical copolymerization using oligoperoxide Cu^{+2} coordinating complexes.

Good plasmid DNA-binding ability of the polymers was determined using agarose gel electrophoresis and zeta potential analysis. The plasmid DNA condensation of the polymers was attributed to their cationic properties, of which zeta potentials were all in the range of 18.3–40.8 mV. The average particle size of the polymer/plasmid DNA complexes was determined at the level of 65.9–95.6 nm. We observed the protection effect of the complexes against DNA degradation by DNase I. It is considered that cytotoxicity of cationic polymers is another important factor of biocompatibility. Low cytotoxicity of carriers was showed using the MTT assay towards 293T, MCF-7 and HeLa cells. Besides, polymers exhibited lower cytotoxicity than that of PEI. We did not found high toxity of the polymers *in vivo*. The carriers demonstrated no mutagenic potencial towards treated cells. It was indicated the low hemolytic activity of studied polymers. The erythrocytes incubated with carriers and PEI were deformed and aggregated only at the effect of high polymers concentration. BG-2 was efficient in crossing cellular barriers due to high lipophilic activity and forming stable and small sized complexes with DNA. Transfection conditions were optimized for plasmid DNA delivery into seven cells lines. BG-2 polymers possessed higher gene delivery efficiency than that of PEI (classical transfection agent). We indicated the increase of p53 and p21 expression in MCF-7 cells using BG-2 carriers.

Novel DMAEM-based polymers are perspective for the use as non-viral gene delivery vectors. They are non-toxic and non-mutagenic. Their application is handy and time saving.

This work was partially supported by the WUBMRC grants.
Keywords: Non-viral gene delivery, polymeric carrier.

MON-446
Biological evaluation of 3-hydroxyflavone in a silver nanoparticles complex

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Applications of nanoparticles are among the most active research areas, mainly in the field of the pharmaceutical and biomedical domains. In this work, biological activity of 3-Hydroxyflavone (3-HF) in a silver nanoparticles complex (SNPs) as well as the contribution of the carrier protein, Bovine Serum Albumin (BSA), to the biological activity of 3-HF, have been investigated. Transmission Electron Microscopy (TEM) analysis showed that the average size of the silver particles is ~ 9 nm, and the core-shell structure of the 3-HF-SNPs has been supported by UV-Vis spectra. The structure, stability, dynamics and conformation of the BSA protein have been investigated by fluorescence, circular dichroism and Atomic Force Spectroscopy (AFM) spectroscopies. Insights on the antioxidant activity of the 3-HF into the BSA – SNPs systems have been investigated using the chemiluminescent system luminol-hydrogen peroxide, in phosphate buffer, pH 7.4. To evaluate the effect of 3-HF, in the BSA-SNPs systems, on the cell viability and on the cell morphology, a mouse fibroblasts cell line, has been used. The results are discussed with relevance to the oxidative stress process.

Keywords: flavones, proteins, silver nanoparticles.

MON-447
Biosynthesis of stable iron nanoparticles in aqueus extracts of *Nicotiana benthamiana* and *Hordeum vulgare*

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Plant extracts contain a potent array of various metabolites, including terpenoids, polyphenols, sugars, alkaloids, phenolic acids, and proteins, which can bioreduce metal ions into nanoparticles. We report the synthesis of amorphous iron nanoparticles from iron salts in aqueous extracts of dicotyledonous (*Nicotiana benthamiana*) and monocotyledonous (*Hordeum vulgare*) plants, and also discuss their characterization using TEM, SAED, DLS, XRD and EELS. *H. vulgare* extracts produced smaller nanoparticles (average diameter of 10 nm) than was observed in *N. benthamiana* (average diameter of 20 nm). Subsequent XPS analysis of the nanoparticles revealed that the binding energies for 2p iron electrons were typical for Fe_3O_4 iron oxide, where iron atoms were contained in combined (II and III) oxidation states. These iron nanoparticles were found to be intrinsically unstable and prone to aggregation: one hour after synthesis the hydrodynamic diameter of the nanoparticles increased by more than 10 times. It was subsequently found that addition of 40 mM citrate buffer pH 3.0 into plant extracts leads to long-term nanoparticle stability. The differences in the sizes of nanoparticles measured using TEM and DLS methods, likely indicates the presence of organic components in their composition. Using AFM analysis, textural differences between the extracts were identified: *H. vulgare* contained smaller nanosized organic aggregates to a higher level than *N. benthamiana*. We suggest that these aggregates might act as nucleation sites, with their different morphologies and numbers likely accounting for the differential modulation of iron nanoparticle formation.

This research was supported by the RFBR grant 14-04-01448 A and Grant of the President of the Russian Federation for supporting of young scientists MK-2072.2014.4
Keywords: Green chemistry, Iron nanoparticles.

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MON-448

Changes in levels of hydrogen peroxide and phenolic compounds in grapevine latent buds during the annual cycle

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The quantitative analysis of phenolic compounds and of hydrogen peroxide (H_2O_2) amounts in the latent buds of the vine (*Vitis vinifiera.* L) showed seasonal variations. These compounds change also as function of the vine development cycle. The total amounts of phenolic compounds are strongly accumulated in the buds in beginning of dormancy phase in mid-August. Never the less, the ending of dormancy phase in mid-November in the buds is associated with increasing of the hydrogen peroxide yield and a considerable drop in the content of the total amount of phenolic compounds. These variations appear to be strictly related to the climatic conditions. This is particularly visible in ending of dormancy phase. This is coinciding with the cold period where the temperature is below 10 ° C for at least 7 consecutive days.

Keywords: dormancy, buds Vitis vinifera, phenolic compounds.

MON-449

Characterization and biocompatibility of synthesized PCL/nHAp bio nanocomposites

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PCL (poly (capro lactone) is a biodegradable polymers that used to both biomedical and industrial applications, Nano hydroxy apatite (nHAp) is an inorganic component that constitutive human skeleton percent 60–70, and based on Ca (calsium) and P (phosphat) components. nHAp is taken advantage of biomedical applications, due to its bioactivity and biyocompatibilty properties. Composite materials consist of two or more different components. Composites, due to they have high strength and low elastic modulus, especially they are preferred for ortopedic applications.

İn our study, we synthesized PCL/nHAp films which used to for biomedical applications or as food packing. PCL was selected to use as a matrix, and nHAp was selected as a filler that used different concentration (1, 2.5 and 5% wt.). PCL/nHAp composites were synthesized as the films by solvent blending method. The films were characterized wit some parameters that are XRD, TG/DTA, FTIR-ATR, TEM, contact angle and uv-visible Spectrofotometer. The films was passed some biological tests like hemocompatibility, antioxidant stress enzyme activities and antimicrobial test. We have detected that physico-chemical properties of PCL were healed to used to as a filler by nHAp. When XRD and FTIR-ATR results have been looked into, all of the synthesized nanocomposites using the different concentration nHAp (%1, %2.5 and %5 wt) were shown an homogen distribution in the matrix (PCL). The optical transmittance spectra of PAA and its bio nanocomposites in the wavelength range from 200 to 700 nm were analysed that blocked uv rays increasing the filler amount. PCL and its bionanocomposites have been detected hemocompatibilty that showing between 0,35 and 1,76% hemolysis. Antioxydant enzyme activities havent been effected treatment with PCL and its bionanocomposites, apart from glutation reductase activity increased a little range.

Keywords: Bio nanocomposite, Biocompatibilty, PCL/nHAp.

MON-450

Codon optimization is a key step for thermostable serine protease expression

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Background: Pernisine is an extracellular thermostable serine protease from hyperthermophilic archeon *Aeropyrum pernix* K1. A lower yield from natural host and expression problems in heterologous host inhibits its characterization and potential application in industry.

Principal findings: Challenges of pernisine overexpression in *Escherichia coli* were overcome by codon preference optimization and DNA synthesis *de novo*. Wild type (pernisine^{wt}) and codon-optimized (pernisine^{co}) were cloned into pMCSGx series of vectors and expressed in *E. coli* cells. Fusion tagged pernisine were purified using fast protein liquid chromatography system equipped with Ni^{2+} chelate and gel filtration chromatography columns. The identity was confirmed with N-terminal sequencing, tandem mass spectrometry analysis and immunodetection. Pernisine^{wt} was not expressed and could not be detected even with immunodetection; meanwhile pernisine^{co} was purified as a proform with a yield of around 10 mg per liter of culture. Recombinant pernisine was heat activated at temperature 90°C for 1 h in buffer 10 mM HEPES pH 8,0 containing 1 mM $CaCl_2$. Proteolytic activity of mature pernisine^{co} was confirmed with zymogramphy at molecular weight 36 kDa. The temperature and the pH optima of the enzymatic activity of the recombinant pernisine, evaluated by azocasein assay, were around 105°C and pH 7, respectively.

Significance: Our findings reveal that codon optimization is crucial for pernisine overexpression in *E. coli*. Recombinant pernisine is activated by autoproteolytical cleavage of its N-terminal proregion consisting of the first 91 aminoacids. Further on, we confirmed that recombinant pernisine retains characteristics of a native one being calcium modulated thermostable serine protease.

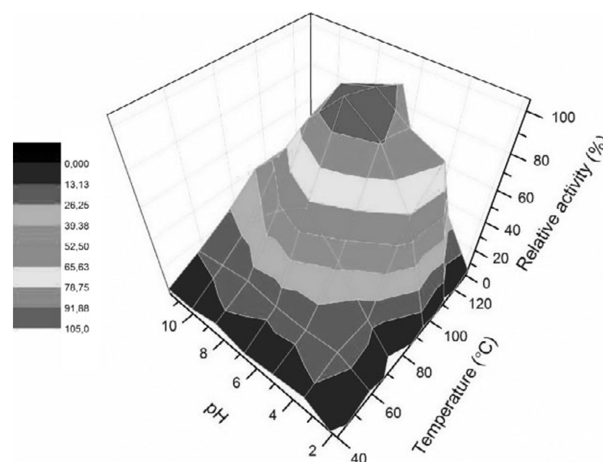

Fig. 1. Recombinant pernisine activity dependence of temperature and pH. Relative activity of recombinant pernisine was incubated at different temperatures from 40–120°C along with six different pH at range from pH 2–12. Average values of pernisine relative activity dependence as a function of temperature and pH are presented in a 3D graph.

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Acknoledgement: This work was founded by the research program P4-0121 (Biochemical and Biophysical-Chemical Characterization of the Natural Compounds), the Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins (CIPKeBIP) and operation research Overproducing Recombinant Pernisine in Bacterial Expression Systems.
Keywords: codon-optimization, pernisine, thermostable.

MON-451
Combinatorial approach for expression level modulation of a multigene pathway: what effects can we really expect?

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Microorganisms are widely used in biotechnologies as microbial factories for the sustainable production of value-added molecules. Most of the chemicals that are actually targeted exist in natural producers, but, unfortunately, in low yield natural producers or even non cultivable organisms. To overcome these limitations, synthetic biology approaches in metabolic engineering aim to design and implement the transposition of full natural or artificial synthetic pathways in heterologous hosts.

It is known that unregulated expression of foreign enzymes can be toxic to the host, in relation with metabolic burden, overconsumption of resources, or accumulation of toxic intermediates. One way to control such imbalances is to regulate separately the expression level of each metabolic step within the biosynthetic pathway. This goal can be achieved at the transcriptional level by varying promoter's strength in front of each gene.

Among the panel of available microorganisms for metabolic engineering, *Saccharomyces cerevisiae* is widely used (Xu et al., 2012). In this host, a list of constitutive promoters is available, associated with their assumed strength in their natural configuration. A previously published study investigated the differences between some of these promoters by associating them with the Green Fluorescent Protein encoding gene and then measuring the produced fluorescence (Lee et al., 2012).

In a context of metabolic engineering, we are wondering if we can expect an expression level that would reflect the strength of the considered promoter, regardless of the open reading frame put immediately below.

To answer that question, we performed a thorough analysis at the transcriptional, translational and metabolic levels, of the real strength of three promoters supposedly different. We worked in the context of heterologous expression of three consecutive steps of the zeaxanthin biosynthesis pathway. The three genes involved were put under the control of a set of three constitutive promoters of *Saccharomyces cerevisiae,* assembled in a combinatorial manner. The resulting 14 strains are currently examined by RT-qPCR and by quantifying the generated products.

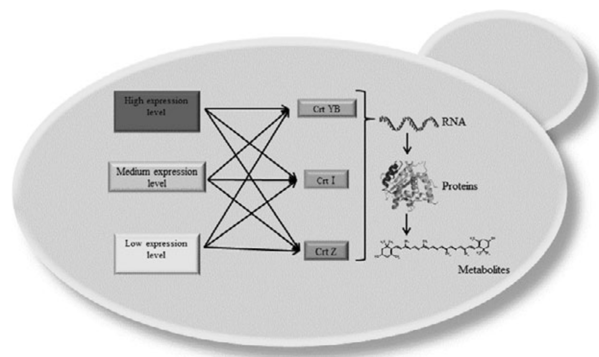

Fig. 1.

Keywords: metabolic adaptation, metabolic engineering, systems biology.

MON-452
Comparative physicochemical studies of human albumin monomer and the cross-linked dimer

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Human serum albumin (HSA) is one of the most important blood protein playing various essential roles. It regulates the osmotic pressure and effectively transports a variety of ligands such as drugs, vitamins, fatty acids, ions due to its specific binding properties. HSA is also used clinically as a drug especially as plasma expander in patients with hypoalbuminemia and can also function as a drug carrier. In order to avoid the risk of transmitting possible allergenic contaminants, recombinant albumin monomeric form (rHSA) is more often used. However, rHSA is readily eliminated from the blood circulation. It is postulated in the literature that cross-linked with 1,6-bis(maleimido)hexane (BMH) HSA dimer shows a longer blood circulation than rHSA monomer and is suited as a drug carrier. Therefore, the main goal of this work is a thorough physicochemical characteristics and binding properties of rHSA albumin monomer in comparison to the synthetic BMH dimeric form. In the first step synthesis of BMH dimer is carried out according to the method described in literature. The purity and stability of dimer is determined by the SDS-PAGE electrophoresis. Subsequently, the electrophoretic mobility and the hydrodynamic diameter of both albumins were determined as a function of pH and ionic strength using the micro-electrophoresis and DLS methods. The obtained results indicate that the hydrodynamic diameter for rHSA monomer and HSA dimer (in 0.15 M NaCl pH 7.4) are 8.0 nm and 11.00 nm, respectively. Next, monolayers of rHSA and BMH dimer are prepared on negatively charged mica surface and characterized using the *in situ* streaming potential method. Protein adsorption is carried out for the range of ionic strength 10^{-2} to 0.15 M NaCl at pH 3.5. It is determined that maximum coverage of albumins increases as a function of ionic strength and reaches 1.3 mg m^{-2} in 0.15M NaCl solution. The study of desorption process confirmed the stability of monolayers in various conditions of pH and ionic strength. The obtained stable albumin monolayers can be used to perform extensive studies of ligand bindings using the precise electrokinetic methods.

Acknowledgements: This work was supported by grant: POIG.01.01.02-12-028/09 and PRELUDIUM 2012/07/N/ST5/02219.
Keywords: protein adsorption, cross-linked albumin dimer, recombinant albumin monomer.

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MON-453
Comparative study between mammal and plant GPI modification mechanism

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GPI (glycosylphosphatidylinositol) is a kind of glycolipid which can anchor proteins to the cell membrane. GPI-anchored proteins (GPI-AP) are known to localize themselves to the microdomain on the cell membrane, called raft, via the endoplasmic reticulum (ER). Human GPI-APs are closely related to incurable human disorders including cancer, Parkinson's disease and BSE (bovine spongiform encephalopathy). Plant proteins which have similar GPI modification systems as mammal GPI-APs, can translocate to the cell wall or the raft on the cell membrane. However, because few plant proteins have been isolated as GPI-APs, the GPI modification mechanism is not clear. CEBiP (Chitin Elicitor Binging Protein), one example of a plant GPI-AP, is known to exist in monocotyledons and dicotyledons as well as other types of plants. In order to protect themselves, plants produce a significant amount of CEBiP which spurs a chitin-oligosaccharides interaction.

Based on this information, plant GPI modification was compared to that of human modification to clarify the plant GPI modification mechanism in this study. The CEBiP sequence was first inserted into a human cell expression vector, pCMV-script. HeLa cells then underwent transfection when the vector entered the cell, this causing the CEBiP gene to be expressed in the HeLa cells. The CEBiP protein could then be observed by confocal laser microscope after undergoing the immunostaining method to make the protein fluorescent. By doing this, the subcellular localization of CEBiP could be evaluated.

Keywords: CEBiP, GPI-AP.

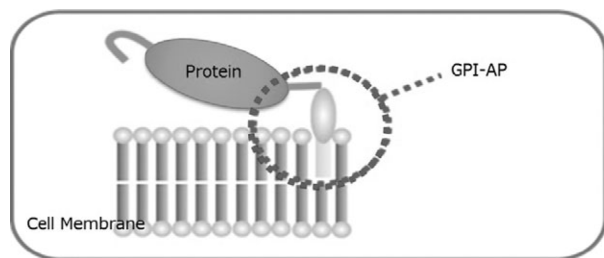

Fig. 1.

MON-454
Computational identification of cis-elements in 5′ regulatory regions of genes associated with carbohydrate metabolism-related events

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Cis-acting regulatory elements are important molecular switches involved in the temporal and spatial expression of a dynamic network of gene activities. This network controls hormone responses, abiotic stress responses and multiple developmental events. In this analysis, a particular emphasis was placed on *cis*-acting regulatory elements present within the 5′ regulatory region of sucrose synthase (SuSy), cell wall invertase (CWI) and sucrose transporter (SUT) gene families in *Arabidopsis thaliana* and *Oryza sativa*. The potential *cis*-acting regulatory elements were predicted by scanning 1.5 kbp of 5′ regulatory regions of the SUT, CWI and SuSy genes translational start sites, using various resources for *cis*-element bioinformatics. *Cis*-elements associated with phytohormone responsiveness, light responsiveness, elicitor responsiveness and abiotic stress were predicted in varying frequencies within the 1.5 kbp of 5′ regulatory region. In addition, *cis*-elements involved in sugar repression, mineral responses, and cold- and light-inducible gene expression were also identified. Some of the predicted *cis*-elements have experimental precedent, but many are novel and encourage further exploration. This analysis provides a basis for elucidating transcription regulatory interactions of SUT, CWI and SuSy gene families in regulation of developmental phase transitions under abiotic stress conditions.

Keywords: carbohydrate metabolism, cis-elements, Gene expression regulation and development.

MON-455
Construction of a prophage-free, hybrid-genome *E. coli* BL21 host strain by directed genome shuffling

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E. coli BL21(DE3) is a widely used host strain for recombinant protein production. While being a superior protein producer, the strain is poorly accessible to genetic engineering (poor transformability by plasmids, unpredictable recombination events, propensity for lysis). On the other hand, K-12 strains are easy to manipulate genetically. Moreover, genome streamlining and elimination of mobile genetic elements, as well as genes involved in generation of mutations were shown to yield further improvements in the applicability of the K-12 host cell. We set out to combine the favorable traits of BL21 and K-12. Taking advantage of the 'clean'-genome (mobile genetic element free, reduced-genome) K-12 derivative MDS42, a BL21 strain-based hybrid-genome E. coli was constructed.

K-12 and BL21 have similar core genomes (99% core sequence identity with notable variations), but possess significantly diverged, laterally transferred genomic islands, including prophages and other mobile genetic elements. Marked segments of 'clean'-genome K-12 MDS42 were sequentially transferred to BL21 by P1 transduction to replace regions occupied by prophages. To further improve engineering, the genomic region responsible for host restriction and modification was removed. To ensure that the resulting cell lines possess the desired characteristics, after each genome re-arranging step a recombinant strain pool was screened for efficient protein-expression and for preserving good growth profiles. The T7 polymerase-expressing construct (important for recombinant protein production) of the original BL21(DE3) strain was replaced by a modified, tightly controlled lac-T7 polymerase system. Altogether, 9 regions of the BL genome were replaced by the corresponding K-12 MDS42 sequence, and all prophages were eliminated from the genome. The resulting, BL21-based strain harbors an estimated 270 kb K-12 sequence, retains the excellent protein producing features, displaying unaltered stress tolerance and increased genomic stability.

Keywords: *E. coli* genetic hybrids, Genome shuffling, Synthetic Biology.

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MON-456
CS2 hydrolase, a rare example of a biological catenane

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Catenanes, structures in which two rings are mechanically interlocked, have been studied widely by the chemical community. Contrary to synthetic catenanes, however, biological catenanes have rarely been observed. Recently the CS_2 hydrolase protein from the thermophilic archaeon *Acidianus*, has been proven to be such a biological catenane.

Because CS2 hydrolase is perhaps one of the few biological catenanes, we are interested in understanding the effect of the quaternary catenane structure of this protein. By employing FFF-MALLS and native mass spectrometry, we have shown that the catenane form can disassemble into its ring structure, whereas the ring does not assemble into the catenane structure under the same conditions. Also, using enzyme kinetics studies, we found that the quaternary protein structure affects its enzymatic activity.

Keywords: catenane, CS2 Hydrolase, enzyme kinetics.

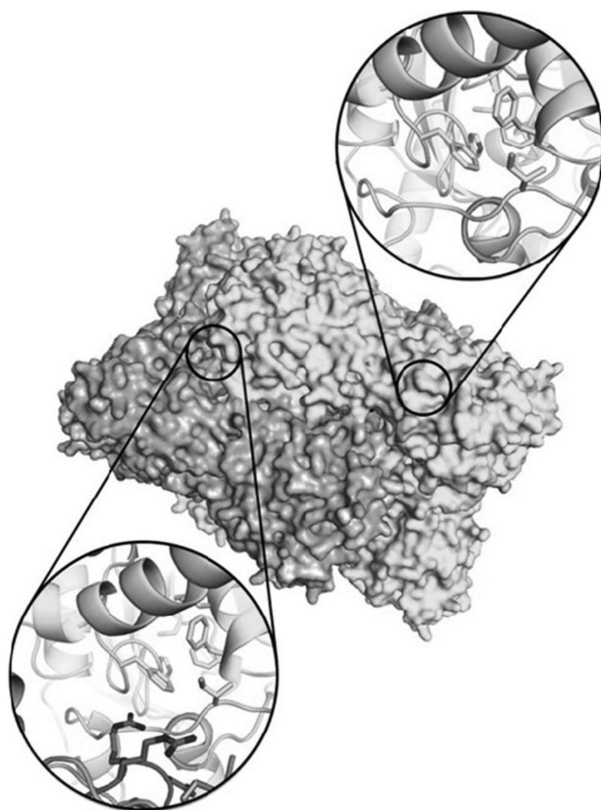

Fig. 1.

MON-457
Cytotoxic enhancement of a bispecific diabody with specificity for EGFR and CD3 by rearranging a domain order

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Bispecific antibodies can be applied to cancer immunotherapy by cross-linking of tumor cells with various immune cells such as T lymphocytes. Recombinant technologies can be used to generate smaller bispecific antibodies, called bispecific diabodies, consisting of only variable domains from two different antibodies. The compact structure of diabodies contributes to low immunogenicity, high tumor-penetration, and the potential for large scale preparation through bacterial expression systems.

We previously reported the marked antitumor effects of a humanized bispecific diabody that targets EGFR and CD3 (hEx3-HL). The domains of bispecific diabodies can be ordered in four different ways and several previous reports have suggested that the order of the variable domain might affect the function of bispecific diabodies; however, only one study has systematically assessed this feature. Here, we rearranged the domains of hEx3-HL to examine the influence of domain order on the function of bispecific diabodies. We successfully prepared homogenous dimers of hEx3 in all four domain configurations. Interestingly, all three rearranged hEx3s inhibited cancer growth more effectively than did the original hEx3-HL, in which both components were in VH-VL order, and the highest effects were observed with hEx3-LH, in which both components were in VL-VH order. In addition, hEx3-LH had comparable *in vitro* growth inhibitory effects to those of the tandem scFv format of hEx3 (hEx3-taFv), which we previously showed to have greater cytotoxicity than does hEx3-HL. Flow cytometry suggested that the enhanced cytotoxicity of hEx3-LH is attributable to structural superiority for cross-linking, similar to that of hEx3-taFv. Furthermore, hEx3-LH inhibited cancer growth in mice more effectively than did hEx3-taFv; this difference may be due to differences in antibody stability. Our results show that merely rearranging the domain order of bispecific diabodies can enhance their effects beyond those with structural format conversion.

Keywords: Bispecific diabody, Cancer immunotherapy, Effective domain order.

MON-458
Design of a remodeling acto-myosin network driving membrane dynamics

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The major aim of our work is to understand the mechanisms behind dynamic organization of the cellular plasma membrane, especially local heterogeneities such as nanometer sized lipid domains (Mayor and Rao, 2004). As reported previously, glycosyl-phosphatidylinositol-anchored protein (GPI-AP) organization in nano-clusters in the plasma membrane is driven by the activity of cortical actin (Goswami *et al*., 2008). A recent theoretical framework proposed by Gowrishankar *et al*. (2012) suggests that the engagement of short actin filaments together with myosin-

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motor like activity at the cytoplasmic leaflet is sufficient to explain all the unusual features of GPI-AP organization.

Here, we present a strategy to reconstitute cortical actin dynamics *in vitro* on supported lipid bilayers. This allows us to explore the role of proteins thought to be involved in actin cluster formation and to test predictions of the theoretical model. In a first step, we investigate how the diffusion of membrane bound actin binding proteins is affected by actin filaments of varying lengths. Then, we increase the complexity of the system by including myosin motors and capping protein, and identify conditions under which actin remodeling, i.e. transient formation of actin asters, occurs. As suggested by observation in cells and by the theoretical framework, short actin filaments (<1 μm) are the main source of fast remodeling events whereas longer filaments create a more static meshwork, which can confine membrane bound particles. In summary, we introduce a new kind of minimal actin cortex, and show how dynamic short actin can drive the organization of plasma membrane particles.

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Keywords: actomyosin contractility, in vitro, nano-clusters.

MON-459
Development of a new protocol for topology detection using NHS cross-linking approach

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Chemical crosslinking in combination with mass spectrometry has been an emerging strategy to study protein-protein interactions. Using microsomes from *Saccharomyces cerevisiae,* we are developping a high throughput method based on crosslinkers to define protein topology. As a first approach, we used the thiol-cleavable, membrane impermeable and lysine-specific crosslinker, 3,3-dithiobis (sulfosuccinimidylpropionate DTSSP) to define the topology of control proteins. In this manner, we have been using the luminal protein Kar2p from BY4742 microsomes. We observed, with this method, a derivatization of Kar2p without any detergent. These results led us to hypothesize that DTSSP could disturb membrane stability due to the derivatization of Phosphaditylserine (PS) and/or Phosphatidylethanolamine (PE). To test this hypothesis, we used the *dpl1Δcho1Δ* double mutant strain affected in the biosynthesis of PE. Using the same method, we observed under optimal growth conditions, a decrease of derivatization of the protein Kar2p from *dpl1Dcho1D* microsomes compare to the WT BY4742. These results suggest a possible interaction between DTSSP and the phospholipids present in the microsomes, which could destabilize and increase membrane permeability.

Keywords: DTSSP, microsomes, topology, PE, PS, Kar2p, dpl1Δcho1Δ.

MON-460
Development of targeted delivery ways for antitumor peptide lactaptin

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Apoptosis is a critical defense mechanism against the formation and progression of cancer. Proteins inducing apoptosis in cancer cells are considered as potential anticancer pharmacological agents. Lactaptin, the proteolytic fragment of human milk kappa-casein, induces apoptosis of various cultured cancer cells. Recombinant analog of lactaptin RL2 was constructed to be expressed in *E. coli*. The anti-tumor potential of RL2 was investigated *in vivo* and *in vitro* [1, 2].

A major problem in systemic therapy is that only a small proportion of administered drug reaches its intended target site. Selective drug delivery to the target tissue or organ could alleviate this problem. Targeted delivery of RL2 will reduce the potential cytotoxic effect on normal cells, diminish the therapeutic dose and enhance antitumor efficacy by increasing the local concentration of the drug within the tumor.

We have chosen peptides as the molecular targeting agents. Targeting peptides display high tissue penetration due to small size, low immunogenicity, high affinity to targets, acceptable stability and ability to be easily conjugated with other agents. To obtain tumor specific peptides we applied a selection method using phage peptide libraries (Ph.D.™- Phage Display Peptide Library Kit, New England Biolabs). Two displayed peptides GLHTSATNLYLH and SGVYKVAYDWQH were selected to mice hepatocarcinoma A-1 (HA-1) *in vivo*. The same phage clones appeared to possess the highest affinity to ascites tumor cells of hepatocarcinoma A-1 and Lung adenocarcinoma *in vitro*. The selected phage clones were studied for the ability to bind other types of tumors *in vivo*. It was also shown the affinity of the selected peptides for Lung adenocarcinoma (A/Sn mice), Melanoma B16 (C57 Black mice), Krebs-2 adenocarcinoma (C57 Black mice) and Lewis lung carcinoma (CBA mice) *in vivo*.

We constructed several plasmid vectors producing fusion proteins united targeted peptides and lactaptin. Here we investigated cytotoxic and anti-tumor effects of targeted protein lactaptin *in vitro* and *in vivo*.

The work was supported by RFBR grant No 13-04-01313-a.

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2. Koval O.A. et al. A novel pro-apoptotic effector lactaptin inhibits tumor growth in mice models // Biochimie, 2012 V.94 (12), p. 2467–2474.

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Keywords: apoptosis, Drug Delivery, targeting peptides.

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MON-461
Differential in vitro inhibition studies of some cerium vanadate derivatives on Xanthine oxidase

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In this preliminary study a new series of some cerium vanadate derivatives have been investigated as new type of inhibitors of Xanthine oxidase (E.C 1.17.3.2, XO). Xanthine oxidase is a superoxide-producing enzyme found normally in serum and the lungs and its activity is concerned with several important health problems such as gout, severe liver damage, vascular dysfunction and injury, oxidative eye injury,and renal failure. Here we present a critical overview of the effects of these novel types agents on XO with comparing the efficacy and safety profiles of allopurinol, the efficient classical inhibitor of XO.

Keywords: Cerium vanadate derivatives, Enzyme inhibition, Xanthine oxidase.

MON-462
Direct tentative identification and characterization of phenolic compounds from crude extracts of buds and internodes in grapevine (*Vitis vinifera* L. Merlot)

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In this study, the crude methanol extracts of latent buds and internodes *Vitis vinifera* L. cv. Merlot were used for the determination of phenolic compounds by a combination of reverse phase HPLC with diode array detection (HPLC-DAD) and mass spectrometry (LC-MS). This method allowed the identification of nine phenolic compounds without purification or fractionation. These nine compounds were divided into three groups: procyanidins, flavonols and stilbenes. Detection by HPLC-DAD at different wavelength of 280 nm to 320 nm allowed the estimation of concentrations of those compounds. This method allowed for the first time both characterization and quantification of polyphenolic compounds in buds of grapevine. The comparison with the results obtained in internodes showed that quercetin, resveratrol tetramer and ε-viniferin are present in similar levels in buds and internodes while six other compounds identified were in higher levels in buds.

Keywords: mass spectrometry LC-MS, Phenolic compounds, Vitis vinifera.

MON-463
Effect of heterologous pre-pro leader sequence on the expression of the human factor IX in cultured mammalian cells

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Hemophilia B is an X -linked recessive bleeding disorder caused by deficiency or malfunctioning of coagulation factor IX (FIX), a vitamin K dependent (VKD) g-carboxylated glycoprotein. Production of recombinant human FIX (rhFIX) in mammalian cells is usually encountered with low secretion efficiency and incomplete g-carboxylation. To improve the rhFIX expression, we replaced the hFIX leader (prepro-)peptide, with those of the human and porcine *Prothrombins* (pProt and hProt, respectively), which are VKD and γ-carboxylated efficiently, to be examined in a human cell line, in transient state. *In silico* analysis of the examined prepeptides predicted their respective secretion potentials as: pProt >hProt>hFIX. Besides, lower minimum free energies of the resulted transcripts of the chimera transgenes suggested for their higher translation rates in comparison with that of the native *hFIX*. In consistent with our *in silico* predictions, improvement of secretion of FIX by the transgenes equipped with either of the pProt and hProt prepro was shown in the human cell line. Biological activities of the expressed rhFIXs indicated in occurrence of γ-carboxylation for the three examined mini-genes products. However, the propeptides of the pProt and hProt appeared more appropriate substrates for the human γ-carboxylase, probably due to their greater turnover. These results confirmed that the total hydrophobicity, rather than the length of the hydrophobic region of a signal peptide is crucial for its secretion efficiency. This study demonstrated the incorporation of a heterologous leader peptide coding region in both transcription and post-transcriptional efficiencies of the linked gene and displayed the feasibility of a leader-peptide replacement approach for the improvement of the expression of the gene of interest.

Keywords: human and porcine Prothrombin, Human coagulation factor IX, Prepro-leader peptide.

MON-464
Efficient strategy for the selection of DNA aptamers specific to recombinant proteins

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We have developed highly efficient technique for selection of aptamers binding recombinant proteins. The recombinant protein of interest is fused to the affinity tag separated from the protein by recognition peptide for a site-specific protease. The fusion protein is produced, purified by affinity chromatography, and immobilized on the solid support (paramagnetic beads, chromatographic media, immunoplates etc.) containing the tag-binding moiety. Beads are further incubated with random DNA aptamer library to afford interaction of aptamers with immobilized target. After extensive washing, the target protein with captured DNA aptamers is cleaved off the support using site-specific protease. Protease-eluted DNAs are further amplified by PCR and proceeded to the next panning round.

Presumably, any affinity tag and any expression system as well as any site-specific protease can be used in this selection strategy. In the present work, the in vivo biotinylated peptide and His tag were used with equal success. SUMO and anthrax lethal factor tested as site-specific protease also showed excellent performance in specific aptamer elution.

Using the described technique, we selected high affinity DNA aptamers to *C. botulinum* neurotoxin light chain and *B. anthracis* lethal factor. The developed strategy helps to reduce the quantity of selection rounds and achieve obtaining of high-affinity aptamers within 2–5 selection cycles while avoiding co-selection of aptamers specific to the solid phase. The work is supported by research grant 14-15-00630 from Russian Science Foundation.

Keywords: aptamer, recombinant protein, selection.

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MON-465
Elucidation of the molecular mechanism of GPI transamidase

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Many proteins anchored to the plasma membrane by Glycosylphosphatidyl-inositol (GPI) exist on the eukaryotic cell surface. The protein modified by the GPI transfer enzyme, GPI transamidase, is called the GPI-anchored protein (GPI-AP). In the endoplasmic reticulum (ER), GPI transamidase recognizes the GPI-attachment signal sequence and then modifies GPI to the ω-site of the protein. The GPI-attachment signal sequence is then separated by GPI transamidase. GPI transamidase is known as a protein complex consisting of GPI8, GAA1, PIG-T, PIG-S and PIG-U. In order to predict GPI-APs, ω-site prediction tools have recently been developed. However these methods have not been successful in clarifying these factors. Therefore, the mechanisms related to the recognition and digestion of the GPI-attachment signal by GPI transamidase were focused on in this study.

The sequence dataset of GPI-AP was extracted from the UniProt KB/Swiss-Prot Release 2014_01. The amino acid propensities around the ω-site and the hydrophobic region of the GPI-attachment signal sequence were calculated. As a result, a large number of serine and threonine were confirmed downstream from the ω-site. Glycine, proline, serine and threonine were also confirmed in the hydrophobic region in the GPI-attachment signal sequences. Based on these facts, the phosphorylation of these residues is thought to be a trigger of the tertiary structural change of GPI-AP. The fact that many small residues were found in the hydrophobic region of the GPI-attachment signal sequence suggests that the polypeptides in these regions form disordered secondary structures in order to interact with GPI transamidase.

Keywords: Bioinformatics, GPI-AP, transamidase.

MON-467
Enzyme linked immuno mass spectrometric assay (ELIMSA)

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Enzyme Linked Immuno Mass Spectral Assay (ELIMSA) may detect and quantify the binding of molecular probes such as ligands and receptors to zeptomole amounts using only common reagents and equipment that may be of great significance to basic biology and medical diagnosis. ELIMSA combines Enzyme-linked immunosorbent assay (ELISA) using a substrate that yields ionizable products that may be measured by liquid chromatography-electrospray ionization and mass spectrometry (LC-ESI-MS) with a high signal to noise ratio to create the transformative new technology. We favourably compared the enzyme alkaline phosphatase (AP) and horseradish peroxidase (HRP) for detection by LC-ESI-MS/MS. Here we show the substrates adenosine monophosphate (AMP) or pyridoxamine-5-phosphate (PA5P) are converted by AP-SA conjugate to adenosine (A) and pyridoxamine (PA) respectively that are resolved well in isocratic LC and ionized very efficiently in an electrospray and showing a linear intensity relationship with AP-SA concentration after log transformation. The A and PA reporter products were observed to show a linear relationship between log ion intensity and quantity to as low as femtomole amounts. Here the enzyme AP bonded to Streptavidin (AP-SA) catalyzed the release of A and PA that showed a direct and linear intensity relationship with AP-SA concentration after simple log transformation as measured by MS. Internal ^{13}C adenosine (A) or pyridoxamine (PA) isotope dilution curves, internal ^{13}C isotope one point calibration, external A or PA dilution curves and external ^{13}C A or ^{13}C PA curves showed close agreement on the linear quantification of the LC-ESI-MS/MS assay. Thus, MS can be used to measure an ELISA that produces A or PA against external protein standards with great sensitivity and accuracy. We applied the system to measure PSA to 1 picogram by ELIMSA with linear and normal results, high sensitivity and high signal to noise ratios that provides a universal system for quantification at vanishingly low concentrations. ELIMSA with the products A or PA was flexible for measuring multiple ions, log linear and quantitative from picomole to zeptomole amounts of the biotinylated probe or prostate specific antigen (PSA) analyzed. The PSA ELIMSA efficiently detected and quantified PSA clearly quantifying samples with <10 pg of PSA that was below the detection limit of the colorimetric or ECL methods. In practical terms ELIMSA showed that all normal patient PSA levels are now within the range of common quantification. ELIMSA allows the direct quantification of enzyme labelled probes to 1 femto gram i.e. ~ 1zeptomole amounts by LC-MS. ELIMSA permitted the routine analysis of quantifying receptor-ligand complexes of biological molecules to attomole amounts or less.

Keywords: ELIMSA, ELISA, mass spectrometry.

MON-468
Evolutionary computation to understand signaling and TFTG network in Leishmania: a mechanistic perspective

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Mathematical modelling of biological processes helps unravel the dynamics associated with transcriptional-translational machinery and the signaling network reconstructed. Perturbation of any biological process through internal or external agents can lead to disease etiology. Leishmaniasis is a protozoan disease that perturbs the immune signalling response eventually affecting the gene regulatory network (GRN) in macrophages that results in an anti-inflammatory phenotype. Through systems biology approach, we are trying to develop a robust GRN that will maintain stable phenotypes under varying conditions. To this end, it is important to understand the inherent stochasticity associated with the network, which can be lowered by introducing a negative feedback loop acting on the system through allosteric changes. The negative feedback loop can also reduce the response time delay that can assist in tuning the system to a desired behaviour. The mechanistics of allosteric changes in gene regulation can help understand and predict the behaviour of the transcriptional control machinery. A transcription factor – target gene (TFTG) network with a negative feedback loop was built and evolutionary trade-offs of the network was mapped. Further insights were also laid down in terms of evolutionary computation of signaling network of Leishmania through genetic algorithm. The resultant morphospace of the infected macrophage may help to decide the tuning factors responsible for changing an anti-inflammatory phenotype to a pro-inflammatory phenotype and there off leading to homeostasis for a disease resolving effect.

Keywords: Leishmaniasis, Mathematical modeling, morphospace.

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MON-469
Evolving highly red shifted congener protein by expanding the genetic code

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Fluorescent proteins are important tools in biotechnology and cellular biology studies. It has been well reported that incorporation of natural and unnatural amino acids into the chromophore residues has shown a variety of spectral variations, but it still failed to offer red fluorescent variant from the wild type Green fluorescent protein. Here, we will introduce the donor-acceptor system into GFP for the generation of new large stock shift red variant. The presence of amino donor in the chromophore and its surrounding region in the stockGFP, it will also acquired the ability to bind selectively and sensitively with metal ions, and resulted in the generation of novel protein-based biosensor. Since, the transition metal ions are plays a crucial role as a metal cofactor in oxidative scavenging mechanisms in human body. Developing novel stockGFP will be an important toolkit to generate novel classes of tailor-made GFP variants, which is not possible through the canonical amino acid modification. This tailor-made stockGFP opened a new door for multiple cell imaging and creation of new protein-based biosensor for metal ion quantification in biological science.

Keywords: green fluorescent protein, expanding the genetic code, non canonical aminoacid.

MON-470
Expression, purification and characterisation of glycosylated influenza H5N1 hemagglutinin produced in yeast.

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Hemagglutinin (HA) plays crucial role in virus infection. HA glycoprotein is synthesized as an inactive HA0 molecule assembled as noncovalently bound homotrimers on the viral surface. This precursor protein is cleaved by trypsin-like proteases to create two subunits, HA1 and HA2 which are linked by a single disulfide bond [1]. After cleavage, at the specific site, these two disulfide-bonded protein domains produce the mature form of the HA protein. The A/swan/Poland/305-135V08/2006 (H5N1- subtype) hemagglutinin gene was cloned and expressed in yeast *Pichia pastoris* (*P. pastoris*). The HA cDNA lacking the C-terminal transmembrane anchor-coding sequence was fused to α-factor leader peptide and placed under control of the methanol-inducible *P. pastoris alcohol oxidase 1* (*AOX1*) promoter. Two *P. pastoris* strains: SMD 1168 and KM 71 were used for protein expression. Recombinant HA protein was secreted into the culture medium reaching the approximately 15 mg/L. The fusion protein with His6 tag was purified to homogeneity in one step affinity chromatography. SDS-Page and MS/MS analysis indicated that the protein is cleaved into HA1 and HA2 domains but linked with disulphide bond. Analysis of the N-linked glycans revealed that the overexpressed HA is fully glycosylated at the same sites as the native HA in the vaccine strain.

1. Skehel JJ, Wiley DC (2000) Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin. Annu. *Rev. Biochem* **69**:531–569

Keywords: Avian influenza, Pichia pastoris, Recombinant hemagglutinin.

MON-471
Folate-targeted cationic liposomes for the efficient delivery of nucleic acids into tumor cells

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Gene therapy is a promising approach for the treatment of cancer *via* the administration of therapeutic nucleic acids (anti-sense oligonucleotides, plasmid DNA, siRNA). Cationic liposomes are widely used as nucleic acid delivery systems due to their ability to protect their cargo against serum nuclease degradation and to efficient transfer nucleic acids through the plasmic membrane. Early we have demonstrated that liposomes composed of spermine-based cationic amphiphiles and DOPE provided the successful delivery of FITC-labeled oligonucleotide, plasmid DNA and siRNA into HEK293 cells with the efficiency significantly higher than that of Lipofectamine 2000. It is known that the introduction of a tumor cell-selective ligand in liposomal formulations can enhance their transfection activity. Folate receptor is overexpressed on the surface of several human cancer cells (ovarian carcinoma, endometrial cancer etc.), therefore, folic acid can be used as a targeting ligand for the improvement of the efficacy of nucleic acid delivery systems.

We have synthesized folate-containing lipoconjugates differed with a length and a nature of the spacer group to study the influence of this structure element on the nucleic acid delivery efficiency. Using folate lipoconjugates targeted cationic liposomes were formulated and their biological activity namely the cytotoxicity and the transfection efficiency was estimated. Targeted cationic liposomes were established to be non-toxic to the different types of eukaryotic cells. To determine the optimal conditions for the targeted delivery folate receptor-bearing cells (KB-3-1 and HEK293 cells) and cells without folate receptors (A549 and HGF cells) were used. Small size slightly positively charged nucleic acid/liposomes complexes were found to penetrate into cells *via* receptor-mediated endocytosis. Besides the presence of lipoconjugate with long polyethylenglycol spacer in these complexes provided the best *in vitro* targeted delivery. Cationic liposomes containing this lipoconjugate were selected as promising candidates for further *in vivo* transfection studies.

This work was supported by Russian Foundation for Basic Research, research project No.13-04-40183 comfi.
Keywords: Folic acid, gene delivery, liposomes.

MON-472
Folding kinetics of outer surface protein A

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Outer surface protein A (OspA) from *Borrelia burgdorferi* has been an excellent model system for studying b-sheet folding and peptide self-assembly. OspA has an unusual dumbbell shape composed of sequential 21 antiparallel β-strands followed by a single α-helix at the C terminus. In the middle of the molecule is a single-layer β-sheet (SLB) segment. Both faces of this SLB segment are exposed to the solvent, and consequently the segment is not associated with a hydrophobic core. Previous equilibrium unfolding studies showed that OspA unfolding follows the three-state mechanism among fully folded (N), C-terminus unfolded (I), and fully unfolded (U) states. Here, we report the folding kinetics of

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OspA studied by a rapid mixing technique. We found that there is a lag phase during the formation of the N state detected by tryptophan fluorescence change. The presence of the lag phase provides strong evidence for the formation of an on-pathway intermediate and we conclude that the kinetic folding of OspA also follows the three-state mechanism with the productive intermediate. The identity of the folding intermediate and the equilibrium intermediate is currently under investigation. We also prepared the N-terminal and the C-terminal domains separately and evaluated their folding properties.

Keywords: None.

MON-473
Generation of extremely sensitive and selective scFv antibodies to protein C

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Introduction: Protein C is the key effector protease of the natural protein C anticoagulation pathway. A lack of protein C lead to excess clotting in veins. Therefore, protein C level measure in plasma is required. For this purpose anti-protein C antibodies are used. However, generation of specific antibodies is complicated since protein C is high homologic with a lot of human plasma proteins. The aim of this study was to select protein C fragment which has low homology to other plasma proteins and obtain antibodies to this fragment.
Methods: Protein C fragment selection was made using bioinformatic databases, tools and algorithms. Peptide that mimic a protein C sequence was synthesized by manual solid-phase method using Fmoc strategy. Monoclonal single chain variable fragment (scFv) antibodies were generated by phage display. Obtained antibodies were tested in ELISA and Western bloting.
Results: Pro^{146}-Leu^{157} protein C fragment was selected because of its capability to form an unique protein C epitope and its high immunogenicity. The scFv library of mouse immunized with Pro^{146}-Leu^{157} peptide was constructed and the protein C specific scFv were selected from the constracted library. The results showed that obtained recombinant scFv antibodies could specific bind both Pro^{146}-Leu^{157} protein C fragment and activated protein C. It was shown that scFv were suitable for protein C detection by ELISA and Western bloting. ScFv bound target antigen in Western bloting under non-reducing conditions. The scFv antibodies dissociation constant was found to be $2x10^{-9}$ M.
Conclusion: Obtained scFv antibodies could be further used as a tool for protein C investigation and for the development of diagnostic system.

Keywords: Protein C, scFv.

MON-474
Genome wide analysis of SF1 and SF2 helicases in archaea

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Helicases represent one of the largest classes of enzymes found in all prokaryotic, eukaryotic organisms and many viruses. These enzymes unwind structured nucleic acids (DNA, RNA) in Adenosine Triphosphates (ATP)-dependent manner and are further grouped into six superfamilies based on their structural motifs and consensus sequences. Superfamily helicases 1 and 2 (SF1 and SF2) play an important role in almost every process of DNA and/or RNA metabolism. All these helicases share a central helicase core domain with characteristic motifs responsible for their ATPase and unwinding activity. Using extensive bioinformatics analysis, we have surveyed the presence of SF1 and SF2 helicase superfamilies in the archaeal genomes. We have identified the sets of SF1 and SF2 helicases in archaea. Our study validated two SF1 family and 14 SF2 families among archaeal sequence genomes. Analysis of the distribution of protein families reveals that some families are ubiquitous among archaeal genomes while other families are specific to each archaeal group. We additionally highlight the conserved motifs and uncharacterized domains in the detected families. We also report the presence of a novel SF2 helicase family specific to archaea that we named ASH. Phylogenetic analysis indicated that ASH is a close homolog of the ski2-like family and analysis of its distribution showed that it is specific to euryarchaeota. Our study provides the first comprehensive analysis of SF1 and SF2 helicases from archaea.

Keywords: Archaea, genomics, helicase.

MON-475
Immunological properties of yeast-derived secreted influenza virus hemagglutinin

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Influenza virus is a pathogen inducing respiratory disease with symptoms varying from mild to severe. The realistic barrier against the disease remains still in vaccination against influenza. It is well established that the virus hemagglutinin is the main antigen, inducing the neutralizing antibodies. The HA gene mutates however fast and requires constant creation of novel vaccine versions acting against the seasonal version of the pathogen. This is rather compelling task as the standard vaccines are prepared from the viruses grown on large scale in chicken embryos. The production of recombinant HA in appropriate expression systems is an alternative approach. Looking for the optimum antigen production method we opted for simple and cheap *Pichia pastoris* system. Here the secretion of the overexpressed polypeptide considerably facilitates the purification of the product. The A/swan/Poland/305-135V08/2006 (H5N1- subtype) hemagglutinin gene was cloned and expressed in yeast *Pichia pastoris* (*P. pastoris*). The HA cDNA lacking the C-terminal transmembrane anchor-coding sequence was fused to α-factor leader peptide and placed under control of the methanol-inducible *P. pastoris alcohol oxidase 1* (*AOX1*) promoter. Recombinant HA protein was secreted into the culture medium reaching the approximately 15 mg/L. The immunological activity of hemagglutinin protein was tested in mice, where rHA elicited high immune response. HA is the main IV antigene inducing the host neutralizing antibodies protein overexpressed in Pichia is a promising candidate subunit vaccine.

Keywords: Avian influenza, Pichia pastoris, Recombinant hemagglutinin.

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MON-476
Intermolecular domain swapping induces intein-mediated protein alternative splicing

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Protein sequences are diversified on the DNA level by recombination and mutation and can be further increased on the RNA level by alternative RNA splicing, involving introns that have important roles in many biological processes. The protein version of introns (inteins), which catalyze protein splicing, were first reported in the 1990s. The biological roles of protein splicing still remain elusive because inteins neither provide any clear benefits nor have an essential role in their host organisms. We now report protein alternative splicing, in which new protein sequences can be produced by protein recombination by intermolecular domain swapping of inteins, as elucidated by NMR spectroscopy and crystal structures. We demonstrate that intein-mediated protein alternative splicing could be a new strategy to increase protein diversity (that is, functions) without any modification in genetic backgrounds. We also exploited it as a post-translational protein conformation–driven switch of protein functions (for example, as highly specific protein interference).

Keywords: 3D domain swapping, intein, protein alternative splicing.

MON-477
Metabolic engineering of *Saccharomyces cerevisiae* for the production of plant derived antioxidants

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The expression of plant metabolic pathways in heterologous organisms such as *Saccharomyces cerevisiae* is an attractive strategy for the production of valuable secondary metabolites.

By engineering a *S. cerevisiae* strain with two plant genes (4 cl-2 from tobacco and hct from globe artichoke) encoding key proteins involved in the biosynthesis of phenolic esters we previously set up a system for the production of two novel phenolic compounds, N-(E)-*p*-coumaroyl-3-hydroxyanthranilic acid (Yeast avenanthramide, YAv I) and N-(E)-caffeoyl-3-hydroxyanthranilic acid (Yeast avenanthramide II, YAv II). These compounds possess structural similarity with a class of bioactive oat compounds called avenanthramides. The antioxidant, anti-proliferative, anti-inflammatory and anticancer activities of oat avenanthramide have been demonstrated both *in vitro* and in animal models

We set up a large scale fermentation system for yeast avenanthramides production following optimization of yeast strain, plasmid constructs, and culture conditions. The production of yeast avenanthramides reached a final yield of 125 mg/L for YAv I and 22.5 mg/l for YAv II.

In order to evaluate the biological properties of yeast avenanthramides, we tested their anti-oxidant properties in distinct cell models, including Mouse Embryonic Fibroblasts (MEF) and the HeLa cancer cell line. In particular, we analyzed the effects of yeast avenanthramides on FoxO1, a member of the FoxO family of transcription factors that has been demonstrated to play a major, highly conserved role in regulating ROS levels by modulating the expression of antioxidant enzymes such as Superoxide Dismutase 2 (SOD2). Real Time PCR and Western Blot analysis suggested that yeast avenanthramides positively regulate the antioxidant defense mechanism through the up-regulation of FoxO1 and SOD2 expression level. Furthermore, we demonstrated that YAv compounds enter the cells, accumulating into discrete cytosolic regions, and may exert an antiproliferative effect by facilitating the down-regulation of Cyclin D1.

Keywords: avenanthramides, metabolic engineering, antioxidant.

MON-479
Mixed organic-inorganic nanocomposites as potential virus inhibitors

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Usually when investigating biospecific interactions by biosensing methods typical approach is the immobilization at the sensor surface of the one reagent (receptor) and detection of its binding to other ones (analytes) one by one, which rest in the solution. However in real situation simultaneous multicomponent interactions are much more typical. Earlier we proposed an approach to the detection of the virus-antibody complex by surface plasmon resonance method based on the refractive index variation within the layer of the virus-antibody complexes depending on the antibody concentration, on the example of tobacco mosaic virus (TMV)[1]. The effectivness of this approach for the multi component mixes was demonstrated on the quantification of the effect of polysaccharide glucuronoxylomannan (GXM) on the interaction between TMV and specific antibodies [2].

Here we consider an approach to the formation of the nanocomposite with potent antivirus ability based on the *Ganoderma adspersum* glycane with embedded gold nanoparticles and its further interaction with TMV and specific antibodies. Au-glycane nanocomposite was obtained by reduction of metal from $HAuCl_4$ saltunder the base condition where glycane play a role of macromolecular reducer and stabilizer. Absorption spectra of product demonstrated the presence of the wide band with the maximum near 560 nm specific for local surface plasmon excitation in gold nanostructures. TEM imaging showed that Au nanoparticles were embedded in glycan matrix with size dispersed in the range from 5 to 30 nm. It was demonstrated, that the incubation of the untreated virus and the virus, firstly preincubated with above mentioned nanocomposite, with antibodies with further observation of the complex immobilization to the sensor surface allows one to state the complexation with the nano-composite changes the binding capabilities of the virus which make it potentially useful as virus inhibitor.

1. Boltovets P.M., Snopok B.A., Boyko V.R., Shevchenko T.P., Dyachenko N.S., Shirshov Yu.M Detection of plant viruses using a surface plasmon resonance via complexing with specific antibodies // Journal of virological methods, 2004. – V121. – P.101-106.

2. P. M. Boltovets, O. M. Polischuk, O.G. Kovalenko, B.A. Snopok A simple SPR-based method for the quantification of the effect of potential virus inhibitors // Analyst, 2013,138, 480–486.

Keywords: organic-inorganic nanocomposites, Surface Plasmon Resonance, tobacco mosaic virus.

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MON-480
Molecular dynamics simulation of 3D structural models of Baeyer-Villiger Monooxygenases with the help of a structural alphabet

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Baeyer-Villiger monooxygenases (BVMOs) are flavoenzymes and belong to the class of oxidoreductases (Alphand & Wohlgemuth, 2010). They catalyze the oxidation of various ketones to esters and lactones (see Figure A for a visualisation of the protein structure, different domains are underlined in colors). During enzymatic oxidation, one atom of oxygen is incorporated between a carbon-carbon bond, whereas the other oxygen atom ends up in a water molecule with the hydrogen atoms originating from the cofactor NAD(P)H. BVMOs' flavin cofactor is crucial for catalysis and is tightly, but not covalently, bound in the active site. These enzymes require the reduction of the flavin cofactor to activate it for molecular oxygen binding (see Figure B for FAD of PAMO from *Thermofibidafusca* in its neighborhood).

We have carefully searched protein databases for new BVMOs related sequences. We had applied strict selection criteria as the flavopotein monooxygenase superfamily is suffering from many annotation problems in the databases. Hence, the 116 proteins we have analyzed are highly characteristic of BVMOs type I family (Rebhemed et al., 2013). We propose efficient structural models of these BVMOs using up-to-date approaches.

Molecular dynamics were performed for each structural model. Interestingly, simulations are analyzed using a structural alphabet, namely Protein Blocks (de Brevern et al., 2000). The latter consists in a library of 16 fragments of 5 residues length able to approximate efficiently every part of protein structures (Joseph et al., 2010). This database of structural models may be useful from a fundamental viewpoint, but also for modelling purposes and therefore, will be freely accessible to the scientific community via a website.

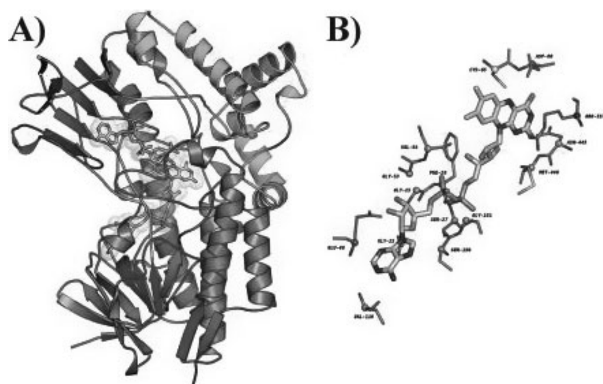

Fig. 1.

Keywords: Bioinformatics tools, protein design, protein engineering.

MON-481
Monolayers of human albumins on colloidal carrier particles and flat surfaces: thorough physicochemical characteristics in terms of electrokinetic methods

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Physicochemical properties of two different types of human albumin were studied, human serum albumin (HSA) and recombinant human albumin (rHSA). HSA is obtained by purification of the blood plasma by Cohn process. The final product received by this method contains up to 10% dimeric forms of albumin. On the other hand, rHSA, which is expressed in Saccharomyces cerevisiae via genetic engineering and molecular biology method, consists of only monomeric species.

In this work, adsorption of HSA and rHSA on colloidal particles surface was studied. Monodisperse, negatively charged polystyrene latex particles, 800 nm in diameter were used as colloidal carriers. The adsorption of proteins was studied via electrokinetic measurements (micro-electrophoresis) and AFM imaging. Adsorption of proteins was carried out at pH 3.5 and 7.4 and ionic strength range of 0.001–0.15 M NaCl. The concentration of polystyrene latex carriers was changed in the range of 40–100 mg L^{-1}.

It was observed that the electrophoretic mobility (zeta potential) of latex monotonically increased with the albumin concentration in the suspension. This was quantitatively interpreted in terms of the three dimensional electrokinetic model. The coverage of adsorbed albumin was quantitatively determined using the depletion method, where the residual protein concentration was determined by the above mentioned methods. These measurements enabled a precise determination of the monolayer coverage of rHSA and HSA on polystyrene latex particles. The maximum coverage of both proteins at pH 3.5 increased with ionic strength and varied between 0.7 mg m^{-2} for 0.001 M NaCl to 1.3 mg m^{-2} for 0.15 M NaCl. These results were interpreted using the random sequential adsorption approach.

In order to evaluate the albumin adsorption mechanism, the experimental results obtained for colloid carrier particles were compared with adsorption on the model mica substrate characterized by uniform charge distribution. These measurements revealed that deep analogies exist between albumin adsorption on colloidal carriers and flat interfaces governed by electrostatic interactions. Therefore, it was concluded that recombinant albumin as a monomeric, free of dimers and less immunogenic protein can be widely used in a wide range of research and practical applications instead of the human serum albumin that can be immunogenic.

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Keywords: albumin adsorption, colloidal carriers for proteins, recombinant albumin.

MON-482
Morphology of cell-penetrating peptide-nucleic acid nanoparticles and their intracellular trafficking

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Nucleic acids hold a great therapeutic potential due to their high specificity and low toxicity. However, the poor permeability of

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plasma membrane to nucleic acids complicates the development of these molecules for therapeutic applications. Cell-penetrating peptides as efficient and non-toxic vectors have been widely used to assist the intracellular delivery of nucleic acids. We recently developed two novel transportan-based CPPs – NickFect 1 (NF1) and NickFect 51 (NF51) for the delivery of nucleic acids.

We show that NFs are highly efficient transport vectors for plasmid DNA (pDNA) and splice correction oligonucleotides (SCOs). NF1 and NF51 form spherical or slightly elliptical (mean aspect ratio <1.4) and monodisperse nanoparticles with pDNA in water that have a size around 40–50 nm. Nanoparticles formed with splice correction oligonucleotide (SCO) had a similar shape and mean size, but showed significantly higher size variation compared to NF-pDNA nanoparticles.

For the detection of plasmid molecules in cells by transmission electron microscopy (TEM) we labeled pDNA with neutravidin-colloidal gold (NA gold) conjugate prior complex formation with CPPs. We show that NA-gold labels efficiently pDNA with more than 80% of gold being associated giving us confidence to use this labeling approach for examining intracellular trafficking of NF-pDNA nanoparticles using TEM.

Using different endocytosis inhibitors and TEM analysis we show that NF-nucleic acid nanoparticles internalize cells via endocytosis. Inside cells, the vast majority of nanoparticles were entrapped inside early and late endosomes, however, a small fraction of nanocomplexes had escaped into cytosol already after 1 h of incubation.

In conclusion, we show that NFs condense nucleic acids to nanoparticles of rather homogenous size, which are taken up by cells using endocytosis. Although a large fraction of nanoparticles are entrapped inside endosomes, the NF assisted delivery of nucleic acids leads to high level of gene expression modulation *in vitro*.

Keywords: gene therapy, nanoparticle characterization, intracellular trafficking.

MON-483
NMR Studies of large protein assemblies using specific isotopic labeling of methyl group

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Large protein complexes are involved in many of the key processes in the cell but their study by NMR has been a real challenge for a long time. Nowadays, it is unequivocally recognized that the strategy of specific isotope labeling of methyl groups in a perdeuterated protein has significantly extended the frontier of liquid state NMR. Production of perdeuterated protein with selective protonation of the methyl groups can be carried out by adding the specifically labeled metabolic precursors in the fully deuterated culture medium before induction of protein expression. Wewill present here our recent development of new precursors and their applications to large protein complex. In the last years, we have exploited metabolic pathways in E. coli and synthesized new isotope-labeled precursors to extend the library of methyl labeling methods. These new strategies allow the stereospecific labeling of the prochiral methyl groups of Leucine and Valine as well as the regioselective labeling of Isoleucine methyl probes. To further improve quality of spectra for protein system with molecular size of several hundred of kilodalton, we have demonstrated that the specific labeling of methyl of Valine groups without Leucine enhances significantly the resolution of spectra. Additionally, new protocols have been developed to extend and optimize specific labeling approaches to methyl groups of Methionine and Threonine residues, as well as Alanine residues. Using these new tools, we have setup robust and scrambling-free protocols to label in targeted proteins any combination of methyl groups. Combined with rapid and cost effective assignment strategies based on mutagenesis, these advances allow to extend the limitation of liquid state NMR, offering the possibility to study more and more challenging protein systems.

Applications to the functional, dynamic and structural studies of a 500 kDa homododecameric protein will be presented.

Keywords: biomolecular nmr, isotopic labeling, large system.

MON-484
Novel inhibitory monoclonal antibodies against tumour-associated carbonic anhydrase XII

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Human carbonic anhydrase XII (CA XII) is a single-pass transmembrane protein with an extracellular catalytic domain. This enzyme is being recognized as a potential biomarker for different tumours. It is involved in tumor progression by acidification of the extracellular milieu and regulation of intracellular pH. CA XII is aberrantly overexpressed in breast, cervix, brain cancers, renal carcinomas. The current study was aimed to generate monoclonal antibodies (MAbs) neutralizing the enzymatic activity of CA XII. Bioinformatics analysis of CA XII structure revealed surface-exposed sequences located in a proximity of its catalytic centre. Two MAbs against the selected antigenic peptide spanning 167–180 aa sequence of CA XII were generated. The MAbs were reactive with recombinant catalytic domain of CA XII expressed either in E. coli or mammalian cells. Inhibitory activity of the MAbs was demonstrated by a stopped flow CO_2 hydration assay. The study provides new data on the surface-exposed linear CA XII epitope that may serve as a target for inhibitory antibodies with a potential immunotherapeutic application.

Keywords: human carbonic anhydrase XII, inhibition of enzymatic activity, monoclonal antibodies.

MON-485
Novel insect-toxin from *Tibellus oblongus* spider venom

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Insect pests act disturbingly to different human activities. Increasing human population would require high amount of food in the future, so large annual reductions in crop yields by insects pose a threat. From other side, spiders are nature-born insect killers. Venom of the most spiders is a complex mixture of differ-

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ent compounds delicately tuned for the aim of paralyzing or immediate killing of insect prey.

In this light, we see the area of spider venom components research having potential in application to production of new generation insecticides.

As the object of this particular research was chosen *Tibellus oblongus* - Middle East spider, completely non-harmful to human. Its crude venom was fractionated by combination of SEC and RP-HPLC. At each step of chromatographic separation, insect-toxicity of each fraction was tested on *Musca domestica* larvae via injections. The most active fractions were being separated until one individual polypeptide component has been found. Novel toxin was named TboIT1. EST-database data to MALDI-MS analysis result comparison together with MS analysis of trypsin hydrolysate allowed determining amino acid sequence. Recombinant production of toxin in *E.coli* provided sufficient amount for toxicity and electrophysiological experiments.

It was determined that recombinant TboIT1 has LD_{50} 12 γ/g (on *M. domestica* larvae). Toxin slows down transmitter release in neuro-muscular junction of *Calliphora vicina* bottle fly. TboIT1 reversibly inhibited evoked EPSC (eEPSC), but did not affect frequency of spontaneous EPSC (sEPSC). Interestingly, inhibition of eEPSC was accompanied by significant change of rise time (rt) or delay (τ) of the eEPSC. Such parameters for sEPSC were unaffected.

Moreover, toxin does not affect any parameter of transmission in frog neuro-muscular junction. These data point out for TboIT1 may being novel selective inhibitor of insect pre-synaptic calcium channels.

Keywords: insect-toxins, spider venom.

MON-486
Optimal number of ribosomal RNA operons in *Escherichia coli* is determined primarily by the stability of environmental conditions

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Background: The copy number of the ribosomal RNA operons (rrn) is a characteristic trait of bacterial genomes. It influences rRNA availability, which in turn, regulates the amount of ribosomes, key players of cellular physiology and economy. It is thought that the rrn operon number reflects the organism's ecological strategy. Bioinformatic and some experimental data suggest that lower copy numbers are favored in bacteria living in low-nutrient, relatively stable environments, and higher numbers (7 in *E. coli*) seem to be associated with fluctuating, feast and famine conditions. It is not clear, however, what the primary determinant of rrn operon copy number is: the benefit of fast growth, the capability of quick adjustment to favorable conditions, or the economic utilization of nutrients.

Results: We constructed isogenic variants of *E. coli* K-12 with 5 to 10 copies of rrn operons, analyzed their growth parameters, measured their RNA and protein contents, and subjected them to pairwise competitions under both fluctuating (serial growth in batch cultures) and stable nutrient influx (growth in a chemostat) conditions. While growth parameters showed only minor changes, competitions revealed a clear pattern: 7–8 copies were optimal under fluctuating conditions, and lower numbers were favored in a stable environment. These patterns persisted at two different growth rates. Interestingly, while the RNA: protein ratio remained constant, an increase in protein and RNA content, accompanied by a slight increase in cell size was observed in the 5 to 8 operon range.

Conclusions: By delineating the effects of nutrient quality and availability, we found that the stability of the environmental conditions is the primary factor determining the optimal number of rrn operons in *E. coli*. Our experiments confirm that the wt rrn copy number of *E. coli* reflects adaptation to fluctuating conditions. However, the results also show that lower rrn copy numbers are beneficial for *E. coli* when environmental conditions are stable. Adjustment of the rrn number to the planned environmental conditions should thus be taken into account when constructing designer bacterial genomes.

Keywords: E. coli, rrn operon number, synthetic biology.

MON-487
Overcoming recombinant protein insolubility: case study of C1q domain of otolin-1

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Otolith and otoconia are calcium carbonate biominerals which are responsible for perception of linear acceleration in fish and land vertebrates, respectively. Apart from calcium carbonate, these biominerals contain an organic matrix composed of proteins, glycoproteins and proteoglycans. Otolith and otoconia differ in size, organic matrix composition and calcium carbonate polymorph. Both biominerals however contain otolin-1, which is a collagen-like protein strongly conserved in variety of species. It contains an N-terminal domain, collagen-like chain and C1q-like globular domain. C1q-like domain of otolin-1 (OtolC1q) may bind calcium and interact with other proteins from organic matrix of otolith and otoconia, and thus otolin-1 may provide a scaffold for calcium carbonate biomineralization.

Human and zebrafish OtolC1q fused with a hexahistidine tag or a solubility enhancing maltose-binding protein (MBP) tag were expressed in Escherichia coli BL21(DE3)pLysS cells. Protein solubility assay showed that OtolC1q fused with hexahistidine tag (His-OtolC1q) was poorly soluble in Escherichia coli in 29°C. In contrast, OtolC1q fused with MBP (MBP-OtolC1q) was well-soluble in these conditions. However, MBP-OtolC1q was resistant to proteolytic cleavage and in non-denaturing conditions, OtolC1q was inseparable from undigested fusion protein. Decrease of bacterial culture temperature to 15°C increased solubility of His-OtolC1q and allowed to purify protein of interest using Co^{2+} affinity chromatography and gel filtration.

This work was supported by a statutory activity subsidy from the Polish Ministry of Science and High Education for the Faculty of Chemistry of Wroclaw University of Technology.
Keywords: biomineralization, otoconia, otolith.

MON-488
Polyanions are a powerful tool for the control of protein aggregation

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The searching for the means of suppression of protein aggregation is an important problem of biochemistry, bioengineering, biotechnology, and even medicine. Thus, production of the recombinant protein in bacterial cells often causes the inclusion

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bodies formation. Inclusion bodies also appear in neural tissues due to neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's diseases and prion infections.

In the present work the influence of polyanion structure and the nature of charged groups on the thermal aggregation of glyceraldehyde-3-phosphate dehydrogenase was investigated. Polyphosphate anions have been found to interact with protein less tightly than polysulfoanions and to do not affect protein structure (in contrast to polysulfoanions, especially relatively hydrophobic polystyrenesulfonate anions). However, they are able to almost completely suppress protein aggregation (though less effectively than polysulfoanions).

Beside of aggregation suppression, polyanions have been shown for the first time to solubilize already formed protein aggregates of different nature. Polysulfoanions solubilized effectively both amyloid and amorphous inclusion bodies as well as amorfous thermal aggregates. The treatment by polysulfoanions of prion protein inclusion bodies resulted in significant decrease of the amyloid structures amount. Solubilization of glyceraldehyde-3-phosphate dehydrogenase thermal aggregates was accompanied by partial recovery of the enzymatic activity. The efficiency of aggregation suppression and solubilization of aggregates has been shown to depend on the polyanion degree of polymerization and hydrophilicity. Thus, amyloid aggregates (the most stable of examined) was solubilized only by highly polymerized polystyrenesulfonate anions, whereas amorphous aggregates can be disassembled even by hydrophobic dextran sulfate.

The developed approach for the protein aggregation control seems to be relevant for extraction of the recombinant protein from inclusion bodies as well as (in the long term) for therapy of the amyloid diseases.

The work was supported by Russian Foundation for Basic Research, project No 14-04-00331.

Keywords: polyelectrolytes, protein aggregate solubilization, protein aggregation.

MON-489
Polysaccharide nanocapsules designed for enhanced daunorubicin delivery; in vitro release and biocompatibility studies

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An ideal drug delivery system apart from its nanoscale size and sustained release, should also have high biocompatibility to enhance cargo bioactivity and to reduce its side-effects. Successful targeting of a cytostatics to the cancer cells using newly designed oil-core polyelectrolyte (PE) nanocapsules can be achieved solely if the nanosystem remains long time in the blood stream enough to accumulate at its site of action. Thus, the fundamental issue for the nanocarrier biocompatibility is evaluating its low cytotoxicity and safe interaction with erythrocytes and macrophages. Hence, the key approach to form optimal delivery nanocapsules consists in using the capacity of some polysaccharide-based PEs to favorable change their macromolecular properties and selective cellular internalization (Bazylińska et al., Soft Matter (2011) 7:6113; Eur. J. Pharm. Sci. (2012) 47:406).

The main purpose of the present study was to investigate which type of polysaccharide-coated nanocapsules may substitute popular PEG-ylated structures and enhance daunorubicin (DN) delivery to the cancer (MC38) cells. Thus we fabricated long-sustained nanocapsules by layer-by-layer adsorption of chitosan-CHIT, λ-carrageenan-CAR or dextran-DEX directly on the nanoemulsion core (created by cationic dicephalic-type N,N-bis [3,3′-(trimethylammonio)-propyl]dodecanamide dimethylsulphate $C_{12}(TAPAMS)_2$ and oleic acid (OA)). The all DN-loaded nanocapsules, with the four or five bilayer of the PEs and with size <150 nm (analyzed by DLS, AFM and SEM), were subjected to *in vitro* release and biocompatibility studies. Drug remaining profiles showed their long-term stability in physiological condition (pH 7.4) and in the presence of HSA. Hemolytic potential determined in whole human blood, does not exceed value of 2% indicating the nanosystem hemocompatibility. The internalization yield by macrophages observed in FACS and interaction with HSA of the nanosystems with the outer DEX layer was about 5-fold and 2-fold lower than those containing CHIT and CAR respectively. Finally, we improved that all DN-loaded systems, are non irritated for MC38 cells, which demonstrate their potential low side effects in comparison to the high cytotoxic free daunorubicin.

Our results provide conclusions that the obtained polysaccharide nanocapsules, primary coated by DEX, might become biocompatible and long-sustained DN delivery systems in potential chemotherapy of colon cancer as a promising alternative to the common PEG-ylated nanocarriers.

This work was supported by statutory activity subsidy from the Polish Ministry of Science and Higher Education for the Faculty of Chemistry of Wrocaw University of Technology.

Keywords: multilayer nanocarriers, anticancer therapy, tumor cells.

MON-490
Production of virus-like particles of porcine circovirus type 2 (PCV2) capsid protein in yeast and their employment for generation of PCV2-specific monoclonal antibodies and PCV2 diagnostics

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Porcine circovirus type 2 (PCV2) is very small (about 17 nm), spherical, non-enveloped virus with icosahedral symmetry classified as a member of the family Circoviridae. This virus is considered to be an important emerging pathogen associated with a number of different syndromes and diseases in pigs such as post-weaning multisystemic wasting syndrome, porcine dermatitis, nephropathy syndrome, porcine reproductive disorders and other all known as PCV2-associated diseases. PCV2 can cause a serious problem to the swine industry and can lead to significant negative impacts on profitability of pork production. PCV2 have circular, single-stranded DNA genomes of approximately 1700 nucleotides, encoding 2 major ambisense open reading frames, ORF1 and ORF2. ORF1 is predicted to encode a replicase protein (Rep) essential for replication of viral DNA. ORF2 encodes the capsid protein (Cap), which plays a key role in the process of virus particle assembly. The Cap protein is highly immunogenic and reacts strongly with the serum of PCV2-infected pigs. Therefore, it is a good candidate antigen for the design of new recombinant vaccines against PCV2 infection and for the development of serological assays. In this study we have demonstrated that PCV2 capsid

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(Cap) protein was efficiently produced in yeast *S.cerevisiae* and formed virus-like particles (VLPs). Yeast-derived PCV2-Cap VLPs mimicked the structure of authentic virus particles and induced production of monoclonal antibodies reactive with virus-infected cells. Moreover, PCV2 Cap VLPs was tested as a recombinant antigen for the development of an indirect IgG PCV2 Cap VLP-based ELISA for the detection of virus-specific IgG antibodies in swine sera. The high sensitivity and specificity of the indirect IgG PCV2 Cap VLP ELISA clearly suggested that developed assay is potentially useful diagnostic tool for screening PCV2–suspected samples.

This research was funded by the European Social Fund (Grant No. VP1-3.1-SMM-10-V-02-017).

Keywords: Antibodies, virus like particles, Porcine circovirus type 2.

MON-491
Protein engineering via assembly of catalytic domains: lessons from a natural bidomain electron transfer protein

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Synthetic Biology aims to reconstruct existing or novel biochemical reactions through the designed and controlled assembly of natural modules bearing defined functions. Among the various exploratory paths targeting new functionalities in biological molecules, the design of multifunctional enzymes is under heavy scrutiny. First, the fixed stoichiometry between catalytic domains guarantees a constant metabolic flux. Second, multidomain enzymes can accelerate subsequent reactions in a metabolic pathway and protect unstable or toxic intermediates due to spatial confinement. Here, I will describe the catalytic and structural mechanisms by which a natural bidomain electron transfer (ET) protein, the NADPH cytochrome P450 reductase (CPR), reaches the optimum catalytic efficiency.

CPR is a bidomain flavoprotein that belongs to the family of diflavin reductases. Structural studies of CPR have evidenced reorganization and rearrangement of the two catalytic domains at the resting state or during catalysis. Through a combined SAXS, NMR, biochemical and theoretical approaches, we have fully described the architectural reorganization of CPR at resting state and evidenced two states (named locked and unlocked) that correspond to the two catalytically active states. Both states are in rapid exchange and the equilibrium is controlled by ionic strength and pH. When the locked and unlocked states are equally populated, CPR presents its maximum catalytic activity. We proposed a kinetic model demonstrating a direct link between the domain movements and ET efficiency during the catalytic cycle of CPR. This model is based on rapid stochastic interdomain interactions, short lifetime of domain interfaces and rapid sampling of the various conformational states present at each catalytic step. The maximal efficiency of the enzyme is obtained when the relative populations of productive conformations match individual catalytic rates. This mechanism is analogous to the cellular metabolic situation where the ratio between enzymes participating in sequential reactions of a metabolic pathway are adjusted to their catalytic rates (slower enzymes will be present at higher concentration than faster enzymes) in order to minimize the accumulation of intermediates.

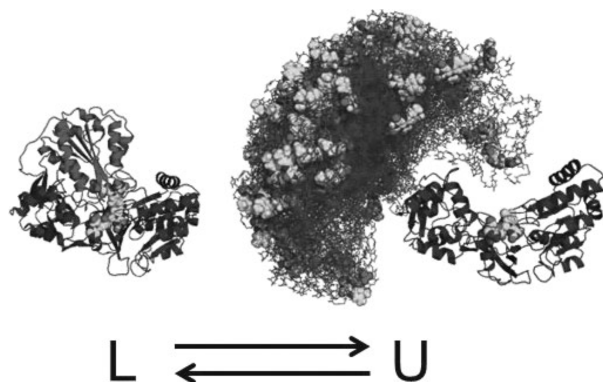

Fig. 1.

Beyond the specific results proposed on our model, I will present possible generalization on how our results could be applied to catalytic domain association in protein engineering: controlling the domain interface stability in multidomain enzymes may represent a direct way to adjust overall enzymatic activity.

Keywords: Domain assembly, protein engineering, Synthetic Biology.

MON-492
Purification and covalent immobilization of benzoylformate decarboxylase with heterofunctional chelate-epoxy modified magnetic nanosupport

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Histidine-tagged benzoylformate decarboxylase (BFD) from *pseudomonas putida* was immobilized on the epoxy-chelate magnetic solid support following a two-step mechanism; first, the protein is physically adsorbed and second, the covalent bonding takes place. This mechanism has been exploited to combine the selectivity of metal chelate affinity chromatography with the covalent immobilization capacity of epoxy supports. In this way, it has been possible to accomplish, in a simple manner, the purification, immobilization, and stabilization of a histidine-tagged benzoylformate decarboxylase. To fulfill this objective we prepared and characterized a multifunctional Co^{+2}-IDA-epoxy functionalized magnetic nanoparticles which are modified with glycidyloxypropyltrimethoxysilane (GPTMS) and iminodiacetic acid (IDA).

The covalently bounded BFD was characterized in terms of its activity and stability for the formation of (*S*)-2-hydroxypropiophenone (2-HPP). The results obtained from the formation of (*S*)-2-hydroxypropiophenone that was performed with this simple and convenient heterogeneous biocatalyst were comparable to that of free-enzyme-catalyzed reaction.

Keywords: Immobilized Benzoylformate Decarboxylase; epoxy-chelate magnetic support; metal-affinity magnetic nanoparticles; selective immobilization; multipoint covalent attachment; one step purification.

MON-493
Rational design of small molecule inhibitors targeting ASK1

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Apoptosis signal-regulating kinase 1 (ASK1) plays important roles in many cardiovascular and neurodegenerative diseases suggesting that small compounds inhibiting ASK1 could possibly be used for the treatment of these pathologies.

To discover the protein kinase ASK1 inhibitors we have performed screening program, using both in silico and in vitro approaches. AutoDock and DOCK software were used to con-

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duct receptor-ligand flexible docking. The best-scored compounds of different chemical classes were taken for the kinase assay analysis.

In vitro observations revealed that derivatives of 2-Thioxo-thiazolidin-4-one exhibited inhibitory activity towards ASK1. The most active compound inhibited ASK1 with IC_{50} = 2 μM. Then, in-depth study of this chemical class was performed using the pre-selected library of 2-thioxo-thiazolidin-4-one derivatives. Ten best-scored compounds were taken for the kinase assay analysis. Compound 2-{5-[5-(3,4-Dichloro-phenyl)-furan-2-ylm-ethylene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propanoic acid (PFTA-1) inhibited ASK1 with an IC_{50} = 650 nM. Our preliminary selectivity studies demonstrated that this ligand seems to be selective inhibitor of ASK1. In silico analysis of the complexes of ASK1 with compounds indicated, that the peculiarity of the PFTA-1 in the comparison to other nine inactive derivatives is its ability to bind simultaneously to the hinge region and the phosphate-binding region of the ATP-binding cleft. Molecular dynamics simulations allowed us to accurately predict binding mode of PFTA-1 inhibitor with ASK1.

The core structure of PFTA-1 was used for developing more potent and selective inhibitors of ASK1. A series of derivatives has been synthesized and evaluated in vitro towards human protein kinase ASK1. It was revealed that the most active compounds inhibit ASK1 with IC_{50} of 200 nM. Structure-activity relationships of 33 compounds have been studied and binding mode of this chemical class has been predicted.

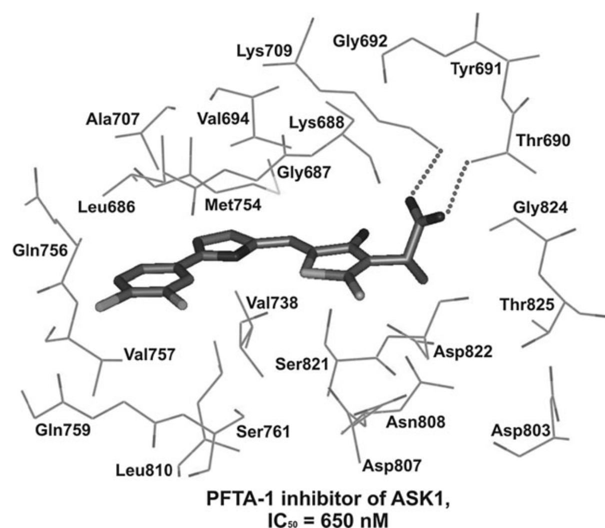

Fig. 1.

Keywords: Apoptosis signal-regulating kinase 1, inhibitor, virtual screening.

MON-494
Recombinant chimeric bifunctional proteins with alkaline phosphatase activity of marine bacterium Cobetia marina for the assessment of lectin-binding patterns

L. Balabanova, V. Golotin, S. Kovalchuk, G. Likhatskaya, A. Bulgakov, O. Chernikov, I. Chikalovets, V. Molchanova, V. Rasskazov
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The strong innate defense system and the absence of the acquired immunity in marine invertebrates have made their lectins are highly specific and selective towards carbohydrates. These are essential to use invertarates' lectins as diagnostic tools for early detection of carbohydrate profiles changes that accompany many pathological processes. The use of mannan-binding C-type lectin from Far-Eastern holothurian *Apostichopus japonicus* (MBL-AJ) and galactose-specific lectin from the mussel *Crenomytilus grayanus* (CGL) have been proposed for recognizing aberrant glycans. The lectin MBL-AJ was successfully applied for the non-invasive assessment of carcinoembryonic antigens (CEA) in cervical specimens for the early differential diagnosis of a malignant condition. The presence and distribution of the lectin CGL ligands, pregnancy specific beta-1-glycoprotein 1 and CEA, were analyzed in the human placental trophoblast pathology and colorectal adenocarcinoma, respectively. For developing the functional tools for the *in situ* identification of glycosylation patterns, the lectins were fused to the highly active alkaline phosphatase of marine bacterium *Cobetia marina* (*Cm*AP) with the use of NcoI/SalI fragment of pET-40b(+) vector and linker $(G_4S)_3$. High efficiency and the facility of the cofactor-independent activity assay, broad pH-stability and a high stability against helate agents as well as monomeric station of *Cm*AP have advantaged rapid monitoring and optimization of various aspects of the recombinant chimeric lectins production. The completely soluble chimeric proteins, comprising the alkaline phosphatase domain which is capable of enzymatic digesting of p-nitrophenyl phosphate and the lectin domain which is able to bind a ligand-bearing target, were produced by *E. coli* Rosetta(DE3) strain in the presence of 0.2 mM IPTG and the temperature of 16 °C for 12 h. The bifunctional chimeras exhibited a high level of the lectin-binding activity towards embryonic alpha-1-acid glycoprotein, CEA, mucin. The 3-D structure modeling and mutagenesis have demonstrated that the lectin and *Cm*AP modules function independently, and revealed the essential amino acid residues for the interaction with ligands. The genetically engineered *Cm*AP-labeled invertebrate lectins will provide an improved method for biomarkers detection.

The work was supported by RFBR grants 12-04-00825, 14-04-00696 and the project 'Far East' 12-I-П6-10.
Keywords: None.

MON-495
Recombinant virus-like particles with inserted segment of viral glycoprotein represent an efficient carrier to generate glycoprotein-specific antibodies

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Monoclonal antibodies (MAbs) against viral glycoproteins have important diagnostic and therapeutic applications. In most cases, the MAbs specific to viral glycoproteins are raised against intact virus particles. The biosynthesis of viral glycoproteins in heterologous expression systems such as bacteria, yeast, insect or mammalian cells is often problematic due to their low expression level, improper folding and limited stability. To generate MAbs against hantavirus glycoprotein Gc, chimeric virus-like particles (VLPs) harbouring a segment of PUUV Gc glycoprotein were expressed in yeast *Saccharomyces cerevisiae* and used as an immunogen. A 99 amino acid (aa)-long segment of Gc protein was inserted into the major capsid protein VP1 of hamster polyomavirus at previously defined positions: either site #1 (aa 80–89) or site #4 (aa 280–289). The chimeric proteins were found to self-assemble to VLPs as evidenced by electron microscopy. Chimeric VLPs induced an efficient insert-specific antibody response in immunized mice. Monoclonal antibody of IgG isotype specific to hantavirus Gc gly-

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coprotein was generated. It recognized recombinant full-length PUUV Gc glycoprotein both in ELISA and Western blot assay and reacted specifically with hantavirus-infected cells in immunofluorescence assay. Epitope mapping studies revealed the N-terminally located epitope highly conserved among different hantavirus strains. In conclusion, our approach to use chimeric VLPs was proven useful for the generation of virus-reactive MAb against hantavirus Gc glycoprotein. The generated broadly-reactive MAb #10B8 might be useful for various diagnostic applications.

This research was funded by the European Social Fund under the Global Grant measure (Grant No. VPI-3.1.-SMM-07-K-03-004).
Keywords: antibodies, chimeric virus-like particles, hantavirus glycoprotein.

MON-497
Revealing new antimicrobial peptides in *Leymus arenarius* based on transcriptome sequencing

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Lyme grass (LG, *Leymus arenarius*) is a perennial weed plant from *Poaceae* family. The distinctive property of LG is its high tolerance to adverse environment factors (AEF) like soil salinification, drought etc.

Earlier we have obtained a number of LG antimicrobial peptides (AMP) using biochemical methods. AMPs are an important component of plant innate immunity system, so their investigation could help in understanding the plant tolerance mechanisms to AEF and pathogens, that will be especially useful for crops. However, biochemical investigations of AMP are laborious and expensive and do not allow investigating all available proteins. Thus the goal of the current research was to analyze the transcriptome of LG sprouts that allowed to investigate the LG RNAs coding for AMPs.

LG transcriptome was obtained by sequencing on Illumina HiSeq 2000 and was assembled *de novo* using various software packages. The AMPs have very low primary structure similarity, but they are characterized by presence of several cysteine residues arranged in rather rigorous order called a 'motif', This is why a novel method was developed for AMP searching. The search for potential AMPs was made by 6-reading frame translation of the transcripts obtained and looking for cystein motifs known to be common for various AMP groups in the resulting amino acid sequences. Also, a filtration step for the sequences with motifs was performed that involved taking only 1 open reading frame, checking the presence of methionine at the beginning of peptide sequence and checking for the presence of signal peptide in a sequence (SignalP program). All these conditions are mandatory for AMPs according to the current data. In addition, the sequences were filtered based on their length (≤150 amino acids).

The data from all assemblies were combined and repetitions were removed. The search was performed for 31 known cysteine motifs. 18964 sequences containing at least 1 motif were found. At the end of filtration step 202 potential AMPs were revealed satisfying the stringent conditions described above. The well-known SPADA software has revealed only 99 proteins in this dataset. Earlier only 10 LG AMPs were obtained by biochemical methods, and all of them were among 202 sequences described above. 55 potential AMPs had significant similarity with cysteine-rich proteins of other members of *Poaceae* family.

A new *in silico* method for AMP search in plant transcriptomes was developed which allowed to reveal novel potential AMPs from LG. The presence most AMPs was confirmed by gene isolation and cloning. The method developed can be applied to any plant transcriptome and its results will greatly facilitate the investigation of plant immune system and obtaining of crops with higher tolerance to AEF and pathogens.

Keywords: Antimicrobial peptide, Bioinformatics tools, Transcriptome sequencing.

MON-498
Revision of genus *Polygonum* L.s.l. in flora of Armenia

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The account of genus Polygonum L. in 'Flora of Armenia' was made more than five decades ago. After that many expeditions have been carried out in different regions of Armenia and a huge herbarium material has been collected. The genus included 5 sections with 20 species. Since then many authors accepted the sections as separate genera on the basis of anatomical, morphological, palynological and molecular data. According to the above mentioned it became clear, that the taxonomy of Armenian representatives of Polygonum s. l. also needs revision. New literature data and our investigations of live and herbarium material (ERE, LE) with specification of the morphological characters, distribution, ecology, flowering and fruiting terms brought us to conclusion, that genus Polygonum s. l. has to be split into 5 different genera (Aconogonon (Meisn.) Reichenb., Bistorta (L.) Scop., Fallopia Adans., Persicaria Mill., Polygonum L. s. s.). The number of species has been reduced to 16 species. For each genus new determination keys has been created.

Keywords: Flora of Armenia, Genus Polygonum.

MON-499
Selection of DNA aptamers to *B. anthracis* lethal factor

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We obtained a panel of DNA aptamers binding to *B. anthracis* lethal factor (LF) with high affinity. LF was produced in *E. coli* as a fusion with SUMO peptide, tagged at the N-terminus by the 16 amino acid *in vivo* biotinylated peptide. The fusion protein was immobilized on streptavidin-coated paramagnetic particles and mixed with random DNA aptamer library followed by washing of the particles to remove unbound DNA. LF with bound aptamers was cleaved off the complex by SUMO protease. DNA was amplified by PCR with 5'-FAM-labeled primer and subsequently treated by 5′ lambda phage exonuclease to obtain single-stranded DNA for further round of selection. After 4 rounds, selected DNA pool was cloned into plasmid vector. Individual aptamers were isolated from the selected pool using PCR with biotinylated primer and tested for LF-specific binding by solid phase assay with neutravidin peroxidase.

Binding constants of the most efficiently binding aptamers were determined by surface plasmon resonance technique. High-throughput sequencing and subsequent analysis of the selected pool using DNA multiple alignment tools, clearly identified 4 aptamer families sharing clearly identifiable primary structure motifs. The developed panel of LF-specific DNA aptamers can be further applied for elaboration of therapeutic and diagnostic tools for prophylaxis and treatment of anthrax.

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The work is supported by research grant 14-15-00630 from Russian Science Foundation.
Keywords: B.anthracis, DNA aptamer, lethal factor.

MON-500
siRNA-bioportide conjugates as a Chronic Myeloid Leukemia gene therapy targeting the BCR/ABL fusion gene

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Chronic Myeloid Leukemia (CML) is a myeloproliferative disorder caused by a single genetic mutation, a reciprocal translocation that originates the BCR-ABL gene with constitutive tyrosine kinase activity. Because there is a specific gene associated to this pathology it is an optimum therapeutic target for RNA silencing therapy (siRNA). We developed a siRNA-based therapeutic approach in which the siRNA is delivered by Dengue Virus Capsid Protein-derived Cell penetrating peptides (CPP), pepM and pepR. Moreover these peptide vectors have dual role in this technology, to deliver siRNA into cells and act as bioportides, cell-penetrating peptides with bioactive intracellular properties by targeting signaling events.

Dengue virus-peptides ability to transfect the positive BCR-ABL^{+} Cell Line (BV-173) was evaluated by confocal microscopy following GFP fluorescence emission after plasmid expression. Anti-BCR-ABL siRNA design was performed using a siRNA design web-tool and the BCR-ABL downregulation kinetics (48 h to 168 h) after transfection by Dengue virus-peptides was evaluated by RT-PCR. The bioportide action of DENV vectors was assessed by genome-wide analysis microarray and further validated by testing BV 173 cell cycle and cell proliferation.

siRNA design for BCR-ABL retrieved 148 potential siRNA sequences, which were reduced to 5 BCR-ABL siRNA for *in vitro* analysis after thorough screening. Positive efficacy of siRNA targeting BCR-ABL was tested using a commercial transfection agent. Significant BCR-ABL gene knockdown were observed for siRNA #3 when delivered by pepM with maximum decrease at 120 h. Both pepM and pepR showed downregulation effects on proliferative CML cancer-related signaling pathways having direct impact on BV 173 cell cycle and proliferation.

With this work we showed the potential therapeutic technology of combining a drug delivery system (CPP) with bioportide properties to deliver functional siRNA into CML cell models. Acting in synergism, these conjugates significantly decreased BCR-ABL gene expression levels, and perturbed leukemogenic cells homeostasis, revealing a potential scaffold to develop an alternative CML therapy.

Keywords: Chronic myeloid leukemia, siRNA-peptide complex.

MON-501
siRNA-Bioportide conjugates as a Chronic Myeloid Leukemia gene therapy targeting the BCR/ABL fusion gene

J. M. Freire[1], I. Rego de Figueiredo[1], B. G. de la Torre[2], A. S. Veiga[3], D. Andreu[2], F. Enguita[3], M. Castanho[3], CML
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Keywords: Chronic Myeloid Leukemia, Dengue virus capsid protein, siRNA-peptide complex, Bioportide.

MON-502
Solution NMR study of viral macro domains

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Macro domains are evolutionarily conserved in eukaryotic organisms, bacteria and archaea, indicating important basic biological function. Furthermore, they are found in nonstructural proteins (nsPs) of several positive-strand RNA viruses, including hepatitis E virus, rubella virus and coronaviruses, as well as alphaviruses. There are limited information on the viral macro domains' but it has been suggested that they act as an ADP-ribose-binding module, while they are also involved in ADP-ribose metabolism and post-translation modification.

In this study we apply NMR spectroscopy to investigate the conformational properties and dynamics of four macro domains: (a) two from New World alphavirus (Mayaro & Venezuelan equine encephalitis virus), (b) one Old world alphavirus (Chikungunya virus) and (c) one from the Hepevirus genus (HEV-1).

The four macro domains are cloned and expressed with Poly (His)tag, in high yields in *E. Coli*. All protein constructs are soluble and, using *E.coli* culture supplements prior to induction in typical (M9) minimal media, the bacteria growth rates and protein yield were generally increased. Initial 1D ^{1}H & 2D ^{1}H-^{15}N HSQC NMR experiments suggest that all four macro domains are folded in solution. Acquisition and analysis of 2D/3D homo/heteronuclear NMR data are underway.

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Keywords: alpha virus, macro domain, NMR Spectroscopy.

MON-503
Spherical virus-like particles generated by thermal denaturation of helical Potato virus X

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Recently the spherical particles (SPs), generated by thermal denaturation and structural remodeling of rigid rod-like tobacco mosaic virus (TMV) were obtained and described [1]. The evidences that SPs are stable, highly immunogenic and biologically safe were provided. The size of SPs is possible to be controlled. The ability to bind target proteins of different nature on the surface of SPs was demonstrated. The immunogenicity of target protein within complexes with SPs was examined by subcutaneous and intraperitoneal immunization of mice. In both cases of immunizations the titers of serum antibodies to target protein increased dramatically. Thus, adjuvant properties of TMV spherical particles generated by thermal treatment of virions were demonstrated [2]. In this work the possibility of thermal transition of helical filamentous Potato virus X (PVX, genus *Potexvirus,* family *Alphaflexiviridae)* virion to structural remodeling particles was examined. The formation of virus-like particles (VLP) with morphology similar to the spherical shape by thermal treatment at 90 °C was demonstrated. In the case of PVX, spherical VLPs thermal transition requires a lower treatment temperature in comparison with TMV. The diameter of the VLPs was in ranges of 50 nm and more. Unlike TMV SPs, the size of structural modified PVX VLPs does not depend on the initial virus concentration. PVX RNA was degraded and completely released following VLPs generation. However no degradation or fragmentation of PVX coat protein occurred upon PVX heating and VLPs formation. The VLPs and native PVX virions densities were compared and significantly difference of density values was registered. The high adsorption property of structural modified PVX VLPs was founded. PVX-generated spherical particles are capable forming compositions with model protein bound to PVX SPs surface. Represented spherical virus-like particles generated by thermal treatment of helical filamentous PVX virions as well as TMV-generated SPs could become a promising platform for the design and assembly of functional active complexes.

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Keywords: functional active complexes, plant virus, virus-like particles.

MON-504
Study of role of light constant domain on catalytic antibodies structural organization and its functionality

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One of the most sophisticated aspects in the design of artificial antibodies is to provide for their proper catalytic function, which requires accurate structural information. Here, the results showing how the antibody domain structure affects its functionality, is presented. The previously designed organophosphate-metabolizing *reactibody* A17 has been re-engineered by replacing its constant κ light chain by the l chain, and the A17 l X-ray structure has been solved at 1.95Å. It has been found that the active center of A17 l, compared to A17κ, is displaced, stabilized and becomes more rigid due to interdomain interactions involving the CDR loops from the V_L and V_H domains. These V_L/V_H domains also display lower mobility as was deduced from the atomic displacement parameters of the crystal structure. The Trp-L92 residue is flipped out to form a lid over the entrance to active center pocket. The antibody elbow angle is decreased to 126°, compared to 138° in A17κ. These structural differences leads to subtle changes in catalytic efficiency and thermodynamic parameters determined with two organophosphate ligands, as well as in affinity for peptide substrates selected from combinatorial cyclic peptide library, between A17κ and A17 l variants. Presented data will be of interest and relevance to researchers dealing with the design of antibodies with tailor-made functions.

Keywords: None.

MON-505
Study on binding of signal recognition particles to signal-peptides

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Many proteins are biosynthesized in ribosomes and have endoplasmic reticulum (ER)-targeting signals. The ER-targeting signals in the N-terminus, called signal-peptides, are separated from mature proteins by signal-peptidase after being transported to the ER. In the early phase of protein translation, biosynthesized signal-peptides appear on the surface of ribosomes, and signal recognition particles (SRP), cytosolic ribonucleoprotein complexes, recognize and interact with signal-peptides. The SRP-signal-peptide-ribosome complex binds to the SRP receptor on the ER membrane and is then transported to the translocons in the ER membrane. By means of these steps, mature proteins are transported first into the ER by translocons and then located to the appropriate subcellular localization based on the transport mechanism of each protein by intracellular vesicular traffic.

Many varieties of signal-peptide sequences exist and have regions with high hydrophobicity (h-region), however, the reasons why signal-peptide sequences are not unique have not been clarified. Nevertheless, SRP can bind signal-peptides which have many varieties of sequences. SRP54, one component of SRP, is thought especially to bind to signal-peptides. As SRP54 has a hydrophobic groove formed between the three alpha helices (h1,

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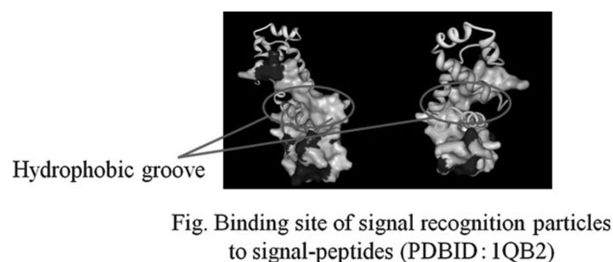

Fig. 1. Binding site of signal recognition particles to signal-peptides (PDBID : 1QB2).

h2 and h3) and the loop stemming from h2 and h3, SRP54 is thought to bind the h-region of each signal-peptide by hydrophobic interactions [1].

In this study, the characteristics of the amino acid sequences upstream from h-regions in signal-peptides were extracted, and the probability of whether or not the sequences were recognized by SRP was examined. The sequence dataset of mammalian proteins which have signal-peptides was first extracted from UniProt Knowledgebase/Swiss-Prot release 2014_01. Based on the fact that the length of the h-region in each signal-peptide was different, the h-region sequences were defined by the annotation of the length of the transmembrane region. Taking into consideration, the propensities, physicochemical properties and hydrophobicity of the amino acids, extracted sequences were classified into clusters. The correlation between the signal-peptide clusters and the subcellular localization of mature proteins was investigated in this study.

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Keywords: Analysis of amino acid sequences, Bioinformatics, signal peptide.

MON-506
The in vitro micro-environment interface: macromolecular crowding meets cell-sheet tissue engineering

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Introduction: Advancements in biology and chemistry have enabled development of scaffold-free substitutes; a technology termed as Tissue Engineering by Self-Assembly (TESA). Despite efficacious *in vitro* and *in vivo* results, few products have been commercialised, primarily due to prolonged culture time. It has been demonstrated that macromolecular crowding (MMC) enhances deposition of extracellular matrix (ECM). Here, we assessed the potential of combining MMC technology with TESA approach.

Materials and Methods: Human fibroblasts, tenocytes and osteoblasts were cultured under various MMC conditions [dextran sulphate (DxS); Ficolls & carrageenan (CR)] in a range of fetal bovine (FBS) and human serum (HS) concentrations. ECM deposition was verified by SDS-PAGE, immunocytochemistry

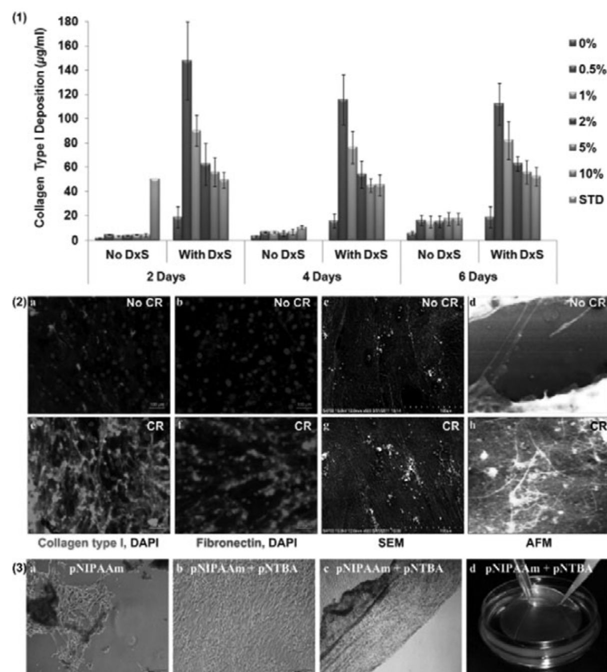

Fig. 1.

(ICC), mass-spectrometry (MS), atomic force (AFM), scanning electron microscopy (SEM). The MMC molecules were characterized by dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA). The cell morphology, cell viability and metabolic activity were evaluated using phase-contrast microscopy, Live/Dead® and alamarBlue® assays respectively. NIPAM based thermo-responsive polymers were developed to facilitate detachment of ECM-rich cell-sheets.

Results: SDS-PAGE demonstrated that MMC significantly increase type-I collagen deposition ($p < 0.001$) at all tested serum (figure-1). ICC, AFM and SEM further confirmed enhanced deposition of fibrillar ECM in presence of MMC (figure-2, a-h). DLS and NTA demonstrated that CR has highest polydispersity among all tested crowders. The cellular morphology, viability and metabolic activity were not affected by MMC. Thermo-responsive coating with Poly(N-isopropylacrylamide) and Poly (N-tert-butylacrylamide) facilitated detachment of ECM rich cell-sheet (figure-3, a-d). Complementary ICC for MS validation confirmed the enhanced deposition of collagens (III, IV, V, VI) and other ECM molecules (laminin, fibronectin, hyaluronic acid, decorin, lysyl oxidase), without changing collagen-VII, elastin, fibrillin-1, transglutaminase-2, α-actin, keratin, tubulin, chondroitin sulphate, keratin sulphate, heparin sulphate, aggrecan, biglycan, CD248 and IL-10.

Discussion & Conclusions: Modulation of the *in vitro* microenvironment with polydispersed macromolecular crowders enhances ECM deposition even under low serum supplementation and facilitates the production of intact ECM rich cell-sheets.

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Keywords: Cell-sheet technology, Extracellular matrix, Macromolecular crowding.

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MON-507

The influence of atrial cardioactive peptides isolated from pigs heart on ECG components under condition of recording of neuronal pulse activity of bulbar cardiovascular center

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Problem statement: Morphological and biochemical studies allowed defining a fact of the peptides synthesis in contractile cells of atria myocardium released to blood, which control and regulate hemodynamic in the organism. Discovery and further intense study of the peptide regulatory system of heart carried out by A.A.Galoyan, R.M Srapionyan. and coworkers, appeared in essence to be an answer to the question about the role of the peptide nature synthesized and released by atria. From the point of view of the above mentioned issue, our goal of primary importance was the isolation, purification, structural and functional characteristics of cardioactive compounds of peptide nature from atria and auricular region of pig heart, which possess exceptionally high specificity in reference to different aspects of functional activity of cardio-vascular system.
Methods: The studied issue is rather than actual, being complicated and requesting high quality platform in methodology. There were used various systems allowing to control the purity level of the preparations including HPLC, mass-spectrometry, etc. The estimation of amino acid sequence was done by steps in an Edman degradation method and NMR-analysis, which resulted in defining the structure of one of the atriopeptides, conditionally named AP-7.
Results: In spite of an intense search on cardioactive compounds the further studies related to cardiopeptides, remain relevant. In turn, of a particular importance are drugs that act on the individual components of the ECG or its complexes related to diseases. Seven samples of cardioactive peptides obtained by us were tested. Sample 1 equally increases the amplitude of P, R, S, T teeth, QRS complex, and somewhat reduces the amplitude of the wave Q. Sample 2 increases the amplitude of all peaks and QRS, dramatically reduces the amplitude of the Q wave and increases the duration of the complexes. Sample 6 unevenly overestimates the amplitude of all teeth, especially the T, with the exception of «Q», the amplitude of which is greatly reduced by the end of the test. Sample 7 unevenly and slightly decreases and overestimates the amplitude of all components, except for Q, the amplitude of which is greatly reduced and overestimated the duration of complexes.
Keywords: atrial peptides, HPLC analysis, PQRST complexes.

MON-508

Transport pathway of GFP protein including signal-peptide and GPI-attachment signal

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Glycosylphosphatidylinositol (GPI) is a post-translational modification molecule which can anchor proteins to the plasma membrane. Proteins modified by GPI, called GPI-anchored proteins (GPI-APs), are localized to microdomains known as rafts via the endoplasmic reticulum (ER). GPI-APs have two signal sequences, signal-peptides (SP) and GPI-attachment signals (GPI-AS). These are N-terminal ER localization sequences and C-terminal signal sequences for GPI modification, respectively. Localization signals are able to transport proteins to specific organelles, one of them being SP which transports proteins to the ER. Most GPI-APs are transported to the ER according to the function of their SP and then modified GPI. GPI-AS is necessary for the post-translational GPI modification and also plays an important role as a raft localization signal.

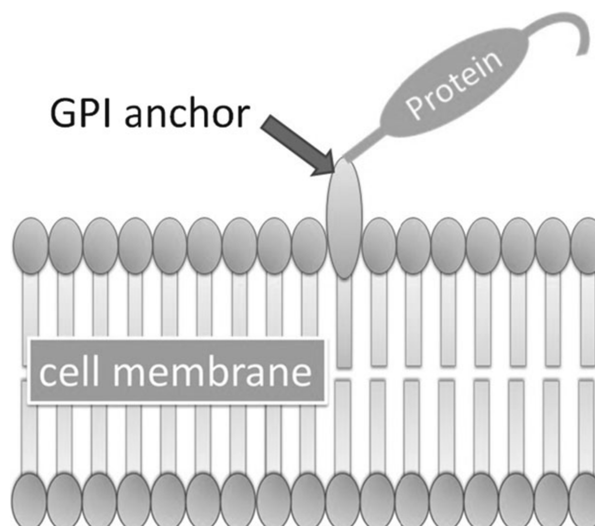

Fig. 1.

When lacking GPI modification, intractable blood diseases and anemia often appear. The relationship between excessive synthesis/loss of GPI-APs and cancers has also been noted, thus GPI modification is thought to have a strong relation with human disease. Therefore the control of GPI modification is expected to be applicable for medical and pharmaceutical fields.

In this study, to understand the sequence patterns for GPI modification in detail, artificial signal sequences were designed based on the amino acid propensities of known SP and GPI-AP sequences. The green fluorescent protein (GFP) which includes two artificial signal sequences, SP and GPI-AS, was expressed in HeLa cells. Subcellular localization of GFP fusion protein was observed by confocal laser fluorescence microscope. As a result, variation in signal sequences has a direct relation to transport pathway patterns. Two signal sequences-GFP/mCherry fusion proteins were expressed in HeLa cells, and the transport pathways of these fusion proteins were monitored by fluorescence observation.
Keywords: Signal sequence.

MON-509

Using AraC variants to analyse protease activity in vivo by means of selecting for growth on arabinose

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The AraC protein is a dimeric transcriptional regulator involved in control of arabinose metabolism in *Escherichia coli*. The structure and function of this protein has been analyzed in great detail by Robert Schleif and co-workers (1) over the last four decades. Each monomer consists of an N-terminal dimerization domain linked to a C-terminal DNA binding domain. In the presence of arabinose, the two DNA binding domains of the AraC dimer bind to the two adjacent direct repeats sites in the pBAD promoter, I1 and I2 and induce transcription of the adjacent araA,

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araB and araD (araBAD) genes. Because the AraC molecule and its function is so well characterized, both as a functional unit in itself but also in various artificial variants it serves as an attractive template for tailoring selection systems in our case based on the ability of *E. coli* to grow on arabinose when combining the function of AraC and the activity of any protease activity as outlined below.

We attached an extra DBD to the C-terminus of the WT AraC monomer (AraC-DBD) and showed that this protein induce transcription of araBAD. We then introduced a H213Y mutation in both DNA binding domains (H213YAraC-DBD), a mutation known to change the properties of other artificial AraC analogs towards being a repressor instead of activator (2) and showed that this fusion protein indeed does not induce transcription of araBAD but probably converts the AraC-DBD protein into a repressor of araBAD. The H213Y modification of the AraC itself (H213YAraC) results in only slightly weaker induction of transcription from pBAD compared to wild type AraC.

However, cells expressing the H213YAraC-DBD variant were able to grow on arabinose although much slower than the AraC-DBD variant suggesting that two H213YAraC-DBD proteins still may dimerize, bind DNA and induce transcription of araBAD, but only to a low extent due to the binding competition between the additional DBD in H213YAraC-DBD and that of another molecule of H213YAraC-DBD.

By inserting protease sites in the linker region connecting the H213YAraC-DBD DNA binding domains we aim to design plasmids that in combination with the relevant protease will allow for fast growth of *E. coli* on arabinose. Results from this work establishing such a system will be presented.

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2. Saviola, B., Seabold, R. R., and Schleif, R. F. 1998. DNA Bending by AraC: a Negative Mutant. Journal of Bacteriology 180 (16) 4227–4232.

Keywords: AraC, Protease Specificity, Selection System.

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CSIII-07 – Education, training and career planning in molecular life sciences

MON-511
A European safety sciences for medicines education and training

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Safety issues are major reasons for discontinuing otherwise promising drug candidates from pharmaceutical development. Being able to better forecast unacceptable adverse events is mandatory to avoid failure in later development phases and unnecessary costs. This requires informed use of knowledge and methodologies in different phases in drug discovery and development by trained safety specialists with a strong focus on integrative and translational strategies.

The IMI project SafeSciMET (www.SafeSciMET.eu) has been initiated to address the needs for a pan-European education and training program for such specialists with a holistic view. The partnership between 16 European universities and 14 leading pharmaceutical companies ensures that cutting-edge knowledge in academia and industry is combined with modern competence-directed learning approaches, which will also include e-learning and distance-learning lectures. In autumn 2014, the successful training program will continue with its third course cycle, delivering 20 modular courses between September 2014 and August 2016. Emphasis lies on integrative, translational and 3Rs aspects of safety assessment, covering the whole process of safety sciences in drug development, from first discovery of an component via pre-clinical and clinical phases to end usage and post-marketing safety issues. The modular setup of the programme allows scientists from industry, academia and regulatory authorities to attend selective courses for building up a continuing professional development portfolio or to follow a pre-defined course package with the possibility to receive an Advanced MSc degree in Safety Sciences issued by the German Excellence University of Constance. In a new collaborative effort, called IMI-TRAIN, and supported by an ENSO grant of IMI, several innovative aspects, such as blended learning and CPD competence development, will be implemented in SafeSciMET.

Highlights of every course are the real-life, industry partner provided case studies that are presented and discussed by industrial experts. By transporting and delivering the European perspective, regulations and methodologies in safety sciences and drug approval, this programme is also highly interesting for safety scientists from countries outside Europe.

Keywords: Education and training, European programme, Safety sciences.

MON-512
Anti-aging effects of vitamin E in perennials

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Compared with our knowledge of aging processes in animals, little is still know about age-related changes in plants, especially in perennials. The aim of this poster is to deepen into our present understanding of the mechanisms underlying aging in perennials, with a special emphasis on the function of vitamin E in various aging processes (seed aging, leaf senescence and meristem aging) by using a physiological, biochemical and molecular approach. We study the function of vitamin E in meristematic tissues of perennial plant by using *Cistus albidus* as model plant. We will determine in these studies the function of vitamin E in meristematic tissues, leaf and flower buds in plants of various ages, with a special emphasis on the role of tocopherols as antioxidants and in cellular signaling, by measuring the hormonal profile an expression levels of JUNBRUNNE1 and ERF1 in the regulation of the vitamin E mediated signaling processes. These studies will undoubtedly help us not only to better understand the biochemistry and physiology of vitamin E in plants but also establish the basis to establish a key anti-aging role for vitamin E in perennial plants at different levels of complexity, including seeds, leaves and meristematic tissues.

Keywords: anti-aging, vitamin E, perennials.

MON-513
Anti-thrombotic activity of Corilagin, an ellagitannin isolated from *Phyllanthus niruri* L.

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Phyllanthus niruri L. is an indigenous plant of tropical areas which is known to possess various therapeutic properties and thereby has been used widely as a traditional remedy in many countries. In our study, we tested methanol, ethanol, hexane and chloroform extracts of this plant to inhibit *in vitro* platelet aggregation. The result showed that all extracts possessed the activity in preventing the formation of platelet aggregates upon platelet activation by ADP. The potency of inhibition is 68.8% ± 6.7, 43.5% ± 6.6, 40.1% ± 5.6, 44.7% ± 2.7, respectively, for methanol, ethanol, hexane and chloroform extracts.

Moreover, using high performance liquid chromatography, we have isolated Corilagin (beta-1-O-galloyl-3,6-(R)-hexahydroxydiphenoyl-d-glucose) as one of the major compounds contained in methanol extract that significantly inhibited ADP-induced platelet aggregation *in-vitro*, with the IC50 of 42.8 ± 0.9 μg/mL.

However, there are only a limited number of reports available so far to explain the anti-thrombotic activity of Corilagin and therefore the mechanism of this inhibitory effect are still not clear. To address this question, we have taken a proteomics approach to investigate protein alterations in the aggregation event with or without the presence of Corilagin. The ultimate goal of this study is to reveal anti-thrombotic properties of Corilagin from *Phyllanthus niruri L.*

Keywords: Anti thrombotic, Corilagin, Phyllanthus niruri L.

MON-514
Bench to bedside molecular functional imaging in translational cancer medicine

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On-going research on the malignant and normal cell biology with understanding of biology of the cancer and carcinogenesis has led

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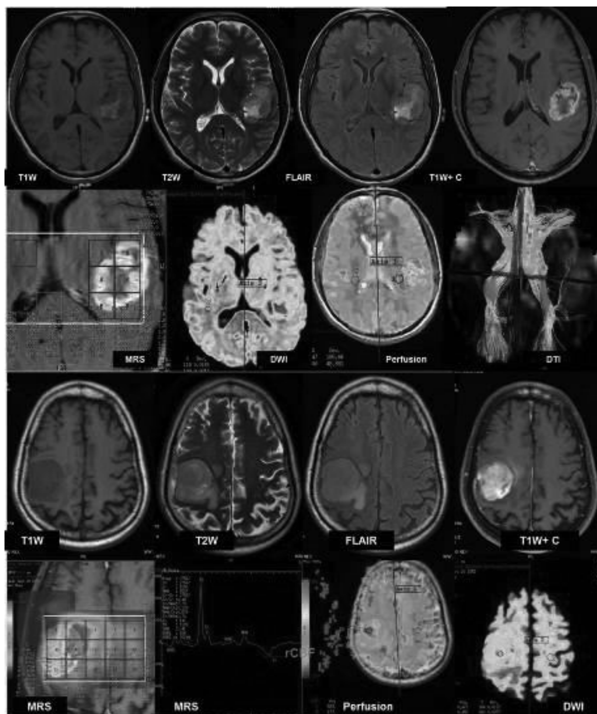

Fig. 1. (A) Glioblastoma Multiforme: molecular status predicted on the bases of the Molecular MRI imaging and correlated with pathology suggests: Meth−, IDH and P53 wild type, EGFR non-amplified, high MIB1 Index, VEGF non-amplified, high Ki-67 index. MRI axial images; the lesion is located in the left pariteal lobe and shows moderate mass effect. The T2 hyperintensity is not homogeneous with a rim of contrast enhancement, low contrast: necrotic radio, high choline levels on MRS, infiltration of the tracts on DWI, High rCBV. (B) Glioblastoma Multiforme: molecular status predicted on the bases of the Molecular MRI imaging and correlated with pathology suggests: Meth+, IDH and P53 Mutated type, EGFR amplified, low MIB1 Index, VEGF amplified, low Ki-67 index. The lesion is located in the right high frontoparietal lobe and shows mass effect and some degree of vasogenic edema. The T2 hyperintensity is more homogeneous. T1 with contrast injection image shows the mixed solid nodular appearance. High contrast: necrotic radio, low choline levels on MRS, displacement of the tracts on DWI, moderate elevation of rCBV.

to development of target specific biological molecules and methods to image the evolution of cancer and image the antitumor effect of these newer therapeutic agents. At the same time there is a paradigm shift happening in the field of cancer imaging from structural imaging to multifunctional imaging at molecular and functional level. Anatomical imaging has been playing a critical role in cancer screening, diagnosis, stating, management, prognosis, assessing success to therapy and follow up. At the same time the evolving molecular functional imaging using molecular targets and ligands has a great potential in translational research, giving specific and quantitate information regarding involved in the screening, diagnoses and management. Molecular functional Imaging has taken a key place in the present era of translational cancer research making an important tool to study targeted receptor therapies, gene therapies and in cancer stem cell research that helps to translate the on-going research into clinical practice. There is need for scientific developments in imaging to match the progresses in the field of the translational research and targeted imaging needs to be developed in close association between the biotechnology, information technology and translational scientists for its best usage. We will review the current role of molecular functional imaging as one of main pillar of translational research that may be translated into clinical applications in the near future for comprehensive cancer management.

Keywords: Molecular Imaging.

MON-515
Developing biochemistry laboratory experiments for different professional and occupational groups: alternative experiments for biochemistry

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There are considerable differences in the way of biochemistry theoretical courses and biochemistry laboratory practicals are taught among different faculties, colleges and vocational schools. In addition programs and curricula vary extensively. Many disiplines of the sciences, life sciences and bioengineering have similar program headings; however presently no agreed upon standardization is available. In order to ensure the compliance requirement (harmonization) of the diploma equivalency for everyone for the European Union Lifelong Learning (LLP) and the Bologna Process, review of the biochemistry laboratory to ensure that needs of private sector, health sector, R&D institutions and finally undergraduate and graduate education are met. Students of biochemistry laboratory, similar to all other fields of study, are often from very diverse backgrounds and different levels of basic knowledge/skills and therefore new teaching methods, program approaches and resource materials are keys and tools to reach the biochemistry harmonization, standardization and competency goals. Initial step could be to elaborate new 'easy-to-implement' experiments with common materials and equipment to chemistry laboratory studies. Along this line, the new approach is expected to bring quality assurance and educational reforms through international cooperation. Biochemistry's interdisciplinary character, placed biochemistry laboratory in a central role. The need for developing new applications using alternative materials leads to modern experimentation to be employed in teaching biochemistry laboratory at different educational establishments. Although biochemistry laboratory education can be implemented in fully equipped laboratories (including molecular biology and biotechnology laboratories); basic biochemistry conceptions and applications that is known to be difficult, can be taught in high quality with the help of principle biochemistry methods/approach and using easily available and low-cost materials, basic laboratory equipment and tools via a new look: These program approaches and new teaching methods facilitate the use of alternative model organisms, promote active student participation, improve students scientific thinking skills, aid innovation, familiarize with scientific research culture. Experiments in the present study were designed and tested in modular form to these aims and are expected to satisfy quality insurance while taking into account the multidisplinary character of biochemistry laboratory studies.

Keywords: biochemistry laboratory education, easy-to-implement experiments, harmonization, standardization.

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MON-516
Didactic sequence for teaching structural molecular biology to various educational levels, through practical and ludic teaching resources

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The understanding of the structure and function of proteins is closely linked to the development of structural biology and an understanding of the whole process involving in 'central dogma of molecular biology', including the importance of structure/function of the molecules involved, is essential for learning this subject. However, students often find difficult to visualize the structures of biomolecules through schematic drawings of figures from textbooks or computer. To help this understanding, we elaborated a didactic sequence, using didactic materials developed by our team, which involved the following practical activities: 1) DNA extraction (from fruits and using domestics reagents, when taught to high school students); 2) building the nucleotides and DNA/RNA structures using the kit of plastic pieces 'Building the molecules life: DNA/RNA'. The kit was used to make also DNA duplication and its transcription to RNA. The translation process used additional materials like as the Amino Acid Disc and the Kit 'Building amino acids and proteins' (primary and secondary structures). In addition, the kit 'Protein Folder' was used with undergraduate and graduate students who could build secondary and tertiary protein structures. This didactic sequence was applied to the following groups in years 2010–2013: a) continuing education courses to basic school teachers (about 1200 teachers); b) undergraduation courses of biological sciences; c) graduation courses of structural biology areas; d) students from high school (more than 2000 students). The courses for teacher included three video conferences, discussion about themes to be proposed for their students, experimental proceedings and two or three face-to-face meetings to build three-dimensional molecules using the kits. In undergraduation courses the students were encouraged to propose and develop a practical activity using the kits. The 'Protein folder' kit was used with graduate students to teach basic and applied concepts on secondary and tertiary structures of different proteins. The courses were evaluated by teachers who pointed out that they became more confident, independent and critical of their own practices, since they had the responsibility in elaborate their teaching strategies as well as evaluating it with their students. The impact in the students learning was made by comparative analysis between a pre- and post-test answered by some of their students (aged 14–17). The results were encouraging, reaching a value of about 76% of correct responses after the activity. The undergraduate and graduate students pointed out that the kits would help them to learn and teach those subjects in a more attractive way, awaking students' curiosity and implementing a dynamic in the classes.

Keywords: didactic resources, protein, structural molecular biology teaching.

MON-517
Identification of phenolic components of *Rosa heckeliana* Tratt roots

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Medicinal plants reveal their therapeutic effects due to high amount of phenolic compounds confined abundantly in roots, fruits, leaves and, flowers. *Rosa heckeliana* Tratt, as a member of Rosa was recently discovered as one of the important medicinal plants used in folk medicine. In this study, the therapeutically notable *R. heckeliana* was selected for the analysis of important phenolic components particularly confined in the roots. The roots were first extracted in methanol, then phenolic constituents were partitioned according to polarity by fractionation method. The method was also supported by the total amount of phenolic compounds. The highest total phenolic content as gallic acid equivalent (8.80 ± 19 μ g/mg) was obtained in the ethyl acetate fraction. Hence, that fraction was further analyzed for the identification of phenolic constituents using a diode array detectors equipped HPLC. Structural information about the compounds was confirmed by high-resolution mass spectrometry. Accordingly, catechin, caffeic acid, epicatechin, p-coumaric acid, scopoletin, ellagic acid, and quercetin were identified in the ethyl acetate fraction. To the best of our knowledge, this is the first detailed effort for the fractionated analysis of phenolic constituents in the roots of *R. heckeliana*.

Keywords: Catechin, HPLC analysis, Rosa heckeliana.

MON-518
Impact and sustainability of hands-on training in the analysis of next-generation sequencing data

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Next-generation sequencing technologies are widely used in the field of functional genomics and applied in an increasing number of applications. For many 'wet lab' scientists, the analysis of the large amount of data generated by such technologies is a major bottleneck that can only be overcome through very specialized training in advanced data analysis methodologies and the use of dedicated bioinformatics software tools.

Here we will discuss the hands-on training activities in the analysis of high-throughput sequencing data that we have developed at the European Bioinformatics Institute and Cambridge University. We will present a snapshot of the audience that we have trained over the last five years, discuss the challenges that we have faced in developing training solutions that fits the needs of this very specialized user community and the best practices that we have embraced to tackle such challenges. We will also provide evidence of the impact such training has had on the research community and discuss our recipe for sustainability.

Keywords: Bioinformatics training, Education, Next-generation sequencing.

MON-519
Multidisciplinary study area molecular nutrition: viewment from nutrition and dietetics postgraduate students

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Background: Nutrition and Dietetics (ND) not only closer to biomedical sciences but also social sciences, since nutrients, phytochemicals, biochemistry, genes and lifestyles interact in human biology. Therefore, it is very important for human wellbeing, quality of life and disease prevention. In this study, it is aim to analyse strengths, weaknesses, opportunities and threats of specializing attempts and efforts of ND postgraduate students on molecular nutrition (MN).

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Method: In this study, the doctoral (Phd) research project on molecular nutrition and the experiences of the ND Phd student were analysed by SWOT Analyse. This analysis was used to determine strengths, weaknesses, opportunities and threats of MN applications of ND postgraduate students.

Results: ND education brings students to combine epidemiological data and molecular mechanisms skills and this gives the strength to transmit the knowledge from molecular based to public. However, lacks at laboratory (lab) applications cause spend much more time and effort to be successful is a weakness. However, at this point while learning new techniques it gives an opportunity to seize new scientific perspectives. Besides that there are some threats. First one is that a few number of ND postgraduate students study on this area since difficulties of the lacks on lab application background. Another threat is that the students who studying on MN can focus much more on lab techniques since improve themselves on lab applications and this can let them away to translation of molecular data to suggestions for public health.

Conclusion: MN which includes nutrigenomics has the potential to lead to evidence-based dietary intervention strategies and related with health, diet, and genomics. This study area requires an understanding of nutrition, genetics, bioinformatics, human physiology, pathology and biochemistry as well as lab techniques and technologies. So, it is often difficult to lead all multidisciplinary information together and translating into prevention and delay of diseases and improve human wellbeing and life quality. According to our previous study, 85.6% of ND bachelor students think that nutritional biochemistry course needs lab applications to be more beneficial. Therefore, there is a need more courses and lab applications on MN in specific topics during ND education at bachelor level. This will give the new scientific perspectives and experiences to students and let them to be much more aware while planning their career. In addition, while dieticians will be encouraged and participated in this research area this will be improved the connection between molecular data and nutritional suggestions to public health.

Keywords: laboratory applications, molecular nutrition, nutrition and dietetics education.

MON-520
Multidisiplinary biochemistry laboratory education; student centered real learning process

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Along with developments in every field in recent decades, need for re-evaluating the systems and reconstructing educational tools has arisen in the field of higher education; and educational reforms were inevitable. Student centered, flexible modules were developed aiming at 'real learning process' for the biochemistry lab. When multidisciplinary character of biochemistry is considered; students from very diverse fields and levels of basic knowledge/skills are required to complete the course and therefore a need for education programmes and resource materials arises. Modular programming for biochemistry laboratory applications/ concepts were designed and tested using one-group pretest posttest design (model). The implementation of the study was realized in Biochemistry Laboratory Course given in third year in Biology Teaching Department. The sample of the study consisted of 43 Biology Teaching students enrolled in their third year of study during the fall semester. All students took the laboratory course for the first time; and had no prior familiarity with biochemistry experimentation. Each module consists of: basic knowledge, materials, necessary equipment, experimental steps, results/discussion. In total three modules were applied to the students. Module 1: Working principles and safety in Biochemistry Laboratory, biochemical calculations. (4 weeks), Module 2: pH meter, acid-base titrations, buffer solutions. (2 weeks), Module 3: Spectrophotometric analysis. (4 weeks). Modules consist of two alternative problem sets and/or experiments presented to the students' choice. In the end of the module, there are self evaluating tools of different question types (open-ended questions, short assays, definitions, pH and solution problems, multiple choice, graphical presentation and interpretation) for the students. Assessment of modular teaching results with the pre- and posttests revealed that there is a significant difference in students' biochemistry academic success after learning with the module ($t(42) = 11.70$, $p = 0.000$). Before learning with the module, average of the students' biochemistry academic achievement points was $X = 9.86$; after implementation of the learning with modules, the mean score increased to 15.02. Modular programming enables active student participation; instructors are mostly limited in guiding the learning process. Each module ensured the dynamic and student centered learning environment where recently preferred new pedagogical strategies were applied. Students with different mental properties were able to learn concepts, knowledge and schemes in similar ways leading to improve biochemistry education.

Keywords: biochemistry laboratory, Modular programming, multidisciplinary health education.

MON-521
Reproducibility in science: how video journals increase research validity and productivity

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Several high-impact studies indicate that an astoundingly low 11–30% of published scientific research is reproducible. The media has implied that scientists are actively practicing poor conduct and falsifying data under the pressure of career considerations. We reject this speculation and instead question the traditional, text-based format of scientific communication. As research methods incorporate new technologies and become increasingly complex, the platform for sharing new techniques remains relatively unchanged. Researchers currently present their dynamic methods as static snapshots manipulated to fit within the limitations of text-based journals. A new generation of science journals is changing that – it employs video technology to capture and share complex research techniques in a dynamic format. Here, we present an overview of the growing field of video publication and discuss its technical challenges, implications for scholarly communication and its adoption by the scientific community. Results from recently conducted case studies will be shared, such as the experiences of research groups at Purdue University and University of Alaska, which indicate that video publications can save a lab up to $15,000 per experiment.

Keywords: None.

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MON-522
EMTRAIN: the European medicines research training network

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Well-trained professionals, who are committed to staying at the leading edge of developments in their field, are the underlying foundation of successful biomedical research. Europe is taking leadership to retain global competitiveness in medicines research and development by building the right environment for innovative education and training. This environment needs to address several challenges. Firstly, lack of mobility across national borders is a major hurdle to Europe's competitiveness: highly qualified professionals may have to undergo retraining to enable them to advance their careers outside of the country or sector in which they received their training. Secondly, there is a perceived gap between the competencies that graduates come out of university with and the competencies that employers need. Thirdly, established mechanisms for maintaining professional competency are being challenged by: the increasingly rapid development of new technologies that unexpectedly displace established ones; the cross-disciplinary, team-working nature of modern science; and

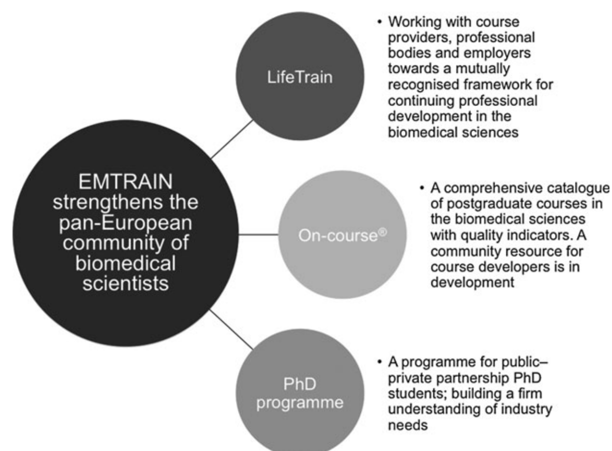

Fig. 1. xxxxxxx.

the need for greater communication and collaboration across traditional boundaries. EMTRAIN is developing a pan-European platform to begin to address some of these issues.

Keywords: Biomedical research, Continuing professional development, Training.

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CSIV-01 – Cancer signalling

TUE-001
Training in data, tools and resources for molecular life scientists

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With the wealth of data being generated by laboratories worldwide, and the depositing of such data into publically accessible databases, there is an increased need for bench biologists to gain the skills required to mine, analyse and interpret this data.

EMBL-European Bioinformatics Institute develops and maintains a variety of data tools and resources for application across the life sciences, but also provides a number of mechanisms for scientists to learn how to become better users of biological data through a range of training opportunities.

Our trainees are diverse, many are from a bench background and at PhD / Postdoctoral level, but whilst they have expertise in their area of scientific interest they are often lacking in computational skill and knowledge of the tools available to them. Our aim is not to produce a new generation of bioinformaticians, but rather to develop a group of scientists who are more confident users of data tools and resources.

We have created a programme that delivers training in three modes – on-site, off-site and on-line. This enables us to provide training to a wide audience, and to provide different focus for courses, from subject specific analysis through to advanced use of a specific resource.

Providing such a programme is challenging due to the diverse nature of our potential trainees, but we present here the approach we take to meeting these challenges and to providing training that enables life scientists to become more confident data users.

Keywords: data analysis, Life scientists, Training.

TUE-002
14-3-3 sigma regulates epithelial-mesenchymal transition through c Jun-Slug aixs

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The role of 14-3-3 proteins has been well established in regulation of cell cycle, DNA damage, regulation of gene expression, cytoskeletal dynamics, regulation of cell to cell adhesion, cancer progression etc.

We have used HCT116 based 14-3-3 σ wild type and null cells to determine the effect of 14-3-3 σ loss in the context of cancer progression. We observed that 14-3-3 σ loss leads to loss of epithelial markers e.g. E-cadherin, cytokeratin, plakoglobin and gain of mesenchymal markers e.g. N-cadherin and vimentin. Re-expression of 14-3-3σ in these cells leads to a reversal of these phenotypes, suggesting that the observed phenotypes are due to loss of 14-3-3σ.

We also observed an increase in cell migration, decrease in cell to cell adhesion and decreased cell to extra cellular matrix adhesion on loss of 14-3-3σ. These results suggested that loss of 14-3-3σ lead to EMT.

Next we determined the expression levels of Snail, Slug, Zeb and Twist as EMT specific transcription factors. We observed increased expression of Slug and Zeb1 in 14-3-3σ null cells as compared to 14-3-3σ wild type cells. We have also identified c-JUN, as an upstream transcription factor which is found to be stabilized at protein level in 14-3-3 σ null cells, whereas, in 14-3-3σ wild type cells c JUN was targeted to proteasomal degradation. c- JUN is known to bind slug promoter and up-regulate Slug at transcriptional level. We have also demonstrated in vitro and in vivo interaction of 14-3-3 σ and c JUN. This interaction might be important for c JUN degradation.

Our results have identified a novel mechanism suggesting presence of a c JUN-slug axis which can promote EMT on loss of 14-3-3 σ. In this conference we will discuss on how 14-3-3 σ regulates c JUN protein stabilty.

Keywords: 14-3-3 Sigma, c Jun and Slug, Epithelial-Mesenchymal Transition.

TUE-003
A genetically encoded biosensor to monitor Tumour Suppressor PTEN function in living cells

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Tumour Suppressor PTEN is a non-redundant phosphatase that is an essential negative regulator of the oncogenic pro-survival PI3K/AKT pathway. The ability to directly monitor PTEN conformation and function in a rapid, sensitive manner is a key step towards developing anti-cancer drugs aimed at enhancing or restoring PTEN-dependent pathways. We developed an intramolecular bioluminescence resonance energy transfer (BRET)-based biosensor, where PTEN is inserted between the energy donor *Renilla* luciferase and the acceptor yellow fluorescent protein. The biosensor retains intrinsic biological properties of PTEN and enables both structure-function and kinetic analyses in live cells. BRET shifts, indicating conformational change, were detected following mutations that disrupt intramolecular PTEN interactions, promoting its targeting to the plasma membrane, and also following physiological activation of PTEN. Using the biosensor as a reporter we unveiled PTEN activation by several G protein-coupled receptors, previously unknown as PTEN regulators. Trastuzumab, an antibody used to treat ERBB2-overexpressing human breast cancers, also produced activation-associated PTEN conformational rearrangement. We propose the biosensor can be used to identify signalling pathways that regulate PTEN or molecules that enhance its anti-tumour activity.

Keywords: Biosensor, PI3K/Akt, PTEN.

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TUE-004
A mitochondrial switch promotes tumor metastasis

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Cancers evolve a subpopulation of tumor cells that metabolically rely on glycolysis uncoupled from oxidative phosphorylation irrespectively of oxygen availability (aerobic glycolysis). Given that most metastases are abnormally avid for glucose (which is the rationale for their clinical detection using FDG-PET) and because clinical data show a positive correlation between lactate production and tumor metastasis, we reasoned that cells performing aerobic glycolysis could constitute a population of metastatic progenitor cells that would remain glycolytic in the blood stream. We found a different metabolic phenotype, though. Indeed, using serial rounds of *in vitro* selection of highly invasive tumor cells (starting from wild-type SiHa human cervix adenocarcinoma cells) and in vivo selection of supermetastatic tumor cells (starting from B16-F10 mouse melanoma cells), we identified a mitochondrial switch corresponding to an overload of the TCA cycle with preserved mitochondrial functions (including ATP production) but increased mitochondrial superoxide production. The switch provided a metastatic advantage which was phenocopied by moderate OXPHOS inhibition associated with mild mitochondrial superoxide increase. Thus, two different events, OXPHOS overload or moderate OXPHOS inhibition, promote superoxide-dependent tumor cell migration, invasion, clonogenicity, and metastasis; demonstrating the central role of mitochondrial superoxide generation in the pathogenesis of metastasis. Consequently, we report that mitochondria-specific superoxide scavenging (using mitoTEMPO or mitoQ) inhibits metastatic dissemination from primary mouse and human tumors, which opens a new avenue for the therapeutic prevention of tumor metastasis.

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Keywords: Metabolism, Metastasis, Mitochondrial dysfunction.

TUE-005
A novel small molecule inhibitor suppresses tumor angiogenesis through targeting vascular endothelial growth factor receptor signlaing

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Angiogenesis occurs during tissue growth and development, and tumour angiogenesis plays an essential in cancer progression. Vascular endothelial growth factor (VEGF) plays a critical factor in the induction of angiogenesis, which therefore has become an attractive target for anticancer treatment. In this study, we evaluated the effect of Wth02, a henna extract derivative, and its inhibitory mechanisms on angiogenesis of human umbilical vascular endothelial cells (HUVECs). Wth02 concentration-dependently inhibited VEGF-induced proliferation, migration and tube formation of HUVECs. Wth02 also suppressed VEGF-induced microvessel sprouting from aortic rings *ex vivo* and suppressed neovascularization of implanted matrigel plugs *in vivo*. Wth02 inhibited VEGF-induced phosphorylation of VEGFR2 and the downstream protein kinases including Akt, focal adhesion kinase (FAK), ERK and Src. Results from in vitro kinase assay further demonstrated that Wth02 suppressed the kinase activity of VEGFR2. Using a colorectal cancer mouse xenograft model, we found that Wth02 treatment caused a decrease in both tumor volume and tumor weight in Balb/C nude mice. Consequently, the inhibitory effect of Wth02 on these pathways resulted in the inhibition of tumor angiogenesis as demonstrated by the decrease of microvessel density in tumor tissues. Altogether, our findings suggest that Wth02 inhibits VEGF- and tumor cells Hep G2 and HCT116-induced angiogenesis and is a potential drug candidate in anti-cancer therapy. Downregulation of VEGFR2-mediated signaling may contribute to its anti-angiogenic actions.

Keywords: angiogenesis, human umbilical vascular endothelial cells (HUVECs), vascular endothelial growth factor (VEGF).

TUE-006
A recombinant protein PinX1 suppresses proliferation of breast cancer cells via telomerase inhibition

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Significantly increased levels of telomerase expression and activity have been observed in nearly 90% of the cancer cells, making it a potential target for anti-cancer drugs. Serval anti-tumor drugs targeting telomere and telomerase such as GRN163L were developed and enter into clinical trials (phase I/II), but there are some disadvantages such as low specify and high toxicity in small molecule drugs, protein drugs become a novel way to anti-tumors because of their high specificity and low toxicity. The PinX1 protein binds directly to the hTERT and suppresses telomerase activity, so PinX1 might be developed as a protein anti-tumor drug. The small HIV Tat peptide of 11 amino acids (YGRKKRRQRRR, TAT) is capable of translocating cargoes of different molecules across the cell membrane. In this study we have generated a recombinant TAT-PinX1-C protein and examined the efficiency and mechanism of the protein in suppression of breast cancer cell growth and tumorigenicity. The protein inhibits telomerase activity in vitro and decreases TRF Length of breast cancer cells, inhibits proliferation via inducing apoptosis and altering cell cycle distribution of breast cancer cells. We will test the anti-tumor activity in xenograft nude mice next. These results suggest the potential of TAT-PinX1-C in targeted therapy of breast cancer.

Keywords: breast cancer, PinX1, telomerase inhibition.

TUE-007
A selective activator of protein kinase C discovered using yeast

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The Protein Kinase C (PKC) is a family of serine/threonine kinases with at least ten isoforms divided into three major subfamilies: classical (cPKCα, βI, βII and γ), novel (nPKCδ, ε, η and -θ) and atypical (aPKCζ and $\lambda\backslash\iota$) [1]. In recent years, PKC isoforms have emerged as striking anticancer drug targets. Particularly, PKCδ

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has been the focus of intense study due to its well-known pro-apoptotic functions [1]. Therefore, the discovery of selective modulators of individual PKC isoforms has proved to be a promising strategy in anticancer treatment. In this way, *Saccharomyces cerevisiae* cells expressing individual mammalian PKC isoforms have been used by our group to search for isoform-selective PKC modulators. In this yeast assay, PKC activators cause growth inhibition, without affecting the growth of control yeast (transformed with the empty vector) [2].

In this work, the yeast assay was used to study the modulatory activity of four semisynthetic diterpenic compounds (C1 - C4), from the Simões' research group (iMed. UL), on mammalian PKCα, βI, δ, ε and ζ. The obtained results showed that C1 had no effect on PKCs, whereas C2 and C3 induced a significant growth inhibition in yeast cells expressing PKCα, βI, δ, ε or ζ, without affecting the growth of control yeast. Notably, C4 significantly inhibited the growth of yeast expressing PKCδ, without interfering with the growth of yeast expressing PKCα, βI, ε or ζ, and control yeast. At the same time, the C4-induced growth inhibition in yeast expressing PKCδ was associated with an increase of DNA fragmentation (apoptosis), without loss of plasma membrane integrity (necrosis). The selectivity and direct activation of PKCδ by C4 was further confirmed using an *in vitro* PKC assay. Moreover, it was shown that C4 was a potent inhibitor of tumour cell growth, an effect associated to the induction of apoptosis.

In conclusion, a putative PKCδ selective activator (C4) was identified in yeast. Promising pharmacological applications may be therefore envisaged for C4, as the first non-peptide selective activator of PKCδ, and as a potential anticancer agent.

[1] Reyland, ME, *Frontiers in Bioscience* **2009**; 14, 2386–2399.

[2] Coutinho, I *et al., Biochem Pharmacol.* **2009**; 78, 449–459.

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Keywords: anticancer, Protein Kinase C, yeast.

TUE-008

Achillea teretifolia Willd. extracts trigger the apoptosis by suppressing bcl-2 expression and activating caspase-3 and bax genes in prostate cancer cell lines

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The species of *Achillea* L. belonging to *Asteraceae (Compositae)*, the largest family of vascular plants, have been widely used by local people as folk or traditional herbal medicines since ancient time and most of them have therapeutic applications. *Achillea teretifolia* Willd. is an endemic plant for Turkey. In this study, its water and methanol extracts were investigated for apoptosis induction on prostate cancer (DU145, PC-3) and gingival fibroblast cells as control group. Normal and cancer cells were treated with IC50 value of the extracts detected by MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay. For the detection of apoptotic and antiapoptotic effects, quantitative real-time polymerase chain reaction (RT-PCR) was used to detect the relative expression levels of *bax, caspase-3* and *bcl-2* genes. All experiments were performed in triplicate. RT-PCR analysis demonstrated that following exposure of DU145 and PC-3 cells to water and methanol extracts of *A.teretifolia*, the level of mRNA expressions of pro-apoptotic proteins (*bax* and *caspase-3*) were significantly up-regulated ($p < 0.05$), whereas the expression of anti-apoptotic proteins (*bcl-2*) were down-regulated ($p < 0.05$). In gingival fibroblast cells, apoptosis induction triggered by *A.teretifolia* extracts was not detected. Furthermore, methanol extract had more powerful apoptotic effect on prostate cancer cells than water extract. As a conclusion, it is the first report showing that *A. teretifolia* extracts triggered the apoptosis in DU145 and PC-3 cancer cells via protein activation of *bax* and *caspase-3*.

Keywords: Achillea teretifolia, apoptosis, cytotoxicity.

TUE-009

Adaptor protein Ruk$_l$/CIN85 overexpression results in the development of stemness characteristics of MCF-7 breast adenocarcinoma cells

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According to modern concept of basic cancer biology, tumors consist of many cell populations with different characteristics and functions. Recent studies indicate that subpopulation of tumor cells with tumor-initiating ability (cancer stem cells, CSCs) play an important role in acquired resistance to chemotherapy and radiation, tumor dissemination and metastasis. A number of agents capable of targeting CSCs in preclinical models are currently entering clinical trials. Beyond all doubt, the further identification of signaling components that regulate biology of these cells will lead to the development of new anti-tumor drugs for comprehensive treatment of oncological diseases. Our previous data demonstrate that overexpression of adaptor/scaffold protein Ruk$_l$/CIN85 in weakly invasive MCF-7 breast adenocarcinoma cells result in their conversion into a more malignant phenotype. The aim of this study was to elucidate the possible role of Ruk$_l$/CIN85 in the development and maintaining of MCF-7 CSCs' population. Overexpression of Ruk$_l$/CIN85 in MCF-7 cells was accompanied by increased manifestation of CSCs' characteristics such as the ability to form mammoshperes, the high number of CD44$^+$/CD24$^-$ cells as well the resistance to chemotherapy drugs. We suggest that enhanced chemoresistence of Ruk$_l$/CIN85-overexpressing cells can be connected with activation of aldehyde dehydrogenase enzyme, ABC membrane transporters, Akt, and mTOR kinases, NF-κB transcription factor and increased resistance to apoptosis. Interestingly, knocking down of Ruk$_l$/CIN85 using specific *shRNA* lentivirus partially reversed the observed effects of Ruk$_l$/CIN85 overexpression on MCF-7 stemness characteristics. The data obtained indicate the involvement of adaptor protein Ruk$_l$/CIN85 in the development and maintaining of stemness potential of breast adenocarcinoma MCF-7 cells.

Keywords: adaptor/scaffold protein Rukl/CIN85, breast adenocarcinoma MCF-7 cells, cancer stem cells.

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TUE-010

Adaptor proteins intersectin 1 and 2 bind similar proline-rich ligands but are differentially recognized by SH2 domain-containing proteins

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Background: Scaffolding proteins of the intersectin (ITSN) family, ITSN1 and ITSN2, are crucial for the initiation stage of clathrin-mediated endocytosis. These proteins are closely related but have implications in distinct pathologies. To determine how these proteins could be segregated in certain cell pathways we performed a comparative study of ITSNs.

Methodology/Principal Findings: We have shown that endogenous ITSN1 and ITSN2 colocalize and form a complex in cells. A structural comparison of five SH3 domains, which mediate most ITSNs protein-protein interactions, demonstrated a similarity of their ligand-binding sites. We showed that the SH3 domains of ITSN2 bound well-established interactors of ITSN1 as well as newly identified for ITSNs protein partners. A search for a novel interacting interface revealed multiple tyrosines that could be phosphorylated in ITSN2. Phosphorylation of ITSN2 isoforms but not ITSN1-S was observed in various cell lines. EGF stimulation of HeLa cells enhanced tyrosine phosphorylation of ITSN2 isoforms and enabled recognition by the SH2 domains of the Fyn, Fgr and Abl1 kinases, the regulatory subunit of PI3K, the adaptor proteins Grb2 and Crk, and phospholipase C gamma. Mentioned SH2 domains were unable to bind ITSN1-S.

Conclusions/Significance: Our results indicate that during evolution of vertebrates ITSN2 acquired novel protein-interaction interface that allows its specific recognition by the SH2 domains of signaling proteins. We propose that these data could be important to understand the functional diversity of paralogous ITSN proteins and establishing their roles in diseases.

Keywords: adaptor protein, protein-protein interactions, tyrosine phosphorylation.

TUE-011

Adenylate kinase 2, an associated activator of DUSP26 phosphatase, regulates FADD phosphorylation for cell growth

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Adenylate kinase 2 (AK2), which balances adenine nucleotide pool, is known as a multi-functional protein. Here we show that AK2 negatively regulates tumor cell growth. AK2 forms a complex with DUSP26 phosphatase, and stimulates DUSP26 activity independently of its adenylate kinase activity. AK2/DUSP26 phosphatase protein complex dephosphorylates phospho-$FADD_{Ser194}$ to regulate cell growth. AK2 deficiency enhances cell proliferation with cell cycle alteration and induces tumor formation in xenograft assay, and this anti-growth function of AK2 is associated with its DUSP26-stimulating activity. Down-regulation of AK2 expression is frequently found in tumor cells and human cancer tissues showing high level of phospho-$FADD_{Ser194}$. Moreover, reconstitution of AK2 in AK2-deficient tumor cells retards cell proliferation. Further, $AK2^{+/-}$ mouse embryo fibroblasts exhibit enhanced cell proliferation with significant alteration in phospho-$FADD_{Ser191}$. These results suggest that AK2 is an associated activator of DUSP26 and suppresses cell proliferation via FADD dephosphorylation, postulating AK2 as a suppressor of tumor growth.

Keywords: AK2, DUSP26 activator, FADD.

TUE-012

ALK rearrangements in Turkey series lung adenocarcinoma

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Abstract Purpose: To establish the frequency of ALK translocation in lung cancer (adenocarcinomas) at a Turkish population level.

Experimental Design: Between 2011–2013, 157 paraffin embedded tumour tissue have been performed by our hospitals. 40 patient chosen from this group were also scanned for EGFR mutations. FISH technique is performed to paraffin embedded tissues were cut 4 micron thick. Vysis ALK Break apart FISH probe kit *[Vysis LSI ALK (2p23) Dual Color, Break Apart Rearrangement Probe; Abbott Molecular]* is used to detect rearrangement of ALK gene.

Results: 157 adenocarcinoma tumor tissue were analyzed. In 20 patients (12.7%) were found positive for ALK. In our study ALK-positive and-negative patients, there is a difference between the 5.69 percent year. The results of ALK-positive patients compared to negative patients showed that younger patients.

Conclusion: In summary, our study is the first study to investigate the ALK status in lung cancer in Turkish patients. Lung adenocarcinoma patients with ALK translocation, which has been reported to be younger age than carrying wild-type allele. According to result ALK breaks observed more frequently in patients at an early age. FISH analysis is the standard method for detecting ALK rearrangements. ALK gene rearrangements in patients with lung cancer, recent studies conducted on personalized treatment increases the success was observed. ALK gene rearrengements by FISH analysis has a leading role to Crizotinib treatment. Thus, wild type EGFR gene results and ALK gene rearrangements plus patients is related to increased survival after crizotinib treatment.

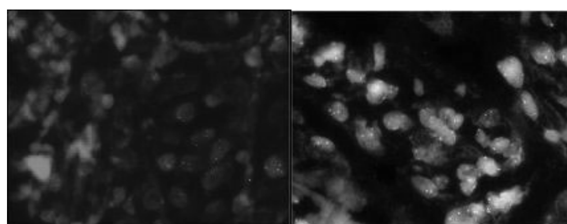

Fig. 1. Fluorescent in situ hybridization (FISH) reveals a split of red and green probe in an *ALK*-positive tumor.

Further studies about ALK gene is important to get more efficient cure result and figure out the molecular mechanism and structure.

Keywords: ALK, Fluorescent *in situ* hybridization, lung adenocarcinoma.

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TUE-013
Alpha 1 syntrophin protein plays a suspicious role in cell migration and carcinogenesis

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We have studied the expression of alpha-1-syntrophin (SNTA1) protein in histologically confirmed human breast cancerous tissue samples. our results suggest a significant increase in the expression of SNTA1 adaptor protein as compared to its expression in the corresponding normal tissue samples. Our results suggested a role for SNTA1 in carcinogenesis and its possibility as a novel diagnostic or prognostic marker in breast cancers. Taking a lead from here and the earlier works we learnt that SNTA1 has also been implicated in the activation of RhoGTP'ase Rac1 protein. However the underlying mechanism has not been explored. We used co-immunoprecipitation assays to show a complex formation, involving SNTA1 and GRB2 proteins, that was shown to significantly increase the active Rac1 levels within human breast cancer cell lines (HBL 100, MCF 7), while SiRNA and ShRNA were used to down regulate the expression of these proteins. Various Rac1 activation assays were performed. Our results showed a significant increase in the activation when SNTA1 and P66shc proteins were overexpressed, whereas their depleation not only effectively reduced the levels of active Rac1 but also showed a negative effect on the migratory potential of human breast cancer cells. Taken together, we propose a possible mechanism of Rac1 activation involving SNTA1 and emphasize its role in breast cancer cell migration, and carcinogenesis.

Keywords: breast cancer, migration, rac1.

TUE-014
AMTB, an inhibitor of calcium permeable ion channel TRPM8 and its effect on breast cancer cells

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Introduction: Calcium channels are involved in a wide range of cellular functions, and studies show that aberrant expression of these channels is a characteristic of some breast tumours. The calcium permeable transient receptor potential cation channel subtype M, member 8 (TRPM8) is reported to be over-expressed in tumours of the prostate; however few studies have evaluated the role of this channel in breast cancer. AMTB is reported to be a selective inhibitor of TRPM8 ion channels. The present study aims to investigate TRPM8 ion channel as a potential therapeutic target in breast cancer using a pharmacological inhibitor of this channel: AMTB (N-(3-aminopropyl)-2-[(3-methylphenyl)methoxy]-N-(2-thienylmethyl)-benzamide hydrochloride (1:1) hydrate).
Aim: To evaluate the effect of pharmacological inhibition of TRPM8 ion channel by AMTB on the proliferation of breast cancer cells.
Methods: Cells from two human breast cancer cell lines: MDA-MB-231 and SK-BR-3 were treated with 0.1 μM – 100 μM of the TRPM8 inhibitor: AMTB (Sigma-Aldrich). The cells plated in 96-well plates were incubated with AMTB for 72 hours and an MTS assay (Promega) was performed to determine cell viability. A high-throughput fluorescent imaging plate reader (FLIPR) membrane potential assay assessed the effect of AMTB on voltage gated sodium channels (Nav). HEK293 cells stably expressing hNav isoforms (Scottish BioMedical) were briefly incubated with AMTB and then activated by veratridine to measure depolarisation of Nav channels. The mRNA expression of TRPM8 and Nav channel subtypes 1.1–1.9 in breast cancer cell lines was determined using real-time RT-PCR (Life Technologies).
Results: AMTB (100 μM) decreased cell viability in both MDA-MB-231 and SKBR-3 cell lines by >70%. However, real-time RT-PCR analysis showed very low (SKBR3) or no (MDA-MB-231) TRPM8 mRNA in both breast cancer cells lines. Analysis of TRPM8 activity using FLIPR membrane potential assay revealed an inhibitory effect of AMTB at Nav channel subtypes 1.1–1.8. Multiple Nav subtype mRNAs were present in breast cancer cell lines: Nav subtypes 1.3, 1.5, 1.7 mRNA were present in MDA-MB-231 breast cancer cells and Nav 1.4, 1.6, 1.9 mRNA was detected in SK-BR-3 breast cancer cells.
Conclusion: The pharmacological activity of AMTB includes blockage of TRPM8 and most subtypes of Nav channels. The effect of AMTB on the proliferation of breast cancer cells may be mainly due to its action on Nav channels. Further studies are required to assess the role of Nav channels

Keywords: breast cancer, calcium channels.

TUE-015
Analysis of association of variations in p53 gene with breast cancer risk in Kazakhstan population

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The association of *p53* gene polymorphisms with breast cancer (BC) varies in different races and nationalities. The aim of this study was to evaluate the associations of polymorphisms in codon 72 exon 4 (Arg72Pro), in intron 3 (16 bp duplication) and intron 6 (G/A) of *p53* gene with BC in Kazakh and Russian ethnic groups in Kazakhstan.

The case-control study included 468 BC patients (267 Kazakhs, mean age 49.1 years, 201 Russians – 51.1 years) and 466 of controls (229 Kazakhs – 49.6 years, 237 Russians – 49.7 years). Genotyping was performed by PCR-RFLP analysis. The Pearson χ^2, p-value (P), Fisher exact test's permutation p-value (P_f), and odds ratio (OR) with 95% confidence intervals (95% CI) criteria were applied to data analysis. Combined effect of allele variations was carried out using algorithm APSampler.

The association of two polymorphisms in intron 3 and intron 6 with BC was shown in Russian group. Significant differences in alleles frequency and genotypes distribution were detected for polymorphism in intron 6 ($\chi^2 = 9.02$, P = 0.003 for alleles; $\chi^2 = 8.64$, P = 0.01 for genotypes); in intron 3 ($\chi^2 = 6.31$, P = 0.01 for alleles; $\chi^2 = 5.90$, P = 0.05 for genotypes) between patients and corresponding controls.

The associations with BC risk were evaluated for 16 bp duplication in intron 3 (OR = 1.83; 95% CI = 1.14–2.93) and for allele A in intron 6 (OR = 1.93; 95% CI = 1.25–2.97) in Russians. Association with BC risk was found by means of APSampler for the combination of allele A in intron 6 with the absence of 16 bp duplication in intron 3 in Russian group (OR = 2.29, $P_f = 5.86*10^{-7}$).

The differences in allele frequencies and genotypes distribution between cases and controls in codon 72 exon 4 (Arg72Pro) in Russian group and in all tested sites in the Kazakh group were not found.

Keywords: breast cancer, p53 gene.

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TUE-016
Analyzing the biophysical properties and elucidating nuclear functions of beta-catenin and its mutants

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The wnt/β-catenin pathway regulates stem cell pluripotency and plays a pivotal role in cell fate decisions during development. The central molecule of this pathway is β-catenin, a multifunctional protein principally involved in two functions: cadherin dependent cell-cell adhesion function at the cell membrane and transcription of wnt-specific genes in the nucleus. β-Catenin, a 781 amino acid long protein has three domains folding in an unknown three dimensional structure with a structured central armadillo domain. Mutations and/or overexpression of β-catenin are associated with many cancers including liver cancer, colorectal cancer, lung cancer, ovarian cancer and malignant breast tumours. In this study we have generated many variants of this protein for understanding its role in cancer development and progression, stem cell biology and also for the biophysical studies. We have cloned and purified β-catenin N-terminal deletion mutants to homogeneity using *E. coli* expression system and we are establishing protein based assays for screening small molecule inhibitors of wnt/β-catenin signalling pathway. β-Catenin interacts with vast array of proteins in the cytosol and many transcription factors in order to regulate various functions. Some of the proteins that interact with β-catenin include Oct4, KLF4 and Sox2, these proteins are known to be critical proteins in stem cell biology. We have generated deletion mutants of the C-terminal domain, also called transcriptional activation domain, of β-catenin in order to analyze their mechanistic studies and their association with Oct4, KLF4 and Sox2. In conclusion, we have generarted many constructs of β-catenin for understanding its biophysical properties and for analyzing its nuclear functions.

Keywords: beta catenin, stem cells, biophysical studies, SOX, KLF.

TUE-018
Anti-angiogenic mechanisms of WMJ-S-001, a novel aliphatic hydroxamate derivative

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The metastatic spread of tumor cells is the leading cause of death for cancer patients. Angiogenesis, one of the major routes for tumor invasion and metastasis, thus represents a rational target for therapeutic intervention. Recent development in drug discovery has highlighted the diverse biological and pharmacological properties of hydroxamate, a key pharmacophore. In this study, we explored and characterized the anti-angiogenic activities of a novel aliphatic hydroxamate, WMJ-S-001, in an effort to develop novel angiogenesis inhibitors. WMJ-S-001 inhibited vascular endothelial growth factor (VEGF)-induced proliferation, migration and endothelial tube formation of human umbilical endothelial cells (HUVECs). WMJ-S-001 suppressed VEGF-induced microvessel sprouting from aortic rings and attenuated VEGF- or HCT116 colorectal cancer cells-induced angiogenesis *in vivo*. In addition, WMJ-S-001 inhibited the phosphorylations of VEGFR2, Src, FAK, Akt and ERK in VEGF-stimulated HUVECs. WMJ-S-001 caused an increase in SHP-1 phosphatase activity, whereas NSC-87877, a SHP-1 inhibitor, restored WMJ-S-001 suppression of VEGFR2 phosphorylation and cell proliferation. Furthermore, WMJ-S-001 inhibited cell cycle progression and induced cell apoptosis in HUVECs. These results are associated with p53 activation and the modulation of p21 and survivin. Taken together, WMJ-S-001 was shown to modulate vascular endothelial cell remodeling through inhibiting VEGFR2 signaling and induction of apoptosis. These results also support the role of WMJ-S-001 as a potential drug candidate and warrant the clinical development in the treatment of cancer.

Keywords: Angiogenesis, Endothelial cells, VEGF.

TUE-019
Antioxidative and cytoprotective effects of proanthocyanidins in mitomycin C – treated cells

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Mitomycin C (MMC) is a quinone based bioreductive drug that alkylates DNA upon metabolic reductive activation. Since it contains a quinone moiety, MMC can suffer a one-electron reduction by a range of cellular oxidoreductases, resulting in the production of semiquinone anion radical. In the presence of oxygen, the semiquinone enters into a redox cycle, which leads to formation of different ROS that interact with cellular macromolecules. This mechanism is generally accepted as not being critical for antitumor activity, but crucial for toxic effects. Therefore, the application of cytoprotective antioxidants may increase the efficacy of MMC, together with decrease of its toxicity towards normal cells. The aim of our study was to compare the viability and antioxidant status of the cells exposed to MMC alone or in combination with proanthocyanidins (PACs) and standard cytoprotective agent - N-acetyl cysteine (NAC).

PACs were extracted from grape seeds by ethylacetate and 10% water, and they represent the mixture of monomers, dimers and small oligomers of catechin and epicatechin. We carried out our experiments in two cell lines – human erythroleukemia (K562) and Chinese hamster ovary (CHO) cells. Cell viability was determined by MTT assay and parameters of oxidant status - activity of antioxidant enzymes superoxide dismutase (SOD), glutathione reductase (GR) and glutathione-S-transferase (GST) were tested by spectrophotometric kinetic methods.

MMC exerted concentration-dependent cytotoxic activity towards both cell lines. Pretreatment with PACs resulted in more pronounced cytoprotection of malignant K562 cells than normal CHO cells. Growth inhibition of CHO cells treated with MMC in combination with PACs showed a biphasic dose response, suggesting the importance of selection of appropriate dose of PACs for desirable cytoprotective effects. PACs and NAC exerted the antioxidative activity and comparative analysis of activities of antioxidant enzymes SOD, GR and GST in malignant and normal cell lines showed diverse modulation of oxidant status of cells when treated with MMC, alone or in combination with PACs and NAC.

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PACs and NAC exert cytoprotective effects in MMC-treated both malignant and normal cell line. Our results indicate the complexity of biological response on PAC-induced effects and further investigation is needed to get more detailed insight into mechanisms of antioxidant defense mediated by PACs.

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Keywords: None.

TUE-020
Identification of KLF17 as a novel epithelial to mesenchymal transition inducer via direct activation of TWIST1 in endometrioid endometrial cancer

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Krüppel-like factor 17 (KLF17), a member of the KLF transcription factor family, is an inhibitor of the epithelial-mesenchymal transition (EMT) and tumor growth. However, the expression, cellular function and mechanism of KLF17 in endometrioid endometrial cancer (EEC; a dominant type of endometrial cancer) remains elusive. Here, we report that among the KLF family members, KLF17 was consistently upregulated in EEC cell lines compared with immortalized endometrial epithelial cells. Overexpression of KLF17 in EEC cell lines induced EMT and promoted cell invasion and drug resistance. In contrast, KLF17 suppression reversed EMT, diminished cell invasion and restored drug sensitivity. We determined whether KLF17 could affect the expression of known EMT/CSC-related genes in EC cells by qRT-PCRs. Overexpression of KLF17 in EC cells dramatically increased endogenous mRNA levels of the EMT inducers TWIST1 and BMI-1, and moderately and significantly increased expression of ZEB1, Snail and Slug. On the other hand, levels of the epithelial markers E-cadherin and CK-18 were significantly reduced compared with control cells. Moreover, the mRNA levels of CSC markers (NANOG, SOX2 and CD133), and chemoresistance-related genes (MDR-1 and MRP-1) were also highly elevated in KLF17 vector-transfected cells. Furthermore, luciferase assays, site-directed mutagenesis and transcription factor DNA binding analysis demonstrated that KLF17 transactivates TWIST1 expression by directly binding to the TWIST1 promoter. Knockdown of TWIST1 prevented KLF17-induced EMT. Consistent with these results, both KLF17 and TWIST1 levels were found to be elevated in EECs compared to normal tissues. KLF17 expression positively correlated with tumor grade, but inversely correlated with estrogen and progesterone receptor expression. Thus, KLF17 may have an oncogenic role during EEC progression via initiating EMT through the regulation of TWIST1.

Keywords: drug resistance, EMT, tumor invasion.

TUE-021
Antitumor effects of antibodies to the E2 extracellular loop of connexin-43 in high-grade gliomas

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We studied the effect of intravenous administration of monoclonal antibodies to the second extracellular loop of connexin 43 (MAbE2Cx43) on the dynamics of glioma growth and survival of experimental animals. Volumometric analysis of glioma via MRI data showed that weekly intravenous administration of MAbE2Cx43 in a dose of 5 mg/kg significantly reduced glioma volume starting from day 21 after tumor implantation. By day 29, the mean volume of glioma in the experimental group (therapy with specific antibodies) was 2-fold lower than in controls. Deceleration of glioma growth in rats receiving MAbE2Cx43 was accompanied by a significant prolongation of rat lifespan (according to Kaplan-Meier test) and even led to complete recovery without delayed relapses in 19.23% animals. The mechanism of tumor-suppressing effects of antibodies can be related to inhibition of specific functions of connexin 43 in glioma cells in the peritumoral zone.

Keywords: connexin 43, glioma, monoclonal antibodies.

TUE-022
AP-1 and miR-16 underlie the differential response of breast cancer cells to tamoxifen upon progestin treatment

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The progesterone receptor (PR) is known to participate in breast cancer cell proliferation and to cross-talk with the estrogen receptor alpha (ERα), yet its role in resistance to anti-estrogen therapy is not fully understood. In the present work, we studied the effects on cell proliferation of the synthetic progestin medroxyprogesterone acetate (MPA) alone or in combination with tamoxifen (Tam), a commonly used selective ER modulator. We found that Tam inhibited MPA-induced activation of the c-Src/MAPK signaling pathway in BT474-HR cells sensitive to Tam antiproliferative effects. Importantly, Western blot and chromatin immunoprecipitation assays revealed that Tam abrogated MPA-induced c-Src/MAPK phosphorylation and recruitment of the transcription factor AP-1 to the cyclin D1 promoter, thus preventing MPA from inducing cyclin D1 transcription and cell proliferation. Interestingly, Tam failed to block these MPA effects in BT474 cells resistant to Tam. Furthermore, we found that PR-dependent activation of c-Src/MAPK leads to the down-regulation of miR-16, a microRNA we have previously shown to act as a tumor suppressor in progestin-induced breast cancer growth (1). This decrease in miR-16 levels correlated with increased expression of cyclin D1. Co-treatment of BT474-HR cells with MPA+Tam restored miR-16 levels, contributing to the repression of cyclin D1 expression. Such inhibitory effects of Tam were not evidenced in Tam-resistant BT474 cells. Taken together these results suggest that, in Tam-resistant tumor cells, Tam fails to abrogate the PR-dependent c-Src/MAPK signaling that triggers the assembly of transcription factor complexes that control cyclin D1 expression directly at its promoter and indirectly through the modulation of microRNA levels, leading to unrestrained cell proliferation. Our results provide insight into a complex synergic mechanism underlying Tam resistance that involves the PR, a receptor not classically studied in the context of ER-targeted therapies, shown here to be a possible therapeutic target to prevent its unrestrained functions in Tam-resistant cells.

Reference

1. Rivas *et al.*, Breast Cancer Res. 2012; 14(3): R77.

Keywords: breast cancer, progesterone receptor, tamoxifen resistance.

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TUE-023
Apoptotic and synergic effect of resveratrol and EGCG combination in DU-145 prostate cancer cells

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Prostate cancer is the second most common cause of male cancer deaths in Unites States. Multiple phytochemicals have been identified as potential anti-cancer agents from commonly consumed fruits and vegetables. Trans-Resveratrol (3,4′,5-trihiydroxy-trans-stilbene, Res) is the most widely studied stilbene found in small fruits. EGCG (epigallocatechinegallate) is the most abundant catechin in tea and is a potent antioxidant that may have therapeutic applications in the treatment of many disorders. Resveratrol and EGCG has been considered as a good anti-carcinogenic agent because of its low toxicity and capability of modulating multiple molecular pathways that play important roles in cell cycle progression, apoptosis. The aims of our study were to find apoptotic effect of Res and EGCG separately in DU-145 prostate cancer cell line and then to find a combination index of the agents, that has synergistic effect, and to evaluate and compare apoptotic effects of the groups. For these purposes cell viability and IC50 values were determined via WST-1 viability assay for 24 hour time point for groups of Res, EGCG and combination (Res+EGCG) in DU-145 prostate cell line. Apoptotic cell ratio was analyzed by Annexin V / Propidium iodide (PI) double staining and cell cyle (PI) was analyzed by flow cytometer. According to WST-1 results; Res and EGCG treatments were increased cell death as a dose dependent manner and IC50 value has been calculated as 141 μM and 58 μM for Res and EGCG respectively. Afterwards synergistic ratio and combination index value (CI) were calculated by using CalcuSyn© software. The combination effect of Res and EGCG was analyzed byChou-Talalay method. Strong synergism CI value (CI<1) was found as 0.519. Apoptosis ratio were found as 2.35%, 1.94% and 14.04% for Res, EGCG and combination groups by flow cytometry According to cell cycle analysis; apoptotic cell ratios were found 12.9% in Res group, 11.8% in EGCG group and 27.6% in combination group. All the results revealed that combination of Res and EGCG has more powerful effect in induction of apoptosis and combination leads to higher amount of apoptotic cell death than Res or EGCG treated groups. On account of Res and EGCG have low toxicity; we suggest that this nutritional combination might be a promising approach for adjuvant therapy of prostate cancer treatment.

Keywords: combination index, EGCG, resveratrol.

TUE-024
Apoptotic effects of methotrexate- loaded nanoparticles in prostate cancer LNCaP cell line

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Prostate cancer is the most prevalent type of cancer in men that causes second rank among of death reason. Androgen hormones and androgen receptor genes, although showing a heterogeneous structure, it is known to have an important role in the prostate cancer development. Lately, Metothrexate (MTX) is used as an anticancer drug for treatment of particularly in prostate cancer. But MTX has short plasma half-life. In recent years, to enhance the efficacy and delivery of MTX and increase the half-life, nanoparticles were used as a drug delivery carrier. Nanoparticles have advantages as targeting in the tumor sites. Here, we used chitosan (CS) nanoparticles to contribute delivery of MTX.

In this study we aimed to evaluate cytotoxic and apoptotic effect of Methotrexate on LNCaP cell line with treated nanoparticles in vitro on the other hand the changes of mRNA expression in related genes with Androgene Receptor (AR) and PSA.

For this study, chitosan nanoparticles were synthesized by us. The cytotoxic effects of MTX-loaded chitosan on LNCaP cells were determined in time and dose dependent manner by using WST-1 analysis. Three groups were formed to treat Methotrexate and Chitosan alone and, in combination to evaluate the cytotoxic effects of MTX-loaded chitosan on LNCaP cells for 24, 48 and 72nd hours. Annexin V (Biovision) was used to determine the apoptotic effects of MTX-loaded chitosan and evaluated by flow cytometry. Quantitative assessment of Androgen Receptor and PSA mRNAs were analyzed by Real Time qRT-PCR.

In vitro cytotoxic studies confirmed that MTX loaded-chitosan was found to have higher cytotoxic effect on LNCaP cells compared with free MTX. IC_{50} value of (MTX)- loaded Chitosan was found 2.635 μmol/ml at 72nd hours. The obtained results indicated that MTX-loaded chitosan induced apoptosis in time-dependent manner as compared to untreated control groups. Especially 33.6% of apoptosis rate was seen at 72nd hours. qRT-PCR assays revealed that mRNA expression levels of AR and PSA genes were downregulated by the effect of MTX loaded-chitosan when compared to untreated control group in LNCaP cells.

In conclusion, these results showed that MTX-loaded chitosan could be more effective than free MTX for prostate cancer treatment. Using nanoparticles as a drug delivery promises more influential therapy to prevent progression of hormone – dependent prostate cancer.

Keywords: Methotrexate, Prostate cancer, Apoptosis, Chitosan, Nanoparticles.

TUE-025
Arf6 promotes proliferation and activates mTOR-downstream signaling of glioblastoma cells

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Small G-protein Arf6 (ADP-ribosylation factor 6) belongs to the ARF subfamily of small GTPases from the Ras superfamily. Arf6 is localized at plasma membrane and various subcellular membranes, regulating vesicular transport, actin cytoskeletal rearrangement, cytokinesis, autophagy and plasma membrane reorganization. Due to the participation in these processes Arf6 significantly contributes to the tumor progression. In particular, it participates in endocytosis and recycling of receptors (such as integrin β1 and E-cadherin) affecting cell adhesion, migration and invasion of cancer cells. Arf6 is also involved in several intracellular signaling pathways mostly through the activation of PLD and PIP5K. At the same time almost nothing is known about the role of this protein in cell proliferation.

Previously published data from our lab obtained on the model of transformed hamster fibroblasts revealed the promitogenic activity of Arf6 mediated through the activation of mTOR signaling.

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Here we studied the impact of Arf6 on cell proliferation as well as on MAPK-Erk1/2 and PLD-mTOR signaling using human glioblastoma cell lines LN229 and U87. For this purpose we obtained stable derivative cell lines with overexpression of wild type Arf6 (Arf6WT), constitutively active mutant Arf6 (Q67L) and with depletion of endogenous Arf6 with two non-overlapping shRNAs.

We found increase of proliferation in LN229 Arf6 (Q67L) and U87 Arf6WT cells whereas both lines with depleted Arf6 demonstrated dramatical lowering of proliferation rate. Analysis of ERK1/2 phosphorylation status revealed decrease of phospho-ERK1/2 as a result of Arf6WT as well as Arf6 (Q67L) overexpression in both LN229 and U87 lines under the condition of EGF stimulation. Correspondingly, depletion of endogenous Arf6 led to upregulation of ERK1/2. Next, we studied the influence of Arf6 on mTOR-depedent signaling using the level of phospho-p70S6K1 as a marker of mTOR activation. We showed that overexpression of Arf6 promotes phosphorylation of p70S6K1 while Arf6 knockdown diminished the level of phospho-p70S6K1.Analysis of extracellular matrix proteinases activity demonstrated decrease of MMP2 activity in LN229 shArf6.

Presented data give the first evidence of Arf6 involvement in regulation of mTOR-downstream signaling in human tumour cells. Additionally, we elucidated the influence of Arf6 on ERK1/2 phosphorylation. We also demonstrated promitogenic effect of this small GTPase on glioma cells.

Keywords: Arf6, glioblastoma, signaling.

TUE-026
BAG-1 forms a complex to allow the phosphorylation of Bad from Ser136 to modulate cell survival in MCF-7 cells

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BAG-1 (Bcl-2 associated athanogene) is a multifunctional protein that interacts with diverse array of cellular targets and modulates a wide range of cellular processes, including proliferation, cell survival, transcription, apoptosis, metastasis and motility. In human cells BAG-1 exists as three major isoforms (BAG-1S, BAG-1M and BAG-1L) derived by alternative translation initiation from a single mRNA, which allows interactions with various molecular targets such as Hsp70/Hsc70 molecular chaperones, components of the ubiquitylation/proteasome machinery, Bcl-2, Raf-1 kinase, nuclear hormone receptors and DNA. Overexpression of BAG-1 isoforms was shown to affect the regulation of proliferation, cell survival and cancer progression. There are a number of possible mechanisms by which BAG-1 may mediate its pro-survival effects. In addition, drug resistance is observed with overexpression of BAG-1, causing cell rescue from apoptosis. Our work aims to investigate how altered BAG-1 expression levels affect cell survival pathways in MCF-7 and MCF-10A cell lines. We first cloned BAG-1L gene to a cloning vector, later transfected MCF-7 and MCF-10A cells with the designed vector and obtained stable cell lines overexpressing our clone. We used BAG-1 siRNA to silence *BAG-1* gene to understand BAG-1 role in cell survival. Western blot analysis and immunocytochemistry were applied to demonstrate relative expression levels of BAG-1, its interacting partners and certain proteins which are important for survival pathway. We performed XTT cell viability assay and growth assay for BAG-1 overexpressed cells to check BAG-1's role in cell survival, and we did Apoptotic Cell Death Elisa assay to show apoptosis rate in *Bag-1* gene silenced cells. We also performed co-immunoprecipitation to show co-existence of certain proteins with BAG-1 protein as a complex. This study revealed that once BAG-1 forms a complex with C-Raf/B-Raf/Hsp70/Akt/Bcl-2, phosphorylation of Bad from Ser136 occurs and this causes Bad's sequestration by 14-3-3, modulating cell survival. We believe that once the localization and molecular mechanism of BAG-1 and its isoforms are found, and the interacting partners are determined, the role of each BAG-1 isoform in cell survival can be understood better.

Keywords: BAG-1, breast cancer, cell survival.

TUE-027
BAG-1 forms a complex to allow the phosphorylation of Bad from Ser136 to modulate cell survival in MCF-7 cells

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BAG-1 (Bcl-2 associated athanogene) is a multifunctional protein that interacts with diverse array of cellular targets and modulates a wide range of cellular processes, including proliferation, cell survival, transcription, apoptosis, metastasis and motility. In human cells BAG-1 exists as three major isoforms (BAG-1S, BAG-1M and BAG-1L) derived by alternative translation initiation from a single mRNA, which allows interactions with various molecular targets such as Hsp70/Hsc70 molecular chaperones, components of the ubiquitylation/proteasome machinery, Bcl-2, Raf-1 kinase, nuclear hormone receptors and DNA. Overexpression of BAG-1 isoforms was shown to affect the regulation of proliferation, cell survival and cancer progression. There are a number of possible mechanisms by which BAG-1 may mediate its pro-survival effects. In addition, drug resistance is observed with overexpression of BAG-1, causing cell rescue from apoptosis. Our work aims to investigate how altered BAG-1 expression levels affect cell survival pathways in MCF-7 and MCF-10A cell lines. We first cloned BAG-1L gene to a cloning vector, later transfected MCF-7 and MCF-10A cells with the designed vector and obtained stable cell lines overexpressing our clone. We used BAG-1 siRNA to silence *BAG-1* gene to understand BAG-1 role in cell survival. Western blot analysis and immunocytochemistry were applied to demonstrate relative expression levels of BAG-1, its interacting partners and certain proteins which are important for survival pathway. We performed XTT cell viability assay and growth assay for BAG-1 overexpressed cells to check BAG-1's role in cell survival, and we did Apoptotic Cell Death Elisa assay to show apoptosis rate in *Bag-1* gene silenced cells. We also performed co-immunoprecipitation to show co-existence of certain proteins with BAG-1 protein as a complex. This study revealed that once BAG-1 forms a complex with C-Raf/B-Raf/Hsp70/Akt/Bcl-2, phosphorylation of Bad from Ser136 occurs and this causes Bad's sequestration by 14-3-3, modulating cell survival. We believe that once the localization and molecular mechanism of BAG-1 and its isoforms are found, and the interacting partners are determined, the role of each BAG-1 isoform in cell survival can be understood better.

Keywords: BAG-1, breast cancer, cell survival.

TUE-028
Bag-1 forms different complexes to play various roles in carcinogenesis

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Bag-1 (Bcl-2 associated athano gene- 1) is a member of an anti-apoptotic Bag family that acts as an adaptor protein to regulate

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a wide variety of cellular processes, including proliferation, survival, transcription, apoptosis, tumorigenesis and motility. To perform these functions Bag-1 has three functionally distinct isoforms (Bag-1L (p50), Bag-1M (p46), Bag-1S (p36) and a minor isoform (p29)) that can interact with a diverse array of molecular targets such as Hsp70/Hsc70 molecular chaperones, components of the ubiquitylation/proteasome machinery, Bcl-2 apoptosis regulator, Raf-1 kinase, nuclear hormone receptors and DNA. In human malignant cells, at certain times Bag-1 interacts with distinctive set of proteins and it can form different complexes. These distinctive set of interaction partners has not been fully demonstrated yet, and thus, the role of Bag-1 in carcinogenesis is not clear. In this study, we aim to identify interaction partners of Bag-1 in MCF-7 breast cancer cells under Bag-1 altered states. We used an affinity purification method to obtain various Bag-1 complexes. After purification of Bag-1 TAP-tagged complexes from MCF-7 cells, duplicates of purified fractions were resolved by 2D gel electrophoresis. We identified several already known interacting partners of Bag-1 as well as undetermined ones. All identified interaction partners of Bag-1 will be discussed in a context of their relation to Bag-1 complex functional mechanisms in MCF-7 cells.

Keywords: BAG-1, CANCER, Tandem Affinity Purification.

TUE-029

BAG3 regulates nucleocytoplasmic shuttling of HSF1 under heat stress

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BAG3 is the only member of BAG family to be induced by stressful stimuli, such as heat shock and heavy metal. Growing evidences have shown that BAG3 regulates cellular adaptive responses against stressful stimuli by regulating apoptosis, development, cytoskeleton organization and autophagy. Considering the association of BAG3 in cellular stress, we hypothesized that BAG3 may associated with HSF1 regulation in response to cellular stress. Therefore, in this study, we investigated the molecular action mechanism of BAG3 under the stress condition. We firstly demonstrated that under the heat stressed condition, BAG3 rapidly co-translocalized to the nucleus with HSF1. Overexpression of BAG3 down-regulated the level of nuclear HSF1 and subsequently decreased the Hsp70 promoter activity by exporting HSF1 to the cytoplasm. In accordance with these results, down-regulation of BAG3 reduced HSF1 export from nuclear to the cytoplasm. $Hsf^{-/-}$ mouse embryonic fibroblast (MEF) cells shows that the translocation of BAG3 upon heat stress is not affected by the absence of HSF1, suggesting BAG3 may act as a HSF1 regulator by nucleocytoplasmic shuttling upon heat stress.

Keywords: BAG3, HSF1, nuclear translocation.

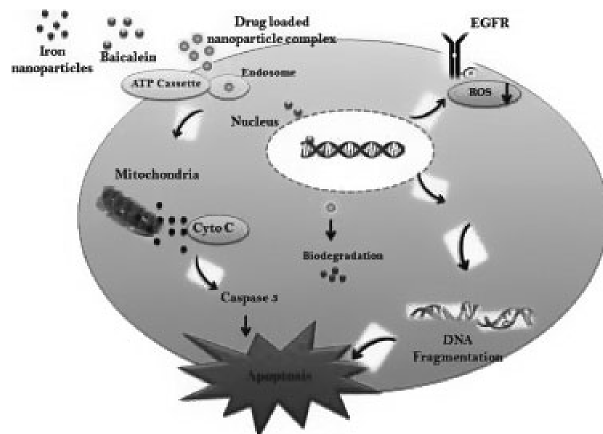

Fig. 1.

TUE-030

Baicalein loaded iron oxide nanoparticles for targeted drug delivery in human breast cancer (MCF-7) cells

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Iron oxide has been widely used in biomedical research because of its biocompatibility and magnetic properties. In the present investigation elucidates the effect of baicalein loaded Fe_3O_4 nanoparticles were determine to establish whether these nanoparticles are effectively deliver the anticancer agent baicalein into the targeted MCF-7 cells. Fe_3O_4 nanoparticles were synthesized by co-precipitation method. A result of X-Ray diffraction indicates highly crystalline and no phase change confirms the purity of spherical Fe_3O_4. The interaction between the magnetite and surfactant was illustrated by FTIR, which confirms that the magnetic nanoparticles were coated by PEG, PVP and baicalein via the amino groups of hexamine. The synthesized Fe_3O_4 nanoparticles are highly monodispersed crystalline nature in a size range of 20 nm confirmed by Transmission electron microscope. The proliferation of MCF-7 cells and their cytotoxicity were evaluated by MTT assay and Western blotting analyses were performed to examine gene transcription and protein expression, respectively. The results showed that iron oxide nanoparticles dramatically enhanced baicalein-induced cytotoxicity and apoptosis in MCF-7 cells. The transcription of caspase-3 and bax gene in the group treated with baicalein and Fe_3O_4 was higher than that in the baicalein and Nanoparticles alone group. Our findings suggest a potential clinical application of a combination of baicalein loaded Fe_3O_4 in Breast cancer therapy.

Keywords: Drug Delivery, Fe3O4 nanoparticles, MCF-7 cells.

TUE-031

Biochemical and structural insights into activation of Arf GTPases by endocytic GEFs

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Small GTPases of the Arf family control many aspects of membrane traffic and organelle structure. They are activated by guanine nucleotide exchange factors (ArfGEFs), which are modular proteins composed of a catalytic Sec7 domain that stimulates GDP/GTP exchange, and other domains that carry out regulatory and membrane targeting functions [1]. Three subfamilies - cytohesins, Brags and EFA6 - are involved in endocytic events in human and promote invasive phenotypes in cancer. They all associate the catalytic Sec7 domain with a downstream PH domain but, except for cytohesins, little is known on how these ArfGEFs couple their subcellular targeting to their activation of Arf GTPases. We present our recent results on BRAG and EFA6 GEFs. Biochemical reconstitution of Arf activation on membranes combined with structural analysis revealed that membranes dramatically affect ArfGEF efficiency [2]. These studies also uncovered an unexpected diversity of regulatory regimes, which suggest that ArfGEFs estab-

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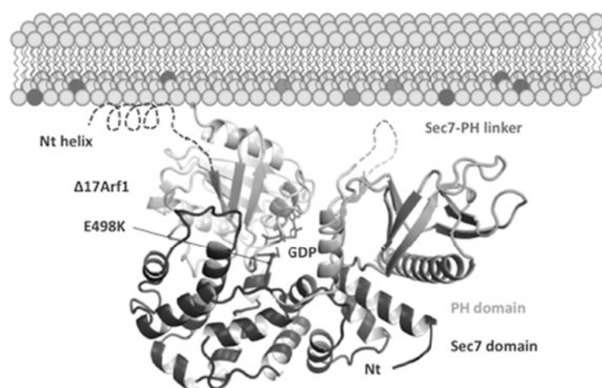

Fig. 1.

lish coupled positive-negative feedback circuits to shape the level and timing of Arf activation in cells. Our study should be valuable for future investigations of the coordination between trafficking pathways and receptor endocytosis and signaling in normal and cancer cells.

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Keywords: Cancer signaling, circuits, GTPase.

TUE-032
Biochemical indicators of cardio- and hepatotoxic actions of novel 4-thiazolidone derivatives and doxorubicin in complexes with PEG-containing polymeric carrier

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The aim of this study was to measure the activity of enzymes which reflects cardiotoxic and hepatotoxic actions in rats of novel synthetic 4-thiazolidone derivatives – 3882, 3288 and 3833 which demonstrated antineoplastic effect in vitro towards 60 lines of human tumor cells tested in frames of the program of screening new anticancer drugs at the National Cancer Institute (Bethesda, USA). Such actions of these compounds were compared with the effect of well known anticancer drug doxorubicin alone and after their conjugation with new polyethylenglycol-containing polymeric comb-like carrier that was synthesized at 2. Among the biochemical indicators of cardio- and hepatotoxic action of anticancer agents, the activity of the following enzymes in rat blood serum showed to be the most informative: aspartate aminotransferase, alanine aminotransferase, α-amylase, gamma-glutamyltransferase, alkaline phosphatase, creatine kinase, lactate dehydrogenase and concentration of total protein, urea, creatinine, calcium and iron ions. 10 times injection of doxorubicin in dose of 5.5 mg/kg of weight caused rats' death, while 3882, 3288 and 3833 substances did not possess such action. Application of the doxorubicin as a complex with polymeric carrier prolonged survival time of animals to 20 days. Thus, the injection of anticancer agents in a complex with polymeric carrier provides a significant decrease in their cardiotoxicity and hepatotoxicity that was confirmed by the corresponding changes in the activity of above mentioned marker enzymes.

Conclusions: Lower antineoplastic activity of synthetic 4-thiazolidone derivatives – 3882, 3288 and 3833 (comparing with such activity of doxorubicin) correlates with lower cardio- and hepathotoxicity of these derivatives in the experimental rats. Complexes of 3882, 3288, 3833 compounds and doxorubicin with polyethylenglycol-containing polymeric comb-like carrier considerably decreases the cardiotoxicity and hepatotoxicity of these anticancer agents in the laboratory rats. That was confirmed by the results of measuring enzymatic activity of aspartate aminotransferase, alanine aminotransferase, α-amylase, gamma-glutamyltransferase, alkaline phosphatase, creatine kinase, lactate dehydrogenase and concentration of total protein, urea, creatinine, calcium and iron ions in blood serum of treated rats.

Keywords: None.

TUE-033
Biochemical profiling of cervical pre-cancer and early cancer biopsies as a way of modelling carcinogenesis

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Cervical intraepithelial neoplasias grade 1–3 (CIN1-3) and cervical cancer (CeCa) constitute virtually an ideal *in vivo* model of virus-induced carcinogenesis: successive stages of cervical neoplasia development are clinically easily detectable, including the very early pre-cancer lesions, and morphologically are relatively well-described. Molecular profiling of biopsy material of CeCa-associated lesions is regarded as one of the most objective approaches to investigation of disruptions in signaling pathways during malignant transformation and natural cancer development. The fact that Human Papilloma Virus (HPV)-induced neoplastic lesions at certain steps of development are potentially reversible raises a fundamental question of defining a "point-of-no-return" – what molecular defects predetermine irreversibility and drive progression of pre-cancer to invasive forms. HPV-mediated mechanisms of dysregulation of the key cellular programs are being thoroughly studied *in vitro*. At the same time, it is assumed that *in vivo* CIN transitions are linked to a sequential redistribution of intracellular signaling pathways and a specific order of overall molecular phenotype changes. Regulation of apoptotic signaling pathways and its disruption are considered to play a significant part in CeCa development. Resistance to apoptosis provides a mechanism of evasion of antiviral/antitumor immune surveillance, and acquiring apoptosis-resistant phenotype at neoplastic locus may be a hallmark or a precondition of irreversible step of CeCa progression, however, experimental data on alteration of activity of components of programmed cell death pathways in pathological tissue samples at different stages of CeCa development are still insufficient. In the present work we examined biopsies of normal and pathological epithelium taken from 94 women with various stages of cervical squamous cell carcinoma progression including severe dysplasia, cancer *in situ* and microinvasive carcinoma. Changes of proteolytic activity and expression level of caspases (-3,-6,-8,-9) – essential mediators of apoptosis – were assessed in these samples with concurrent evaluation of apoptotic activity in circulating lymphocytes. We revealed a) stage-specific pattern of caspase activity, b) caspase-specific redistribution of activity, c) heterogeneity of pre-invasive stage at the level of caspases, d) general inhibition of caspase activity in carcinoma specimens suggesting accumulation of defects in apoptotic program and formation of resistant pheno-

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type. The work was supported by grants no. 11.G34.31.0052 and NK14-04-32098/14.
Keywords: apoptosis, caspases, cervical neoplasia.

TUE-034
Biochemical signalling of Ste20 kinase family proteins with Ras family adaptor proteins

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Mammalian Sterile 20 kinase (MST) proteins belong to a large family of serine / threonine kinases that are involved in regulation of apoptosis and signalling of mitogen activated protein kinase pathway. Proteins belongs to this family possess an N-terminal catalytic kinase domain followed by a C-terminal SARAH (for Salvador/RASSF/Hippo) domain. The C-terminal region of MST kinase regulates its kinase activity via formation of complex with partner adaptor proteins RASSF (for Ras association domain family). These RASSF proteins are scaffolding proteins, which contain both Ras association (RA) domain via which they interact with Ras-GTP, followed immediately by the SARAH domain. The GTP-Ras activated form regulates downstream signal transduction pathways and misregulation of the GTP-Ras pool causes uncontrolled cell proliferation and leads to cancer. The GTP-Ras-RASSF-MST complex formation route is therefore proposed as a safeguard mechanism by which the misregulated activated Ras complex is sequestered and apoptosis is triggered.

Here in the present work we study the binding profile of RASSF adaptors (RASSF1, RASSF5) with various isoforms of MST (MST1 and MST2) by surface plasmon resonance technique (SPR) using GST kinetics. Our studies reveal that the SARAH domain of both MST1 and MST2 binds to RASSF1 and RASSF5 SARAH region with KD values ranging from 0.3 to 2.1 μM. The addition of RA domain to RASSF1 SARAH faintly decreases its binding affinity towards MST1-SARAH, but enormously decreases the binding to MST2-SARAH domain, where the KD value increases by tenfold (15 μM). On the other hand, RA domain containing RASSF5 has the opposite effect and it prefers MST2 (KD value of 1.2 nM) as oppose to MST1. Therefore, we conclude that while the SARAH domain of these adaptors binds indiscriminately to both MST1 and MST2, the RA domain regulates the interaction between these two proteins at the cellular level.

Furthermore, we performed radioactive kinase assays towards the two substrates of MST1, (Fork box protein O) FoxO and Histone H2B in presence of these adaptor proteins. The results show that the RASSF family of proteins have differential effect towards both the substrates. It negatively regulates the kinase activity of MST1 towards its cytosolic substrate FoxO. However, the effect is opposite for H2B that resides in the nucleus, with its activity being positively regulated. Additionally we observed that MST1 is able to phosphorylate the RASSF family proteins of proteins within their Ras binding domain. We propose that this phoshorylation may have implications towards stabilization of the GTP-Ras-RASSF-MST that controls apoptotic signalling.
Keywords: apoptosis, differential regualtion, Mammalian Sterile 20 kinase.

TUE-035
Bisphenol A binds to ras proteins and competes with guanine nucleotide exchange: implications for GTPase-selective antagonists

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We show for the first time that bisphenol A has the capacity to interact directly with K-Ras and that Rheb weakly binds to bisphenol A and 4,4′-biphenol derivatives. We have characterized these interactions at atomic resolution suggesting that these compounds sterically interfere with the Sos-mediated nucleotide exchange in H- and K-Ras. We show that 4,4′-biphenol selectively inhibits Rheb signaling and induces cell death suggesting that this compound might be a novel candidate for treatment of tuberous sclerosis-mediated tumor growth. Our results propose a new mode of action for bisphenol A that advocates a reduced exposure to this compound in our environment. Our data may lay the foundation for the future design of GTPase-selective antagonists with higher affinity to benefit of the treatment of cancer because K-Ras inhibition is regarded to be a promising strategy with a potential therapeutic window for targeting Sos in Ras-driven tumours.
Keywords: None.

TUE-036
C/EBPβ expression is an independent predictor of overall survival in breast cancer patients by MHCII/CD4-dependent mechanism of metastasis formation

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CCAAT-enhancer binding protein beta (C/EBPβ) is a transcription factor playing a critical role in mammary gland development and breast cancer progression. Loss of C/EBPβ increases metastatic dissemination of mouse mammary tumor cells. However, the mechanism, by which C/EBPβ expression affects metastasis formation remains unknown.

To determine the relationship between C/EBPβ and survival of breast cancer patients, C/EBPβ expression was evaluated in 137 cases of human breast cancer and correlated with overall survival by Kaplan-Meier analysis. Additionally, the mouse 4T1 tumor model was used to elucidate the mechanism linking C/EBPβ with metastasis formation.

Here we report that decreased C/EBPβ expression is associated with shorter overall survival of breast cancer patients. We also demonstrate that in the murine 4T1 model loss of C/EBPb affects tumor growth and morphology, as well as promotes metastatic spread to the lungs. Immunohistochemical analyses showed that C/EBPβ inhibition leads to increased expression of MHCII, followed by accumulation of CD45, CD3 and CD4-positive lymphocytes in the tumors. Inflammation involvement in C/EBPβ-mediated metastasis formation was confirmed by DNA microarray and experiments on CD4+ cell-deprived nude mice. Additionally, anti-CD3 and anti-CD4 treatments of C/EBPβ-silenced tumor-bearing mice resulted in reverting C/EBPβ effect on tumor growth.

Altogether, C/EBPβ is a predictor of overall survival in breast cancer patients, and affects tumor growth, morphology and lung metastasis formation in murine 4T1 model. The mechanism of

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metastasis formation, we have discovered, involves immunological response depending on C/EBPβ-mediated activation of MHCII and accumulation of CD4 + lymphocytes in the tumor.
Keywords: breast cancer, inflammation, metastasis.

TUE-037
CD157/BST1 regulates cell adhesion and spreading through the interaction with the heparin-binding domains of fibronectin

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CD157/BST1 is a glycosylphosphatidylinisotol-anchored glycoprotein belonging to the ADP-rybosil cyclase gene family expressed in myeloid, endothelial, mesothelial cells and in epithelial ovarian cancer cells. In leukocytes, CD157 controls migration, adhesion to extracellular matrix (ECM) proteins and diapedesis. To accomplish these functions, CD157 establishes structural and functional partnership with specific members of the integrin family. In ovarian cancer, CD157 enhances cell motility and invasion of surrounding tissues, ultimately increasing tumor aggressiveness by promoting mesenchymal differentiation. To exert these receptor activities, CD157 interacts with an unknown ligand. The crucial role of CD157 in cell adhesion and migration, and its functional partnership with integrins led us to hypothesize that the non-substrate ligand of CD157 might be found in the ECM. Using solid phase and Surface Plasmon Resonance binding assays, we demonstrated that CD157 binds to fibronectin, fibrinogen, laminin and collagen I but not to vitronectin or to the polysaccharide components of ECM (such as heparin and hyaluronan). We identified the CD157 binding site within the N-terminal and C-terminal heparin-binding domains of fibronectin (HBD1 and HBD2). The CD157-HBD binding is mediated by the protein core while the glycosidic chains contribute to stabilize the interaction. Molecular docking analysis performed to predict the geometry of the interaction between CD157 and HBD1 or HBD2 of fibronectin suggested that a high number of residues (outside the catalytic domain in CD157) are involved. The CD157-ECM interaction demonstrated using purified proteins was confirmed in non-tumorigenic mesothelial Met-5A cells. Indeed, i) membrane CD157 expressed by Met-5A cells binds fibronectin and its HBDs, and ii) anti-CD157 antibodies significantly reduce cell adhesion to selected ECM proteins. Moreover, knockdown of CD157 expression in Met-5A cells reduced cell spreading and remarkably decreased the fibronectin-mediated phosphorylation of FAK, SRC and Akt tyrosine kinases. These morphological and functional changes resulted in impaired cell adhesion. It is reasonable to envision that the broad interaction of CD157 with several ECM proteins may be responsible for many of the biological effects exerted by CD157 in different physiological (e.g., leukocyte trafficking) and pathological contexts (e.g, inflammatory diseases and cancer), where the composition of the ECM dictates the final outcome.
Keywords: CD157, cell adhesion, extracellular matrix.

TUE-038
CDK8 promotes DNA replication but is dispensable for cell proliferation in vivo

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CDK8 is the most highly conserved metazoan CDK, as is its cognate cyclin, cyclin C (97% identity between Xenopus and Human). CDK8, which is essential for mouse pre-implantation development and is implicated in colorectal cancer. CDK8 is mainly thought to regulate transcription, where it functions in a sub-module of the Mediator complex. Here, we find that CDK8 is expressed in early stages of Xenopus embryogenesis when transcription is silent, it is recruited to chromatin and promotes, but is not essential for, DNA replication. To evaluate its role in cell proliferation and differentiation in somatic tissues *in vivo,* we have generated a conditional CDK8 knockout in the mouse intestinal epithelium. Induction of CDK8 knockout by tamoxifen feeding is efficient, but this does not affect intestinal morphology, cell proliferation or differentiation. Furthermore, CDK8 conditional knockout MEFs continue to proliferate and show no defects in cell cycle. Taken together we conclude that CDK8 is a multifunctional chromatin protein kinase that promotes a replication competent state, but it is not essential for cell division. The absence of a striking phenotype of loss of CDK8 in these experiments suggests functional redundancy. We have found that CDK19, a kinase conserved among vertebrates, highly related to CDK8 but which has no known function, is coexpressed with CDK8 in the intestinal epithelium and its levels increase upon CDK8 removal. We are undertaking TALEN-mediated knockout of CDK19 in wild-type and CDK8 knockout backgrounds to evaluate this potential synthetic lethality and compare the functions of these two highly related kinases.
Keywords: CDK8, colon cancer, Proliferation.

TUE-039
CEACAM19, the latest member of the CEACAM family of cancer-related cell adhesion molecules, represents a novel biomarker for lung cancer prognosis

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The carcinoembryonic antigen-related cell adhesion molecule (*CEACAM*) subfamily is robustly related to lung tumor progression. *CEACAM19*, the newest member of this tumor-related family (identified by Scorilas *et al*, 2003), which also hosts the well-established cancer biomarker CEA, has been recently associated with aggressive breast cancer. In this study we comprehensively profile the expression of *CEACAM19* in non-small cell lung cancer (NSCLC), a disease with unmet clinical needs regarding prognostic indicators.

Tissue samples from a total of 121 NSCLC patients were analyzed in the present study. RNA was isolated and purified on a QIAsymphony SP workstation, including DNAse I digestion. The amount and quality of total RNA were assessed by a NanoDrop 2000c spectrophotometer and an Agilent 2100 bioanalyzer, respectively. Samples with an RNA integrity number (RIN)> 6 were considered for subsequent analysis. Following reverse transcription and after rigorous quality control, *CEACAM19* mRNA

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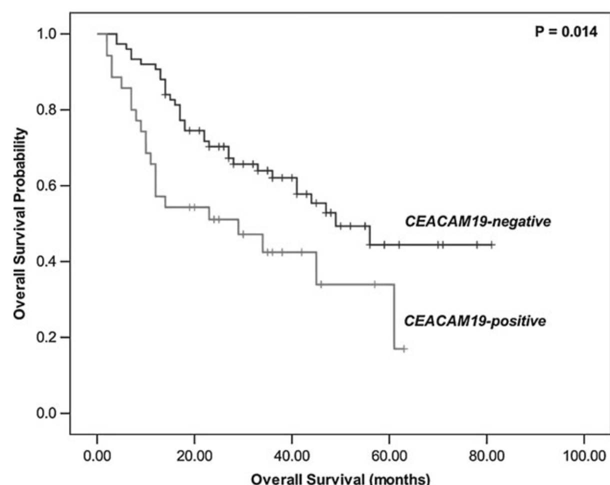

Fig. 1. Kaplan–Meier OS curves for CEACAM19 expression in NSCLC patients.

levels were quantified *via* an optimized qPCR method. SPSS Statistics software was used for biostatistical analyses.

CEACAM19 mRNA levels were slightly elevated in poorly differentiated compared to well differentiated tumors. Most importantly, patients stratified as *CEACAM19*-postitive exhibited inferior overall survival (OS) intervals compared to *CEACAM19*-negative ones (P = 0.014). The 5-year OS probability was 17.0% for *CEACAM19*-postitive patients and 49.3% for *CEACAM19*-negative ones. Univariate logistic regression corroborated the prognostic relevance of *CEACAM19* expression (HR = 1.96, 95% CI = 1.13–3.40, P = 0.016). Multivariate logistic regression analysis, adjusted for important clinicopathological parameters, including TNM stage, histotype, chemotherapy/radiotherapy administration, identified *CEACAM19* mRNA levels as a novel independent biomarker of poor prognosis for NSCLC patients (HR = 2.07, 95% CI = 1.11– 3.87, P = 0.023). The prognostic significance of *CEACAM19* was retained in the subgroup of low-risk patients (P = 0.041).

Improved risk stratification procedures are urgently needed for NSCLC. *CEACAM19* can aid in the decision-making for the management of NSCLC patients, since it has been identified by our study a biomarker that can provide important prognostic information which is independent of the currently used conventional indicators such as the TNM staging system.

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Keywords: Biomarker, CEACAM19, lung cancer.

TUE-040
Cell lineage-dependent activation of NF-κB by human T-cell leukemia virus type 1 Tax1

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Human T cell leukemia virus type1 (HTLV-1) is shown to be a causative agent of adult T cell leukemia (ATL), an aggressive malignancy. HTLV-1 produces the transcriptional modulator Tax1. Tax1 exerts its activities through activation of cellular transcription factors, among which NF-kB is demonstrated to be important for T cell transformation. There are two sub-pathways in the NF-kB system, canonical and non-canonical pathways. Though both pathways have been demonstrated to be activated by Tax1, we found in this study that Tax differentially activated NF-kB sub-pathways in a cell lineage-dependent manner.

Luciferase reporter plasmids with an immunoglobulin gene NF-kB binding element (IgkB site) and an OX40 ligand gene NF-kB binding element (gpkB site) were used to monitor NF-kB activities in response to Tax1. Expression of Tax1 activated IgkB and gpkB sites in hematopoietic cell lines such as Jurkat (human T cell leukemia cell line) and K562 (human erythroleukemia cell line). Interestingly the gpkB site was not activated by Tax1 in non-hematopoietic cell lines such as MG63 (human osteosarcoma cell line) and REF56 (rat embryonic fibroblast cell line) unlike the IgkB site, and exogenous introduction of RelA activated the gpkB site in non-hematopoietic cell lines. We found that hematopoietic cell lines activated the canonical and non-canonical pathways of NF-kB in response to Tax1, while only the non-canonical pathway was activated by Tax1 in non-hematopoietic cell lines. As TNF alpha stimulated NF-kB in non-hematopoietic cell lines, the canonical pathway system was functional in those cells. Tax1 however did not induce translocation of RelA to the nucleus, resulting in failure in RelA binding to the gpkB site. Chromatin immunoprecipitation assays revealed that non-canonical pathway components RelB and p52 bound the IgkB site, but the gpkB site showed p50 binding only in those cells. These results indicate that the gpkB site may be preferentially activated by the canonical pathway at least in hematopoietic cell lines tested. Importantly HTLV-1 Tax1 activates NF-kB in more restricted conditions than we thought, presumably accounting for pathogenesis of HTLV-1 infection.

Keywords: HTLV-1, leukemia, NF-kB.

TUE-041
Cellular internalization of Bortezomib loaded CS MNPs by cervical cancer cells

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Use of nanotechnology in cancer treatment offers exciting opportunities, including the possibility of destroying tumors with minimal damage to healthy tissue by novel targeted drug delivery systems. Chitosan coated superparamagnetic iron oxide nanoparticles (CS MNPs) were *in-situ* synthesized by ionic crosslinking

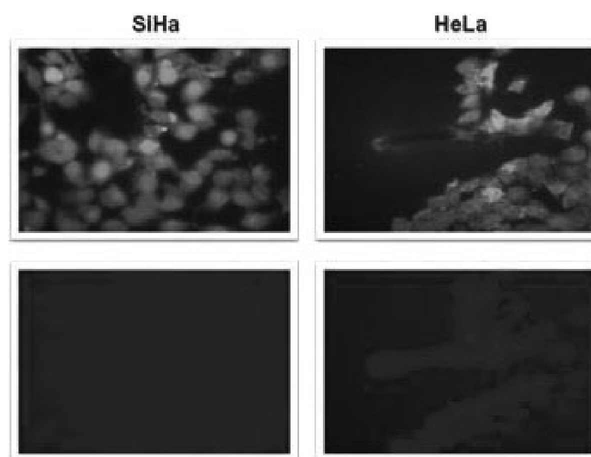

Fig. 1. Cellular internalization and localization of FITC labeled Bortezomib loaded CS MNPs on SiHa and HeLa cell lines.

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method as nanocarrier systems (Unsoy *et al*., 2012) and loaded with the anti-cancer drug Bortezomib (Velcade®).

Bortezomib loaded CS MNPs were labeled with FITC and visualized inside the cells by fluorescence microscopy on DAPI stained cervical cancer cell lines (SiHa and HeLa). Internalization of drug loaded nanoparticles can be clearly identified by the intensity of fluorescent color in the cells. FITC bounded Bortezomib loaded CS MNPs are successfully taken up by the cells and localized both in the cytoplasm and the nuclei of the cells (Figure 1).

Therefore, synthesized and Bortezomib loaded CS MNPs can be effectively enter to the cells and show its antiproliferative activity by inhibiting proteasome on cervical cancer cell lines.

Keywords: Bortezomib, cervical cancer, nanoparticle, FITC.

TUE-042

Cellular internalization of polyhydroxybutyrate coated magnetic nanoparticles in SKBR-3 cell lines

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Biodegradable polymeric nanomaterials gained importance in biomedical and bioengineering research such as drug delivery and targeting, tissue engineering, cancer diagnosis and therapy. Polyhydroxybutyrate (PHB) is a nontoxic, biodegradable, biocompatible polymer, and hence is suitable for medical applications. In this study, PHB coated magnetic nanoparticles (PHB-MNPs), were produced for targeted delivery of anticancer agent in cancer chemotherapy. PHB-MNPs were synthesized by in situ precipitation method. PHB-MNPs were incubated with breast cancer (SKBR-3) cell lines in 6 well plates and their photographs were taken with time intervals during the incubation to determine their cellular internalization. The cellular internalization of PHB-MNPs was demonstrated by fluorescence microscopy. It was revealed that these nanoparticles are efficiently internalized by the SKBR cells, and seem to be suitable for biomedical applications.

Keywords: PHB, magnetic nanoparticles, cellular internalization, SKBR-3 cell line.

TUE-043

Characterization of 7-dehydrocholesterol cytotoxic effects on melanoma cell lines

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Ultraviolet radiation is the main cause of skin cancers, and melanoma is the most serious form of tumor. Surgery is standard treatment for localized melanoma, while there is no therapy for advanced-stage of melanoma and its metastasis, because of the high resistance of melanoma cells to various anticancer therapies. Human skin is an important metabolic organ in which occurs photo-induced synthesis of vitamin D3 from 7-dehydrocholesterol (7-DHC). The 7-DHC, the precursor of cholesterol biosynthesis, is highly reactive and easily modifiable to produce 7-DHC-derived compounds. The intracellular levels of 7-DHC or its derivatives can have deleterious effects on cellular functionality and viability.

In this study we evaluated the effect on melanoma cell lines by 7-DHC as such and for this aim much care to minimize 7-DHC modifications was used. We found that from 12 to 72 hours of treatment 82-86% of 7-DHC entered into the cells, and the levels of 7-DHC-derivative compounds were not significant. At same time ROS production was significantly increased already after 2 hours and, after 24 hours, a reduction of cell viability was observed. Indeed, after 48-72 hours a pro-apoptotic effect of 7-DHC was detected. The cytotoxic effect of 7-DHC was associated with an increase in Bax levels, decrease in Bcl-2/Bax ratio, reduction of mitochondrial membrane potential, increase in apoptosis-inducing factor levels, unchanged caspase-3 activity, and absence of cleavage of PARP- 1. These findings could explain the mechanism through which 7-DHC exerts its cytotoxic effects. This is the first report in which the biological effects found in melanoma cells are mainly attributable to 7-DHC as such.

Keywords: 7-dehydrocholesterol, Melanoma cells, Reactive Oxygen Species.

TUE-044

Characterization of 7-dehydrocholesterol cytotoxic effects on melanoma cell lines

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Keywords: 7-dehydrocholesterol, Melanoma cells, Reactive Oxygen Species.

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TUE-045
CHI3L1 and TSC22 proteins modulate oncogenic properties of cell under oxidative agents

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Aims: Reactive oxygen species (ROS) are universal messengers of the extra- and intracellular signaling. However, the significance of ROS and antioxidant systems in tumor initiation and progression is complicated and contradictory. The inefficiency of the last years' anticancer therapies that used antioxidants to prevent progression of tumour could be attributed to the higher resistance of malignantly transformed cells to reactive oxygen species. The main goal of the present work was to reveal whether constitutive expression of *CHI3L1* oncogene leads to the resistance of malignantly transformed cells to oxidative damage. We performed this investigation to investigate the vulnerability of CHI3L1 oncogene-transformed cells to the reactive oxygen (ROS); to characterize proliferation of 293 cells stably producing CHI3L1 (293_*CHI3L1* cells) and to identify the involvement of key signaling pathways in reply to oxidants. As we showed earlier *CHI3L1* oncogene is overexpressed whereas *TSC22* oncosuppresor is down-regulated in most malignant human brain tumours – glioblastomas. So we aimed on the endorsement of TSC22 tumor suppressor protein capability to retard the proliferation and restore sensitivity of CHI3L1-producing cells to oxidants.
Methods and Results: 293 cells stably producing CHI3L1 (293_*CHI3L1*) cells had 2.7-fold higher rate constant for H_2O_2 scavenging than 293_pcDNA3.1 cells as it was shown by measurement of DCF fluorescence. Their resistance to ROS, at least partially, could be supported by the elevated level of reduced glutathione in 293_*CHI3L1* cells measured by MCB fluorescence. Ectopic expression of *TSC22* oncosuppresor diminished CHI3L1 production, retarded proliferation, and decreased the viability of CHI3L1-producing cells under oxidative stress according to the results of MTT test.
Conclusion: Our data indicate that altered expressions of *CHI3L1* oncogene and tumor suppressor gene *TSC22* contribute to ROS resistance suggesting a novel oncogene-dependent mechanism of chemoresistance in glioma.
Keywords: oncogene, glioblastoma, tumor suppressor.

TUE-046
Chitosan-coated magnetic nanoparticles as vehicles for the delivery of the antitumor drug gemcitabine

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Breast cancer is a heterogeneous group of diseases that cause cells in the body to change and grow out of control.

Gemcitabine, a nucleoside analogue, has indicated experimental and clinical antitumor activity in a variety of human tumors, including breast cancer. After integration of the gemcitabine to the DNA, DNA polymerase could add just one nucleotide to the DNA chain, which leads to termination of chain elongation, and forces the cell to apoptosis. However, gemcitabine has a short biological half-life and the plasma level of this drug can quickly drop below the effective threshold level.

Tumor-specific delivery of anticancer drugs will maximize the efficacy of the drug and minimize side effects and reduce systemic toxicity. Among the various drug delivery systems, chitosan has gained considerable attention due to its biocompatibility, biodegradability, and non-toxicity.Chitosan is a natural linear polysaccharide and it has playing an important role in controlling the release of drugs and proteins.

The aim of this investigation is to produce nanoparticles with magnetic core which actively target the drug-loaded nanoparticles to the tumor site by an externally applied magnetic field.

In this study, chitosan-coated magnetic nanoparticles (CsMNPs) were in situ synthesized in the presence of chitosan and sodium tripolyphosphate (TPP) molecules.

Gemcitabine loading in CsMNPs was performed by mixing various concentrations of the drug with CsMNP in order to optimize the loading efficiency.The loading capacity were determined by measuring the amount of remaining drug in the supernatant by UV spectrophotometer.

The drug release and stability of gemcitabine-loaded CsMNPs were determined at pH 4.2, pH 5.2, and pH 7.2.

The cytotoxicity of gemcitabine-loaded nanoparticles and drug alone was assessed by XTT assay on breast cancer cell lines.

Successful binding of gemcitabine to CsMNPs were indicated using XPS, zeta-potential, and FTIR. UV spectophotometric measurements also validated the gemcitabine loading in these particles.

The drug unbounded chitosan nanoparticles were found to have minimum cytotoxicity in the XTT analysis. *In vitro* cytotoxicity of gemcitabine-CsMNP complex is increased significantly when bounding CsMNP comparedto unloaded with the free drug toxicity.

In the present study, a new nanocarrier systemfor gemcitabine delivery was reported which showed enhanced antitumor activity on breast cancer cell lines. Binding to the chitosan nanoparticles significantly enhanced the bioavailability and increased the accumulation of the gemcitabine in cancer cells, and caused apoptosis.
Keywords: Antitumor therapy, Chitosan, Gemcitabine.

TUE-047
Circulating growth factors in patients with colorectal cancer and colorectal polyps

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Early detection of cancer is a cruical factor for a successful therapy and increased survival rate in patients with colorectal cancer. Predictive potentials of circulating insulin like growth factor 1 (IGF-1), transforming growth factor beta 1 (TGFβ1), epidermal growth factor (EGF) and extracellular domain of c-erb-B2 (co-receptor of EGF), were examined in the present study.

Patients with colorectal cancer (n = 31), patients with colorectal polyps (n = 23) and age-matched healty volunteers (n = 30) were included by the study. Blood samples were obtained just before the operation and in the 30th postoperative day. Serum levels of IGF-1, TGFβ1, EGF and c-erb-B2 were measured by ELISA kits.

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The preoperative IGF-1 level in the colorectal cancer group was significantly higher than those in the colorectal polyp group and control group, whereas preoperative EGF level was significantly higher in the both colorectal cancer and colorectal polyp groups compared to control group. The preoperative TGFβ1 level in the colorectal cancer group was found to be higher than those in the colorectal polyp group and control group. In the colorectal cancer group, postoperative IGF-I level was found to be decreased but this decrease was not statistically significant. The postoperative EGF level was significantly lower in the colorectal cancer group compared to its preoperative level but it was still higher than those in the control group. In the colorectal polyp group, the postoperative EGF level significantly decreased to level of the control group. In the both colorectal cancer and colorectal polyp groups, postoperative TGF β1 levels were signicantly lower compared to their corresponding preoperative levels. There was no significant difference between study groups for preoperative and postoperative c-erb B-2 levels. A significant correlation was found between tumor size and TGF- β1 in the colorectal cancer group ($r = 0.378$ $p < 0.05$).

It was concluded that serum TGF β1 level might be a good predictor for diagnose and prognostic follow up in colorectal cancer patients. High levels of EGF and TGF β1 in patients with colorectal polyps may provide awareness to take precautions.

Keywords: colorectal cancer, epidermal growth factor, transforming growth factor beta 1.

TUE-048
CK1δ affects cancer cell adaptation to hypoxia by controlling HIF-1α/ARNT complex formation

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Hypoxia-inducible factor 1 (HIF-1) is a key regulator of cellular response to hypoxic conditions. It acts as a heterodimer, consisting of two subunits, HIF-1α and HIF-1β (or ARNT). ARNT is constantly expressed while HIF-1α stability is regulated by oxygen levels. HIF-1α is additionally controlled by oxygen-independent mechanisms including activating modification of its C-terminal part by ERK and inactivating phosphorylation of S247 at its N-terminal part by CK1δ. Previous biochemical and *in vitro* experiments have suggested that phosphorylation by CK1δ reduces the affinity of HIF-1α for ARNT[1]. To corroborate these results in living and/or intact cells, we have applied fluorescence recovery after photobleaching (FRAP) and *in situ* proximity ligation assay (*in situ* PLA) methods to study the formation of the HIF-1α/ARNT complex under conditions that stimulate or inhibit CK1δ-dependent modification of HIF-1α. Analysis of cells expressing GFP-tagged forms of HIF-1α with FRAP shows that a Ser247 to Ala247 mutation or treatment with a CK1-specific inhibitor slows down the nuclear migration of HIF-1α indicating increased formation of a DNA-binding complex. This hypothesis is further supported by *in situ* PLA in cells grown under hypoxia (1% O_2), which shows that complex formation by endogenous untagged HIF-1α and ARNT is impaired when CK1δ is overexpressed but enhanced when CK1δ is inhibited. These findings have biological significance as CK1δ inhibition enhances cancer cell proliferation under hypoxic conditions. Taken together, our data suggest that CK1δ impairs cancer cell adaptation to hypoxia by inhibiting HIF-1α/ARNT heterodimerization.

Co-financed by the European Union (ESF) and Greek national funds (Education and Lifelong Learning-NSRF, Program THALIS).

Reference

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Keywords: CK1δ, HIF-1α, Hypoxia.

TUE-049
Clinical Relevance of TMPRSS2-ERG fusion marker for prostate cancer

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Objectives: The novel *TMPRSS2: ERG* gene fusion is a common somatic event in prostate cancer that in some studies is linked with a more aggressive disease phenotype. Thus, this study aims to determine whether clinical variables are associated with the presence of *TMPRSS2: ERG*-fusion gene transcript in Indian patients of prostate cancer.
Methods: We evaluated the clinical variables with presence and absence of *TMPRSS2: ERG* gene fusion in prostate cancer and BPH association of clinical patients. Patients referred for prostate biopsy because of abnormal DRE or/and elevated sPSA were enrolled for this prospective clinical study. TMPRSS2: ERG mRNA copies in samples were quantified using a Taqman chemistry by real time PCR assay in prostate biopsy samples ($N = 42$). The T2:ERG assay detects the gene fusion mRNA isoform TMPRSS2 exon1 to ERG exon4.
Results: Histopathology report has confirmed 25 cases as prostate cancer adenocarcinoma (PCa) and 17 patients as benign prostate hyperplasia (BPH). Out of 25 PCa cases, 16 (64%) were T2: ERG fusion positive. All 17 BPH controls were fusion negative. The T2: ERG fusion transcript was exclusively specific for prostate cancer as no case of BPH was detected having T2: ERG fusion, showing 100% specificity. The positive predictive value of fusion marker for prostate cancer is thus 100% and the negative predictive value is 65.3%. The T2: ERG fusion marker is significantly associated with clinical variables like no. of positive cores in prostate biopsy, Gleason score, serum PSA, perineural invasion, perivascular invasion and peri prostatic fat involvement.
Conclusions: Prostate cancer is a heterogeneous disease that may be defined by molecular subtypes such as the *TMPRSS2: ERG* fusion. In the present prospective study, the T2:ERG quantitative assay demonstrated high specificity for predicting biopsy outcome; sensitivity was similar to the prevalence of T2:ERG gene fusions in prostate tumors. These data suggest that further improvement in diagnostic accuracy could be achieved using a nomogram that combines T2:ERG with other markers and risk factors for prostate cancer.

Keywords: Gene Fusion, Prostate cancer.

TUE-051
Cobalt inhibits HIF-2-dependent gene expression by blocking HIF-2α/USF2 interaction in liver cancer cells

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Cellular responses to oxygen play an important role in many aspects of physiologic homeostasis. Central to these responses is the hypoxia-inducible factor (HIF) transcriptional control system. Although HIF-1 is usually considered as the principal mediator of hypoxic adaptation, several tissues and different cell types

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express both HIF-1 and HIF-2 isoforms under hypoxia or when treated with hypoxia mimetic chemicals such as cobalt. However, the similarities or differences between HIF-1 and HIF-2, in terms of their tissue- and inducer-specific activation and function, are not adequately characterized. To address this issue, we investigated the effects of true hypoxia and hypoxia mimetics on HIF-1 and HIF-2 induction and specific gene transcriptional activity in hepatic (Huh7 and HepG2) and non-hepatic (HEp2 and Saos-2) cancer cell lines.

Both hypoxia and cobalt caused rapid induction of both HIF-1α and HIF-2α proteins in all cell lines tested. In liver cancer cells, hypoxia induced erythropoietin (EPO) expression and secretion in a HIF-2-dependent way. Surprisingly, however, EPO expression was not induced when cells were treated with cobalt. In agreement, both HIF-1- and HIF-2-dependent promoters (of PGK and SOD2 genes, respectively) were activated by hypoxia while cobalt activated the HIF-1-dependent PGK promoter, but not the HIF-2-dependent SOD2 promoter. Interestingly, the negative effect of cobalt on HIF-2 activity was not observed in non-hepatic cancer cells. Unlike cobalt, other hypoxia mimetics, such as DFO and DMOG, stimulated both types of promoters. Furthermore, cobalt impaired the hypoxic stimulation of HIF-2, but not HIF-1, activity without affecting the HIF-2α nuclear accumulation. This specific effect of cobalt on HIF-2 activation was accompanied by reduced interaction of HIF-2α with upstream stimulatory factor 2 (USF2), a HIF-2-specific co-activator.

These data show that despite similar induction of HIF-1α and HIF-2α protein expression, HIF-1 and HIF-2 specific gene activating functions respond differently to different stimuli. Also, this study suggests that the operation of oxygen-independent and gene- or tissue-specific regulatory mechanisms involve additional transcription factors or co-activators.

Keywords: erythropoietin, hepatoma, HIF-2.

TUE-052
Cobalt inhibits HIF-2-dependent gene expression by blocking HIF-2α/USF2 interaction in liver cancer cells

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Cellular responses to oxygen play an important role in many aspects of physiologic homeostasis. Central to these responses is the hypoxia-inducible factor (HIF) transcriptional control system. Although HIF-1 is usually considered as the principal mediator of hypoxic adaptation, several tissues and different cell types express both HIF-1 and HIF-2 isoforms under hypoxia or when treated with hypoxia mimetic chemicals such as cobalt. However, the similarities or differences between HIF-1 and HIF-2, in terms of their tissue- and inducer-specific activation and function, are not adequately characterized. To address this issue, we investigated the effects of true hypoxia and hypoxia mimetics on HIF-1 and HIF-2 induction and specific gene transcriptional activity in hepatic (Huh7 and HepG2) and non-hepatic (HEp2 and Saos-2) cancer cell lines.

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These data show that despite similar induction of HIF-1α and HIF-2α protein expression, HIF-1 and HIF-2 specific gene activating functions respond differently to different stimuli. Also, this study suggests that the operation of oxygen-independent and gene- or tissue-specific regulatory mechanisms involve additional transcription factors or co-activators.

Keywords: erythropoietin, hepatoma, HIF-2α.

TUE-053
Co-exposure of human kidney cells to two carcinogenic mycotoxins (Ochratoxin A and Fumonisin B1): molecular mechanisms

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Fumonisin B_1 (FB_1) and ochratoxin A (OTA) are mycotoxins classified as possible human carcinogens (IARC, 1993). They coexist in human food and have been shown to act synergistically in stimulating nephrotoxicity in pigs (Diaz et al, 2001; Pfohl-Leszkowicz & Manderville, 2007; 2012). In this study the combined impact of the toxins in cultured human kidney cells (HK2) was analysed. Parameters common to both FB_1 and OTA (cytotoxicity and activation of mitogen-activated protein kinase, MAPK), and features characteristic of the individual mycotoxins (DNA adduction by OTA and release of arachidonic acid (AA) by FB_1) were measured. Treatment of HK2 cells with the combined toxin mixture (FB_1 + OTA) was found to stimulate cell proliferation. Under analogous conditions FB_1 was weakly cytotoxic while OTA showed no impact on cell viability. The conditions for cell proliferation by (FB_1 + OTA) were then examined for DNA adduction, release of AA and activation of MAPK. OTA-mediated DNA adduction, as measured by ^{32}P-postlabeling, occurred at a quicker rate in the presence of FB_1, with maximum levels being detected following 2 h incubation; with OTA alone adducts were not observed until 7 h incubation. Release of AA by FB_1 was inhibited by OTA, although levels of AA, prostaglandins (PGs) and leukotrienes (LTs) were higher for the combined toxin mixture than for OTA alone. Western blot analysis for MAPK activation showed the mycotoxins to synergistically enhance MAPK activity. Our data suggests that stronger nephrotoxic damages to human kidney are likely from combined exposure of (FB_1 and OTA) versus the individual mycotoxins, as resistance to apoptosis and stimulation of cell proliferation are key events in the development of tumors

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Keywords: Carcinogenesis, Kidney, mycotoxin.

TUE-054
Combined regulation of carbonic anhydrase 9 (CA9) expression via hypoxia and cytokines in hepatocellular carcinoma cells

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Our aim was to study the expression of carbonic anhydrase (CA) 9 in human hepatocellular carcinoma (HCC) cells. We studied CA9 protein, CA9 mRNA and hypoxia-inducible factor-1 alpha (HIF-1α) protein levels in Hep3B cells exposed in different parallel approaches. In one of these approaches, HCC cells were exposed to extreme in vitro hypoxia (24 h 0.1% O2) without or with interleukin (IL)-1, IL-6, tumor necrosis factor alpha (TNF-α) and transforming growth factor-beta (TGF-β) stimulation for the same hypoxic exposure time or exposed to normoxic oxygenation conditions without or with cytokine stimulation.The tumour cell line analysed showed a strong hypoxic CA9 mRNA expression pattern in response to prolonged severe hypoxia with cell-line specific patterns and a marked induction of CA9 protein in response to severe hypoxia. These results were paralleled by the results for HIF-1α protein under identical oxygenation conditions with a similar expression tendency to that displayed during the CA9 protein expression experimental series. Continuous stimulation with the cytokines, IL-1, IL-6, TNF-α and TGF-β, under normoxic conditions significantly increased the carbonic anhydrase 9 expression level at both the protein and mRNA level, almost doubling the CA9 mRNA and CA9 and HIF-1α protein expression levels found under hypoxia. The findings from these experiments indicated that hypoxia is a positive regulator of CA9 expression in HCC, and the four signal transduction pathways, IL-1,IL-6, TNF-α and TGF-β, positively influence CA9 expression under both normoxic and hypoxic conditions. Our findings may potentially be considered in the design of anti- cancer therapeutic approaches involving hypoxia-induced or cytokine stimulatory effects on expression. In addition, they provide evidence of the stimulatory role of the examined cytokine families resulting in an increase in CA9 expression under different oxygenation conditions in human cancer, especially HCC, and on the role of the CA9 gene as a positive disease regulator in human cancer.

Keywords: Cancer, Carbonic Anhydrase 9 (CA9), Hypoxia.

TUE-055
Comparative expression of KLF4 in normal and tumor laryngeal tissues

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The kruppel like factor KLF4, overexpressed in many cancers,is involved in carcinogenesis by acting as a tumor promoter or tumor suppressor depending on the cell type and context.Overexpression of this factor is often related to the progression and tumor agressiveness and metastatic potential.

In this study, we avaluated by immunohistochemistry the expression level of KLF4 in 15 larynx cancer biopsies from different stages compared to normal tissue.the SPSS statistical analysis revealed a significant difference in the expression of KLF4 between healthy tissue and tumor tissue.The existence of a significant difference in the mean expression of KLF4 between tumor stages (I,II,III,IV)was demonstrated.No correlation was observed between the expression of KLF4 and each studied epidemiological factor(age,alcohol, all of them were heavy smokers)

These preliminary results suggest that KLF4 may be considered as a possible biomarker of larynx cancer

Keywords: immunohistochemistry, KLF4, larynx cancer.

TUE-056
Comparative study of the sensitivity of chondrosarcoma to DNA damaging agents

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Chondrosarcomas are rare tumors, potentially fatal. No effective treatment exists since chondrosarcomas are chemoresistant and radioresistant. Current treatment consists to tumor resection which may lead to amputation. The aim of this work is to study the resistance mechanisms of chondrosarcoma to different DNA damaging agents.

Different chondrosarcoma cells lines with different origin and grade were used. Their sensitivity to DNA damaging agents (X-ray irradiation or cisplatine treatment) was evaluated by carrying out survival curve obtained after cell counting or clonogenicity assay. Cell cycle was studied by flow cytometry after staining cells with propidium iodide. Apoptosis investigated by monitoring the Apo 2.7 release by flow cytometry (marks of early stages of apoptosis).

Cisplatin and irradiation induces different responses following the treatment. Some chondrosarcomas are more sensitive than others. DNA damage treatments lead to an increased sub-G1 fraction correlated to increased Apo 2.7 expression of the sensitive cell lines. These data confirm apoptotic death of cells to cisplatin and irradiation of the sensitive chondrosarcomas.

Our study shows that chondrosarcoma cell lines have different responses to DNA damaging agents. Chondrosarcomas cells lines sensitive to cisplatine aren't the same than these sensitive to irradiation. However, treatments induce apoptotic death of the sensitive chondrosarcomas cells lines. It is therefore essential to use multiple chondrosarcomas (cells lines or primary cultures) with different grade or origin in order to investigate the response of these tumors to conventional (radiotherapy, chemotherapy) or emerging treatment as hadrontherapy.

Keywords: chondrosarcoma, cisplatin, X-ray.

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TUE-057

Comparison of two polymeric nanocarriers for targeted CpG-ODN delivery: chitosan- and poly (amidoamine) dendrimer-coated magnetic nanoparticles

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The field of gene delivery has experienced rapid growth due to its application in the treatment of various diseases such as cancer.

Unmethylated cytosine-phosphate-guanosine oligodeoxynucleotides(CpG-ODNs) have antitumor activity via interaction with Toll-like receptor 9.Successful transfer of CpG-ODNs to the tumor site is dependent on loading them in a suitable nanocarrier to protect CpG-ODN from serum nucleases and facilitating its bioavailability.

Poly(amidoamine) (PAMAM) dendrimers and chitosan have been proposed as biocompatible polymers for gene delivery. PAMAM is a dendrimer with surface amine groups which form stable complexes with ODNs.On the other hand, chitosan is a biodegradable,and biocompatible polymer with minimum toxicity.

Active magnetic targeting strategies applied on nanoparticles enhance the intracellular drug concentrations in cancer cells and avoid toxicity in normal cells (Fig. 1).

In this research, the aim is to synthesize chitosan- and G5PAMAM dendrimer-coated nanoparticles (CsMNP and DcMNP), to be used in targeted cancer therapy and to compare them in four aspects, CpG-ODN loading efficiency,complex stability, CpG-ODN release behavior,and cytotoxicity.To achieve this purpose, in situ synthesis of CsMNP was performed. For synthesizing G5DcMNP, magnetite was produced with coprecipitation method and coated with PAMAM dendrimer.

The CsMNPs and DcMNPs were mixed with CpG-ODN and their ability of binding was evaluated. Moreover, these nanoparticles were characterized and compared to each other in terms of cytotoxicity, particle size, and CpG-ODN release.

DcMNPs showed greater ability to form a complex with CpG-ODN and showed more suitable physicochemical properties as delivery system. The sufficient surface amine groups enable DcMNPs to interact with DNA through charge-based interactions.Lower loading efficiency of the chitosan may also depend on other factors,such as the chemical structure, nanoparticle size, and composition.

DcMNP is found to be more toxic compared to CsMNP, due to the amine groups on the PAMAM dendrimer surface, which can be reduced by surface modification.The availability, noncytotoxicity, ease of modification are the advantages of CsMNPs.For DcMNPs, the interest arises from their ease of synthesis with controlled size, and high transfection efficiencies.

In conclusion, these results suggest that DcMNPs seem to be a better candidate with higher loading of CpG-ODN which make it suitable for targeted gene delivery.Using DcMNPs as a suitable nanocarrier may lead the CpG-ODN a powerful agent for tumor therapy.

Keywords: Chitosan polymer, CpG-oligodeoxynucleotides, PAMAM dendrimer-coated magnetic nanoparticles.

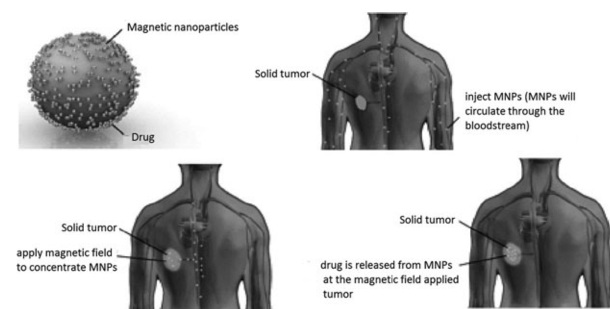

Fig. 1. Anticancer drug-loaded magnetic nanoparticles are guided by external magnetic field after the administration (Biophan Technologies, 2013).

TUE-058

Conjugation of gemcitabine to PAMAM dendrimer-coated magnetic nanoparticles for targeted delivery

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Magnetic iron oxide nanoparticles (MNPs) as carriers for delivering chemotheraptic drugs to tumors have been described well.Efforts in implementing magnetic particles for medical applications are based on their lack of toxicity, biodegradability, biocompatibility, and absorption. In addition, controllable size and targeting ability to the desired site by an external magnetic field are the advantages of MNP.

A new model of MNPs coated with Polyamidoamine (PAMAM) dendrimer could overcome the side effects of chemotherapy. Modifiable surface functionality, monodispersity and nanosize of PAMAM make these dendrimers attractive candidate for drug delivery (Fig 1).However, biomedical applications of PAMAM dendrimers limited due to their toxicity associated with the terminal-NH2 groups and multiple cationic charges. Surface engineering by modifying the surface of dendrimers with various functional groups is a satisfactory strategy for producing less toxic PAMAM dendrimers.

Gemcitabine (2′, 2′-diflurodeoxycytidine) being an analogue of deoxycytidine, is an effective anticancer drug against several solid tumors, including breast cancers.

However, clinical benefits of gemcitabine are limited due to some aspects, such as short plasma half-life after intravenous administration resulting from rapid metabolism by cytidinedeaminase, which represents a major disadvantage of this anticancer compound. Gemcitabine hydrophilicity is another limitation of using this drug, since it can not cross the plasma membrane and not accumulate into the cancer cells. Additionally, when the drug is administrated alone, significant damage to non-cancerous tissues is caused due to the lack of target specificity. This results in

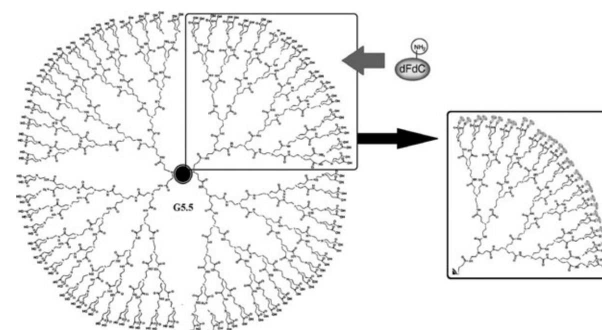

Fig. 1. Gemcitabine (dfdc)-loaded DcMNP.

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serious side effects such as hair loss, anemia, vomiting, and loss of appetite.

The aim of this study is to obtain an effective targeted delivery system for gemcitabine. MNPs were synthesized by co-precipitation method. The different generations of PAMAM dendrimer coated magnetic nanoparticles (DcMNPs) were conjugated with gemcitabine.

The results show that best loading efficiency was obtained for generation 5.5 of DcMNPs. The FTIR and XPS analysis supported the conjugation of drug and dendrimer. TEM images show nanoscale size of the nanoparticles before and after conjugation. The zeta-potential analysis indicated a decreased negativity of surface charge in drug bound dendrimer. Thus, it was shown that Gemcitabine was conjugated onto the surface of PAMAM coated magnetic nanoparticles successfully. Further in vitro studies have indicated higher cytotoxicity of drug bound nanoparticles on breast cancer cell line compared to free Gemcitabine.

Keywords: Gemcitabine, magnetic nanoparticles, PAMAM dendrimer.

TUE-059
CRBP1 could be a new tumor suppressor gene in cervical cancer

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Aims: Cervical Cancer (CC) is one of the most important health problems in women. CC shows genetic changes at chromosome region 3q21. This region contains the Cellular Retinol Binding Protein 1 gene (*CRBP1*). This protein has been implicate as a key element in the development of other types of cancer. The main goal of the present work was to determine the molecular alterations of *CRBP1* and its relationship to CC.

Methods: To determine the molecular alterations of *CRBP1* gene in CC; twenty-six CC and twenty-six healthy cervix samples were evaluated for: 1) Copy number gain by real-time PCR analysis, 2) expression levels by an immunohistochemistry assay on tissue microarray, and 3) the methylation status of the *CRBP1* promoter region.

Results: The increase in *CRBP1* copy number was observe in 10 out of the 26 CC samples analyzed, while healthy cervices samples showed no changes in the copy number. In addition, there was a lack of expression of the *CRBP1* gene in an important number of the CC samples (17/26), and the *CRBP1* gene promoter was methylate in 15/26 of the CC samples. Interestingly, there was a significant association between the lack of expression of the *CRBP1* gene and its methylation status. Conclusions: The data indicates that, both activating and inactivating changes in the *CRBP1* gene could be significant events in the development and progression of CC, and a lack of expression of the CRBP1 protein could be related with to the development of CC. We believe that there is enough evidence to consider the *CRBP1* gene as a tumor suppressor gene for CC.

Keywords: CRBP1, Expression, suppressor gene.

TUE-060
Cx43 expression in endothelial cells determines prostate cancer cell diapedesis†

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Metastatic niche is defined as the microenvironment of cancer cells at the place of their prospective extravasation. Efficiency of cancer cell diapedesis is determined by intercellular communication between cancer and endothelial cells, which among others depends on the function of connexin (Cx)43. Using an in vitro model of "metastatic niche" based on co-cultures of prostate cancer (DU-145) and endothelial cells (HUVEC), we assessed the role of Cx43-dependent mechanisms in the regulation of endothelial cell mobilization during prostate cancer cell diapedesis. The involvement of Cx43 in DU-145 cell diapedesis and endothelial mobilization was illustrated by the attenuation of transendothelial migration of DU-145 cells and of DU-145-induced HUVEC motility observed upon Cx43 silencing in cancer cells. Concomitantly with HUVEC mobilization, an induction of Cx43 expression was observed in endothelial continuum proximal to DU-145 cells. Apparently, it sensitized the endothelium to Cx43-mediated signals from cancer cells and facilitated their transendothelial migration. Cx43 silencing in DU-145 by siRNA, but not chemical block of GJIC, reduced this effect, which indicates the involvement of GJIC-independent Cx43 signaling in this process. Moreover, peripheral blood monocytes modulated DU-145-induced Cx43 expression changes in HUVEC in response to DU-145 cells, suggesting the involvement of paracrine loops within the metastatic niche. Because the effect of DU-145 and monocytes on Cx43 expression was partially eradicated by ERK1/2 inhibitor PD98059, ERK1/2-dependent pathway seems to be involved in the regulation of Cx43 expression in HUVEC. When extrapolated to in vivo situation, these data explain how Cx43 confers selective advantage on the cells during their invasion and confirm the significance of Cx43 for prostate cancer cell diapedesis.

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Keywords: Cx43, metastasis, prostate cancer.

TUE-061
CYLD downregulation in human hepatocellular carcinoma cells antagonizes p53-mediated apoptosis

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Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide, since its highly chemoresistant nature renders chemotherapy with conventional cytotoxic agents ineffective. Shedding light to the molecular mechanisms and signaling pathways involved in hepatocarcinogenesis, will lead to novel successful therapies.

CYLD is a deubiquitinating enzyme that was originally identified as the tumor suppressor of familial cylindromatosis. CYLD's deficiency has been shown to promote the development of several types of malignancies including HCC. Hepatoma cell lines display significantly lower CYLD expression compared to primary human hepatocytes, while its inactivation in murine hepatocytes can lead to fibrosis, inflammation and cancer. Therefore, loss of

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CYLD expression may contribute to HCC development and progression, highlighting CYLD as a new target for therapeutic intervention. *Cyld*'s silencing involves DNA methylation, histone deacetylation and direct recruitment of Snail to *Cyld*'s promoter whereas, recruiment of NF-κB, SRF and BAF57 to *Cyld* promoter promote transcription. However, the mechanisms of *Cyld's* silencing in HCC remain still ellusive.

We previously identified the tumor suppressor p53 as a putative transcriptional regulator of *hCyld* and demonstrated that both its exogenous expression and treatment of wt-p53 HepG2 cells with drugs like doxorubicin and etoposide, upregulate CYLD's protein and mRNA levels. ChIP analysis reveals now that CYLD's upregulation upon etoposide treatment is not employed via direct binding of p53 to three putative p53-binding sites on *hCyld* promoter,. Since p53 is frequently mutated in HCC and p53-dependent apoptosis primarily determines the efficacy of chemotherapy, we investigated further the role of CYLD in p53-mediated apoptosis. We treated HepG2 cells with p53 Activator III, RITA, a specific acivator of p53 that disrupts the MDM2-p53 complex and recorded an early upregulation of CYLD followed by a gradual decline, where apoptosis was evident. Using viability assays and RNAi silencing we show that *Cyld's* knockdown in HepG2 cells antagonizes RITA induced decline in cell survival. Our experiments thus far reveal a possible implication of CYLD in p53-mediated cell death of HCC cells that may be of potential therapeutic importance.

Keywords: CYLD, Hepatocellular carcinoma, p53-mediated apoptosis.

TUE-062
CYLD down-regulation promotes oncogenic phenotypic characteristics in breast cancer cells

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The cylindromatosis gene (*Cyld*) is a tumor suppressor that is mutated in familial cylindromatosis, a rare autosomal dominant disorder associated with numerous benign skin adnexal tumors. However, a number of reports have indicated a role for CYLD in growth suppression of different types of cancer including breast cancer. CYLD is a deubiquitinating enzyme which hydrolyzes preferentially K63-linked and linear polyubiquitin chains. It is a known regulator of several signaling pathways including the NF-kB, MAPK and Wnt/β-catenin pathways. This study investigated the role of CYLD in the growth characteristics of breast cancer cell lines. We assessed mRNA and protein expression profiles of CYLD in two basal (MDA-MB-231, HCC1937) and two luminal (MCF7, T47D) breast cancer cell lines and in a non-tumorigenic mammary epithelial cell line (MCF10A). All breast cancer cells exhibited significantly reduced CYLD mRNA expression compared with MCF10A cells. Next, stable monoclonal cell lines with varying levels of CYLD downregulation were established by lentiviral mediated expression of short-hairpin RNAs (shA, shB), and one non-silencing RNA control (NS). CYLD downregulation resulted in statistical significant enhancement of oncogenic characteristics as measured by cell viability (MTT assay), cell migration (wound healing assay) and anchorage-independent growth in semi-solid substrate. At the molecular level CYLD downregulation enhanced JNK activation whereas additional signaling pathways are currently being analyzed in breast cancer cell lines with downregulated CYLD expression. Our findings indicate that downregulation of CYLD contributes to multiple phenotypic characteristics of breast cancer cells.

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: ARISTEIA-1921-EMBRACE. Investing in knowledge society through the European Social Fund.
Keywords: breast cancer, CYLD, signaling.

TUE-063
Cysteine protease cathepsin X regulates the function of gamma enolase in malignant progression

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Gamma enolase (neuron specific enolase) has been used as a tumor marker in neuroendocrine tumors, however, its role in cancer is not well understood. As a glycolytic enzyme it can be involved in increased aerobic glycolysis in cancer cells and thus their enhanced proliferation. On the other hand, it can act as a prosurvival factor, as demonstrated for neuronal cells, where gamma enolase can activate PI 3-K/Akt and MAPK/ERK signaling pathways. This effect is achieved through the active site at the C-terminal part of the molecule. Gamma enolase prosurvival activity is regulated by cysteine protease cathepsin X, which sequentially cleaves amino acids from the C-terminal end. In clinical practice, total gamma enolase, including the uncleaved (active) and the C-terminally cleaved (inactive) forms is evaluated in cancer samples. The levels of active uncleaved gamma enolase may provide additional information on patient's status.

To prove that, we performed a prospectively accrued multicenter observational cohort study on 264 patients with colorectal cancer. Blood samples were collected before preoperative large bowel endoscopy. Uncleaved gamma enolase was measured in sera by ELISA and the total gamma enolase by ECLIA (Elecsys NSE assay). Univariate survival analysis showed that the increased levels of total ($HR = 1.40$, $p = 0.0004$) and uncleaved gamma enolase ($HR = 1.11$, $p = 0.025$) were related to poor prognosis. In multivariate analysis however, only total gamma enolase levels had independent, significant ($p = 0.03$) value. Additionally, in stage IV patients we observed that total gamma enolase levels may predict response to palliative chemotherapy.

The levels of total and uncleaved gamma enolase were evaluated also in the CaCo2 cell line. The uncleaved form of gamma enolase was significantly upregulated, when the cells were cultured in serum-starvation medium. Its upregulation was even more evident in the presence of the cathepsin X specific inhibitor AMS-36 or in cells silenced for cathepsin X by siRNA.

Our results demonstrate that besides glycolytic activity of gamma enolase its prosurvival function is important for progression of malignant diseases. Cathepsin X, a protease impairing the prosurvival function, represents a possible tool for regulation of malignant processes.

Keywords: cancer signalling, cathepsin X, gamma enolase.

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TUE-064
Cytotoxic effect and apoptosis induction by *Cerastes cerastes* and *Macrovipera mauritanica* venoms and evaluation of their antimicrobial profile

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Viperidae venoms contain a variety of pharmacologically active substances which are recognized for their therapeutic properties. The present study aims to highlight the antibacterial and antitumoral activities of Moroccan viper Cerastes cerastes and Macrovipera mauritanica venoms and their purified fractions.

The antimicrobial potential of both venoms and their fractions was evaluated against strains of bacteria and yeast by the disk diffusion method. The results showed that both venoms and their toxic fractions have dose-dependent antibacterial activity against the majority of Gram positive and negative bacterial strains used, with the exception of Pseudomonas aeruginosa and yeasts tested which have been found to be resistant.

Venoms and fractions were evaluated for their cytotoxicity and their capacity to activate apoptosis in the cancer cell line P815. The results of the MTT assay have shown a differential cytotoxic effect between both venoms and their fractions. Both venoms appear to inhibit the proliferation and cell adhesion in a dose and time dependent 24, 48 and 72 h after incubation. Morphological changes were also observed along the post-treatment period compared with untreated control cells. The DNA fragmentation test and cells marking with Annexin V-Biotin were found positive for both venoms indicating the involvement of apoptosis in the inhibition of cell proliferation.

In conclusion, this work provides the first experimental evidence showing the antibacterial and antitumor potential of Moroccan viper venom in vitro. Further studies are needed to elucidate the molecular mechanism implicated in these activities.

Keywords: Antibacterien activity, antitumoral activity, viperidae venom.

TUE-065
Deciphering triple negative breast cancer driven pathways via multi-omic networks

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Triple negative breast cancer (TNBC) constitutes approximately 15% of breast cancers that is characterized by lack of expression of both estrogen receptor (ER) and progesterone receptor (PR) together with absence of human epidermal growth factor 2 (HER2) over expression. TNBC has attracted more attention due to its aggressiveness such as large tumor size, high proliferation rate and metastasis. The absence of efficient molecular targets is still challenging in the treatment of TNBC patients. To understand the complexity of TNBC, comprehensive and integrative transcriptomics and proteomics analyses were performed including 4 publicly available gene expression datasets, which encompassed 201 non-TNBC tumors compared with 157 TNBC tumor samples. Differentially expressed genes (DEGs) were identified through statistical analysis. 23 down-regulated and 17 up-regulated DEGs (called core DEGs) were detected. The protein-protein interaction networks of associated core DEGs were constructed and down-regulated interaction network consisted of 672 edges between 615 nodes and up-regulated interaction network included 837 edges between 755 nodes. The highly connected proteins (hubs) on which different pathways converge were ascertained by applying the degree and betweenness criteria. The down-regulated GATA3, FOXA1, MYB and up-regulated ATF5, CEBPB, FOXC1, MYC, STAT5A, RELA transcriptional factors from the hub list were utilized to reveal a potential transcriptional control mechanism of TNBC which leads to TNBC driven molecular pathways such as JAK-STAT and TNF signaling pathways. These approaches not only provided novel candidate biomarkers of TNBC but also pointed out the TNBC driven pathways that can be used to design targeted therapies.

Keywords: Triple negative breast cancer, transcriptional regulatory network, protein-protein interaction network.

TUE-066
Dependency of translationally controlled tumor protein degradation on mammalian target of rapamycin complex 1/polo-like kinase-1 signaling in human lung cancer cells

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TCTP protein is highly expressed in human cancer cell lines of various tissue origins as well as in tumor tissues and sera of cancer patients. However, the signaling mechanisms underlying TCTP overexpression in cancer are still unknown. Based on a preliminary study in which mammalian target of rapamycin (mTOR) might be involved in the regulation of TCTP expression in lung cancer cell lines, the role of mTOR complex 1 (mTORC1) in TCTP expression was investigated. The protein levels of TCTP in various lung cancer cells were decreased without changes in mRNA levels when mTORC1 activity was selectively inhibited. mTORC1 inhibition induced protein degradation of TCTP through the ubiquitin-proteasome system without affecting TCTP translation. PLK1 activity was required for TCTP protein degradation by mTORC1 inhibition. Furthermore, Selective inhibition of polo-like kinase-1 (PLK1) activity completely blocked the increased TCTP ubiquitination upon mTORC1 inhibition. Ser46 and Ser64 were necessary for proteasomal degradation of TCTP by mTORC1 inhibition. TCTP phosphorylation at Ser46 depended on PLK1, indicating that mTORC1 down-regulated PLK1 activity. In addition, the levels of TCTP expression were proportional to malignancy in a set of four cell lines comprising a human lung carcinogenesis model. The mTORC1/PLK1 signaling was also required for the reported inhibitory effect of sertraline and thioridazine on TCTP protein expression. These results demonstrate that mTORC1/PLK1 signaling is essential for the increased expression of TCTP in lung cancer and provide the first evidence, to our knowledge, that mTORC1 can negatively regulate PLK1 activity.

Keywords: mTOR, PLK1, TCTP.

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TUE-067
Design and development of alpacas VHH-antibodies specific to CD47 protein on cancer cells

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In the end of twentieth century antibodies with uncommon structure were detected in mammals of Camelidae family (which include camels, llamas, alpacas) along with traditional type of IgG antibodies. These antibodies were designated VHH (variable heavy-heavy) and have an antigen-binding pocket containing heavy chain variable region and lacking light chain.

Recombinant VHH-antibodies have several advantages over single-chain antibodies derived from conventional molecules of IgG such as simplicity of genetic manipulation, high expression in different systems, good solubility, high stability in a wide range of conditions and better tissue penetration due to smaller size (15–30 kDa). Structural properties of VHH-antibodies facilitate in construction of immune phage libraries which has great potential for precise engineering of antibodies specific to target proteins. Variable domains of VHH-antibodies may be used in particular as a framework for design of synthetic libraries since only three CDR-fragments of VHH-antibody are required for high affinity binding.

It is known that CD47 protein is expressed at high level on the surface of many cancer cells. CD47 interacts with SIRPa receptor which is present on macrophages resulting in phagocytosis inhibition. Thus cancer cells can escape their recognition by the host immune system. CD47-SIRPa interaction is considered as a promising target for cancer therapy. One approach comprises of using of monoclonal antibodies which block CD47 protein allowing macrophages to engulf cancer cells. However, antibodies with properties suitable for clinical application are still under development. Our aim is to develop a new high affinity anti-CD47 VHH-antibody which has a better blocking action, smaller size and minimal toxicity. To achieve this goal we immunized alpacas with extracellular fragment of human CD47 protein, isolated mRNA from PBMCs and generated PCR-fragments with VHH sequences to obtain VHH library. Further we performed selection of specific antibodies using phage display technology. Now we are studying the characteristics and functional activity of such VHH-antibodies. We suggest that these new anti-CD47 VHH-antibodies might prove perspective and suitable for cancer therapy.

Keywords: CD47, phage display, VHH single domain antibodies.

TUE-068
Design and synthesis of nitric oxide releasing acridone carboxamide derivatives as reverters of doxorubicin resistance in MCF7/dx cells

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Objective: In the present investigation, based on our earlier observations we have designed new NO donating acridones as MDR modulators, capable of releasing NO in controlled manner into P-gp and/or BCRP that could reverse the doxorubicin resistance in cancer.

Methods: We prepared an essentially complete set of *N10*-substituted acridone-4-carboxamides whose amide fragments were formed by the alkyl groups or aryl ring systems bearing exocyclic groups at different ring positions. The *in vitro* cytotoxic effects against Drug sensitive and resistant human cancer cell lines [MCF7, (P-gp and BCRP), MCF7/dx (P-gp expressed) and MCF7/mr (BCRP expressed)] are studied by using SRB assay. Selected molecules were also screened for their cytotoxic properties against SW1398, WIDR and LS174T cell lines. Doxorubicin accumulation and efflux studies were performed and *in vitro* levels of formed nitrate/nitrite were determined by the colorimetric assay.

Results & Conclusion: NO releasing acridones showed activity against MCF-7/wt (0.7–3.1 μM), MCF7/mr proved to be sensitive towards the compounds 1, 2, 3, 7, 10, 11 and 16 with IC_{50} ranges of 0.7–2.3 μM. MCF7/dx sensitivity was observed with compounds 1, 10 and 11 with IC_{50} range of 1.9–2.9 μM. Compounds 1, 10 and 11 showed significant cytotoxic activity against SW1398, WIDR and LS174T cell line. The results indicate NO-acridones are potent against both the sensitive and resistant cells and significantly correlated with rate of *in vitro* nitric oxide release. The results of accumulation studies showed that intracellular accumulation of doxorubicin is significantly dependent on the rate of nitric oxide release.

Keywords: Acridones, Nitric oxide, Multidrug Resistance.

TUE-069
Detection of BRAF mutation in colorectal cancer patients and compare the tumors and normal tissues with PNA clamp qPCR

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Colorectal cancer (CRC) is one of the most common malignancies in the world and a heterogeneous disease with multiple underlying genetic mutations resulting in different phenotypes. *BRAF* plays an important role in the activation of RAS–RAF–MEK–ERK signaling pathway, is one of the targeted genes in colorectal cancers. Most known BRAF mutation in colorectal cancer occurs when valine is substituted for glutamate at codon 600 on chromosome 7, referred to as the V600E mutation. V600E mutant B-Raf not only plays a important role in uncontrolled cellular proliferation through spontaneous ERK signalling, but also controls apoptosis via NF-κB signaling. Uncontrolled NF-κB signaling also related with angiogenesis, invasion and metastatic processes. The aim of this work was to assess BRAF mutations in the Turkish population with CRC. We also compared the clinicopathological data from CRC patients with the mutational status of in their cancer and normal tissues to determine a relationship to outcomes.

A total of 40 paraffin-embedded colorectal cancer and normal specimens were obtained from department of pathology in Cerrahpasa Medical Faculty. Ten-micrometer-thick tissue sections were placed on a glass slide. The tissue sections were dehydrated in graded ethanol solutions and dried without a cover glass. DNA was extracted from the tissues with 50 μl of extraction buffer at 55°C overnight. The tubes were boiled for 15 min to inactivate the proteinase K. Investigate the possibility of *BRAF* mutations, PNA clamp qPCR was performed using the PNA Clamp BRAF Mutation Detection kit (Panagene Inc., Daejeon, Korea) according to the manufacturer's instructions. The PNA probe was designed to hybridize completely to the wild-type *BRAF* allele. PNA probe hybridization securely inhibited amplification of the wild-type *BRAF* allele, whereas the PNA/mutant allele hybrid was unstable due to a base pair mismatch and there-

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fore did not inhibit extension by Taq polymerase. The Ct was automatically calculated from PCR amplification plots in which fluorescence was plotted against the number of cycles.

A BRAF mutation increases the risk of mortality in colorectal cancer patients over twofold. In this study, we explore BRAF mutations in the colorectal tissues, not only tumors but also normal, which may play a role in carcinogenesis in some cases, and this will require further large sample sizes study.

Keywords: BRAF, Colorectal Cancer, QPCR.

TUE-070

Detection of Pirh2-interacting proteins using GST-pulldown followed MALDI-TOF mass spectrometry

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Ubiquitin-dependent proteasomal degradation is one of the major pathways of non-lysosomal protein degradation in the cell. The ubiquitination process involves several enzymatic reactions and includes the following enzymes: E1 — activating, E2 — conjugating, and the third — E3 — ligating enzymes. E3 ligases determine the specificity of ubiquitination reaction, i. e. what target protein will be subjected to the covalent modification by ubiquitins.

Pirh2 (RCHY1) is the E3 ubiquitin ligase that promotes ubiquitination and subsequent proteasomal degradation of the p53 tumor suppressor protein. Phosphorylation of human p53 at serine 15 occurs after DNA damage and this leads to reduced interaction of p53 with its main negative regulator Mdm2. However, Pirh2, unlike Mdm2, targets the activated form of p53 after DNA damage, when the activity of Mdm2 is attenuated by post-translational modifications.

Beside its interaction with p53, Pirh2 interacts with other proteins involved in cell cycle regulation, DNA-damage response (DDR) and apoptosis such as Cdk9, p27, PolH.

Overexpression of Pirh2 is frequently detected in many clinical tumor tissues including hepatocellular carcinoma (HCC), prostate cancer, and lung cancer. Despite evidence of PIrh2 participation in tumorigenesis its specific role in this process remains unclear and requires further investigation. Our work is aimed toward better understanding of the molecular mechanisms of tumorigenesis involving Pirh2 protein.

For extraction and identification of proteins interacting with Pirh2 we carried out GST pull-down assay followed by MALDI-TOF mass spectrometry. To this end, a GST-Pirh2- expression vector was generated and the respective protein was purified from *E. coli* lysate. Subsequently, we incubated GST-Pirh2 with nuclear extract prepared from p53-negative H1299 cells treated with 1 μM Doxorubicin for 6 h to induce DNA Damage. Bound proteins were then separated by 1D SDS-PAGE gel electrophoresis and then analyzed by means of MALDI-TOF mass spectrometry.

Analysis of mass-spec peaks revealed about 30 novel significant proteins interacting with Pirh2. Several identified proteins are known to play role in splicing processes: hnRNP M, hnRNP K, Aly/REF etc. Some other proteins are involved in apoptosis regulation: DDX5 (p68), cytochrome c etc. Such proteins as DDX3, VDAC, PRKDC, and several others have shown to directly participate in tumorigenesis. These interactions certainly need further validation by other means. However, this work provides the ground for investigating the role of Pirh2 in apoptosis and cancer.

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Keywords: cancer, Pirh2, protein interactions.

TUE-071

Detrimental and cell death effects of organic selenium treatment on human glioblastoma cells

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Glioblastoma multiforme (GBM) is caused by the central nervous system-derived glial cells and is the most common (50-60%) form of primary brain tumor. The aim of this study was to investigate *in vitro* effects of selenium on human glioblastoma multiforme cells.

In the present study, GMS-10 and DBTRG-05MG human GBM cell lines were used as a model to examine, in the selenomethionine treated and non-treated groups; proliferation, cytotoxicity, DNA fragmentation and apoptosis. The organic source of selenium, selenomethionine stimulated effects on cell proliferation and cytotoxicity, as these were assessed with WST-1 and lactate dehydrogenase (LDH) tests, respectively. DNA fragmentation was using cell death ELISA kit and apoptosis was determined by Annexin V staining.

This is the first study to examine SeMet effects on cell growth and death in GMS-10 and DBTRG-05MG cells. According to the results of our research, cells respond to seleno-L-methionine in a dose-dependent and time-dependent manner for both cell lines. As a result of WST-1 test cell proliferation increased at 50 and 100 μM doses, which were considered as low doses by us. With the analyzis of the DNA fragmentation, SeMet induced apoptosis after 72 h incubation on these GBM cells. In this study, SeMet, in the *in vitro* environmental conditions, has decreased proliferation and has shown cytotoxicity in a dose and incubation period dependent manner in both DBTRG-05MG and GMS-10 cells and also SeMet induced apoptosis on these GBM cells. Our results suggest that SeMet may be key therapeutic target for GBM. This study will yield further studies focusing on the possibility of using SeMet against some types of GBM.

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Keywords: Selenium; Selenomethionine, Glioblastoma multiforme, Proliferation, Cytotoxicity, Ki-67, apoptosis.

TUE-072

Development and optimization of a novel assay for studying protein kinase CK2 enzyme interactions using quartz crystal microbalance with dissipation monitoring (QCM-D)

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Protein kinase CK2 is a ubiquitous, highly pleiotropic and constitutively active kinase that phosphorylates mainly serine/threonine residues. It has been observed, that an elevated CK2 activity underlies global human diseases, with special reference to cancer and tumor pathologies. Six major hallmarks for cancer have been reported and protein kinases, especially CK2, can contribute to each of these steps. Therefore, screening for selective CK2 inhibitors is of great importance. The standard method for study of CK2 and most protein kinases activity is based on a radiometric filtration binding system, involving the use of radioactive ATP as a phosphate donor. Radioactivity methods require special han-

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dling and involve high cost of waste disposal. Therefore, availability of non-radiometric technique is highly desirable.

The aim of this study is to develop and optimize a novel assay for investigating enzyme inhibition and protein-protein interactions using CK2 as a model substrate. This technique might serve as a powerful tool for fast and sensitive evaluation of potential anticancer agents. Our research includes a full analysis of the mechanism and kinetics of kinase-catalyzed phosphorylation and enzyme inhibition by known and novel CK2 inhibitors.

In our studies we use a quartz crystal microbalance with dissipation monitoring (QCM-D), a unique tool which can be applied for studying biomolecular interactions. Its main advantage is ultra-sensitivity and ability to simultanously measure frequency and dissipation changes in real time. In other techniques, applied for studying enzyme assays, only the result of the reaction is investigated. Here, it is possible to monitor changes in mass and viscoelastic properties of the adsorbed layer throughout the whole process. This gives novel insights into the structural and kinetic properties of the reactions investigated.

Our studies of CK2 activity have shown that QCM-D is a suitable device for this assay, as it allowed us to determine all enzyme-reaction kinetic parameters. Moreover, we have investigated the influence of inhibitors on CK2 activity and measured the inhibitor-CK2 interactions kinetic parameters. For this assay we have used two types of quartz crystal sensors: i) Au surface and ii) modified with Cu^{2+} chelated ions for HisTag label capturing. Finally, the influence of CK2 inhibitors on colonal cancer cells HCT 116 was analyzed using a QCM-D device equipped in a window module enabling simultaneous optical access to the sensor surface.

The study was co-financed by NCN grant No 2011/01/B/ST5/00849 and by Warsaw University of Technology.

Keywords: Protein - protein interactions, protein kinase CK2, Quartz Crystal Microbalance with Dissipation Monitoring.

TUE-073
Differential regulation of pyrimidine nucleotide biosynthesis by Rheb and mTOR

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Rheb small GTPases, which consist of Rheb1 and Rheb2 (also known as RhebL1) in mammalian cells, are unique members of the Ras superfamily and play central roles in regulating protein synthesis and pyrimidine nucleotide synthesis by activating mTOR. Although extensive studies have been carried out on Rheb activation of mTOR, little is known about other downstream effectors of Rheb. As multiple effectors have been identified for each of many small GTPases, it is reasonable to assume that Rheb may also activate multiple downstream effectors. We analyzed proteins co-immunoprecipitated with Rheb and found that Rheb binds to CAD (carbamoyl-phosphate synthetase 2, aspartate transcarbamoylase and dihydroorotase) protein, a multifunctional enzyme required for the *de novo* synthesis of pyrimidine nucleotides. CAD binding to Rheb is specific to GTP-bound active form of Rheb and is dependent on the effector domain of Rheb. Immunostaining reveals that Rheb recruits CAD to the lysosomes, while CAD is mislocalized when HeLa cells are treated with a farnesyltransferase inhibitor, which blocks the membrane targeting of Rheb. *In vitro* assays showed that Rheb increased CPSase activity of CAD. Although it has been reported the CAD phosphorylation at S1859 by S6K1, a downstream effector of Rheb-mTOR signaling, is a key event in regulating pyrimidine biosynthesis, the phosphorylation of CAD did not alter its enzymatic activity in our hands. In addition, elevated levels of intracellular pyrimidine nucleotides were observed in TSC2-knockout cells, which persisted even when the cells were treated with rapamycin. These results suggest that Rheb regulates *de novo* pyrimidine nucleotide synthesis by binding and activating CAD, independent of mTOR.

Keywords: mTOR, pyrimidine biosynthesis, Rheb.

TUE-074
Differential sensitivity of human gliomas to nonpathogenic oncolytic enteroviruses

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Background and Aims: Viral oncolysis is a promising approach for the therapy of human malignant tumors. However, therapeutic response of a patient to a particular oncolytic virus strain is difficult to predict because of the substantial genetic variability within cancer types. A development of a diagnostic test for confident personalized prediction of the response would facilitate the success of therapy. Malignant gliomas are generally more susceptible to viruses, as tumor cells commonly have defective antiviral mechanisms. Non-pathogenic eneroviruses can efficiently replicate and kill some malignant glioma cells, but the efficiency may vary. One of the critical determinants of the efficiency is linked to host cell receptors that are used for entry of the virus. The goal of the study was to develop a diagnostic method for prediction of individual gliomas sensitivity for oncolytic enterovirus treatment.

Material and Methods: Standard stocks of four non-pathogenic enteroviruses (Polio Type 1 vaccine, ECHO-12, Coxsaxkie B2 and B5) were examined for self-replication in human glioblastoma cell lines U87, U251, A172 and in normal asctrocytes cell culture. Organ cultures of 15 biopsies from patients with low- and high-grade primary and recurrent gliomas were also tested for viral replication in 48 hours after infection with their stocks. Cell viability was measured by the metabolic XTT test. Relative expression levels of viral receptors were obtained using RT-PCR.

Results:

1 Human astrocytes, as nontumor cells, were not susceptible for any of the viruses from the panel. By contrast, the glioblastoma cells lines displayed differential sensitivity to the viral strains.
2 Organic cultures demonstrated individual sensitivity to the viruses in wide range.
3 The sensitivity is strongly correlated with relative expression levels of enterovirus receptors, such as CD155, CD112, CD55, ICAM-1 and CAR.

Conclusion: We suggest a rapid RT-PCR based test as a novel personalized approach for selection of the most effective oncolytic virotherapy for individual glioma patients treatment.

Keywords: oncolytic enteroviruses.

TUE-075
Double role of CacyBP/SIP N-terminal domain in the MAP kinase signaling pathway

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CacyBP/SIP plays an important role in various cellular processes such as cells differentiation and cytoskeleton re-organization through the interaction with its binding partners: S100A6, Siah-1, actin, tubulin and tropomyosin [Schneider and Filipek, 2011, Jurewicz et al., 2013]. It has been also shown that CacyBP/SIP

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participates in down-regulation of the MAP kinase cascade *via* dephosphorylation of ERK1/2 [Schneider and Filipek, 2011; Kilanczyk et al., 2011].

In this work we show that apart from the C-terminal domain, also the N-terminal domain of CacyBP/SIP is capable of ERK1/2 binding and dephosphorylation. That phenomenon is related to the presence of a double Kinase Interacting Motif (KIM) in CacyBP/SIP sequence, one in the C-terminal domain, previously reported [Kilanczyk et al., 2009], and second in the N-terminus, a newly found. Both domains exhibit comparable phosphatase activity, slightly lower than the full-length protein but modification of very conservative residues in the N-terminal KIM motif, K25 and R26, utterly abolishes the activity of CacyBP/SIP. Furthermore, we found that the N-terminal domain of CacyBP/SIP participates in homodimerization of this protein. Mutants D11-E14 with disturbed dimerization efficiency do not exhibit significant loss of activity. This may suggest that the activity does not depend directly on the oligomerization state but rather on the functionality of the KIM motif and other factors, such as post-translational modifications, which affect also dimer formation.

To support the *in vitro* analyses we determined the level and localization of CacyBP/SIP in neuroblastoma NB2a cells. Since the MAP kinase cascade is activated during oxidative stress, NB2a cells were treated with H_2O_2. Interestingly, we have found that after H_2O_2 treatment the CacyBP/SIP level increases and the functional monomer-dimer equilibrium is altered. Under control conditions CacyBP/SIP is present in the dimeric form mainly in the cytoplasm of NB2a cells. Even though under oxidative stress the protein translocates to the nucleus, the dimer is not found in that compartment. Thus, it seems that dephoshorylation of ERK1/2, which takes place in the nucleus, is catalyzed by the monomer of CacyBP/SIP, which is a prevalent active form of the protein in this compartment.

Concluding, we showed, using *in vitro* methods and experiments on cells, that the N-terminal domain of CacyBP/SIP is engaged in two processes – protein homodimerization and down-regulation of ERK1/2 signaling pathway. Changes in CacyBP/SIP oligomerization state, imposed by the N-terminal domain, are strongly dependent on its subcellular localization and phosphatase activity toward ERK1/2.

Keywords: CacyBP/SIP, dimerization, ERK1/2.

TUE-076
Doxorubicin induces G2/M arrest, Ca2+ release, oxidative stress and apoptosis in human leukemia Jurkat cells at clinical doses and dose rates

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Doxorubicin, an effective anticancer drug, has benn reported to induce either apoptosis or necrosis in various cancer cell lines, in function of dose and treatment duration. We investigated the antiproliferative effects of doxorubicin at clinical doses (50–100 nM) and duration (24 h, 72 h) in human leukemia JUrkat cells. Doxorubicin induced G_2/M arrest and significant apoptosis (up to 54% after 72 h of exposure), as assessed by flow cytometry analysis of the DNA content. However, cellular viability determined by flow cytometry on propidium iodide labeled cells was 1.6% after the 72 h treatment with 100 nM doxorubicin, indicating a significant fraction of necrotic cells as well. Neither quercetin nor menadione or their combination at clinical concentrations (10–15 μM) enhanced doxorubicin-induced apoptosis, despite their apoptigenic effects reported in the Jurkat cell line [1, 2]. Fluorimetric measurements on fura-2 loaded cells showed that doxorubicin (7.5 μM) induced Ca^{2+} release at slow rate and elicited a three-fold, progressive increase in cytosolic Ca^{2+} concentration over the next 55 min. from drug addition. However, since the fluorescence of internalized doxorubicin displayed a considerable and rapid increase after extracellular addition, this result indicates a sustained but indirect activation of the Ca^{2+} release channels. The observed Ca^{2+} increase was partly mediated by ryanodine receptors, so that the presence of the specific inhibitor, ryanodine (200 μM), consistently inhibited the doxorubicin-evoked Ca^{2+} signal. We also found by spectrofluorimetry that doxorubicin at clinical doses and dose rates generates oxidative stress, which is enhanced by menadione (10-15 μM), but is alleviated by both quercetin (10-15 μM) and the quercetin-menadione combination. However, these agents did not improve cellular viability following the doxorubicin treatment. In addition, quercetin did not affect the cell cycle distribution of doxorubicin treated-cells, whereas menadione and the quercetin-menadione concentration apparently abolished the doxorubicin-induced without further progression through the cell cycle phases.

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Keywords: None.

TUE-077
D-propranolol anti-tumoral effects in B16F10 mouse melanoma

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We recently reported that D-propranolol, used as a phosphatidic acid (PA) phosphohydrolase (PAP) inhibitor to increase cellular PA levels, may underlie a novel anti-tumoral strategy (Shaughnessy et al. FEBS J-2014). The evidence was based on a perturbation of EGFR endocytic traffic leading to both an accumulation of empty/inactive and ligand-activated receptor in recycling endosomes and a decreased proliferation/survival rate in tumoral cell lines bearing EGFR as an oncogenic trait. Here we study the effect of D-propranolol *in vitro* and *in vivo* using the B16F10 melanoma mouse model. *In vitro,* D-propranolol-treated B16F10 cells, which express low levels of EGFR, showed a reduced proliferation rate and increased apoptosis. *In vivo*, tumor progression in C57BL/6 mice inoculated with B16F10 cells was significantly decreased by intraperitoneal (IP) treatment with D-propranolol once a day. Tumors showed an increased total mass of EGFR and c-MET (hepatocyte growth factor receptor) in D-propranolol-treated mice. Both receptors were internalized under D-propranolol treatment. These results provide further evidence to consider PAP inhibition with D-propranolol as a plausible anti-tumoral approach, counteracting the tumorigenic contribution of growth factor receptors through the protein trafficking machinery. (Financed in part by CONICYT Doctoral Scholarship to RS and CONICYT PFB12/2007 to AG).

Keywords: endocytosis, melanoma, phosphatidic acid.

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TUE-078
eEF1A1 and eEF1A2 hetrodimerization by confocal microscopy and fret analysis

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The eukaryotic translation elongation factor 1A (eEF1A) catalyzes the first step of the protein synthesis elongation cycle [1]. Two isoforms of the protein (eEF1A1 and eEF1A2), sharing 92% amino acid homology, are known expressed in a tissue specific manner: eEF1A1 is widely expressed in almost all tissues whereas eEF1A2 is normally expressed only in non-proliferative tissues (brain, heart, skeletal, muscle). Beyond their canonical role in protein synthesis, both isoforms have been reported to be involved in other non-translational functions. eEF1A1 isoform seems to play a pro-apoptotic role whereas eEF1A2 appears to protect from apoptosis suggesting its potential function as putative oncogene [2]. Previous studies identified eEF1A Raf-mediated phosphorylation sites (S21 and T88) during survival response mediated by epidermal growth factor receptor (EGFR). However phosphorylation of S21 occurs only in presence of both eEF1A isoforms, suggesting that the eEF1A isoforms can heterodimerize. A docking model supported the formation of a possible heterodimer between eEF1A1 and eEF1A2. In particular, this model showed that the M-domain of one isoform was in contact to the G-domain of the other and vice versa. The heterodimer formation somehow could induce a conformational change in one or in both eEF1A isoforms that allows the phosphorylation of S21 [3]. The heterodimerization hypothesis was investigated by carrying out sensitized emission FRET experiment that involves the non-radiative transfer of energy from an excited state donor fluorophore to a nearby acceptor and the energy transfer efficiency ($FRET_{eff}$) was evaluated using FRET and colocalization analyzer ImageJ plug-in software [4]. HeK293 cells were transfected with pcDNA3.1 construct containing the human eEF1A2 cDNA ingineered with a C-terminal His_6-tag. FRET measurements via confocal microscopy, was carried out using mouse anti-His antibody and rabbit anti-human EF1A-1 antibody. Confocal analysis showed colocalization of the eEF1A isoforms within the cytoplasm with a stronger merge signal at the level of plasma membrane. FRET analysis indicated a specific interaction between endogenous eEF1A-1 and transfected eEF1A1-2 at the level of the plasma membrane. These results strongly confirmed that the eEF1A isoforms might interact by possibly forming a dimer that undergo to phosphorylation regulated by C-Raf [3].

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Keywords: Elongation Factor 1A, FRET, Protein - protein interactions.

TUE-079
Effect of down-regulation of STEAP1 by siRNA in cell cycle and apoptosis of human LNCaP prostate cells

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Introduction: The six transmembrane epithelial antigen of the prostate 1 (STEAP1) is over-expressed in several types of tumors, particularly in prostate cancer. STEAP1 can be found in the plasma membrane of epithelial cells, at cell–cell junctions, but also in the cytoplasm with less intensity. Although its physiological functions are yet to be ascertained, STEAP1 appears to take part on intra- and intercellular communication. Several studies have pointed out that STEAP1 has a great potential as an immunotherapeutic target, highlighting its role on prostate pathophysiology. It is well known that sex steroid hormones, namely dihydrotestosterone (DHT), are not only involved in maintenance of prostate physiology but also promote proliferation of prostate cancer cells. In addition, it was recently demonstrated that DHT is involved in regulation of STEAP1 gene. This way, we hypothesized that the effect of DHT on cell proliferation and apoptosis may be dependent on STEAP1 levels in cancer cells.
Materials and Methods: LNCaP prostate cancer cell line was cultured under an atmosphere of 5% CO_2 and 37°C. To access the effects of DHT and STEAP1 on cell proliferation and apoptosis, cells were transfected with STEAP1 siRNA and either treated with 0 or 10 nM DHT for 48 h. Control group included non-transfected LNCaP cells and treated with 0 nM DHT. Cell cycle and apoptosis analysis was performed by flow cytometry, using propidium iodide and FITC annexin V staining, respectively. Cell proliferation was also investigated through MTS assay.
Results and Discussion: STEAP1 gene silencing with a specific siRNA appears to induce cell cycle arrest and decrease proliferation when compared to control group. Hormonal treatment with DHT induces proliferation in the non-silenced cells, as it was previously described by others. When comparing siRNA transfected cells treated with DHT with non-transfected cells, it appears that DHT impairs cell proliferation, diminishing the number of cells on S-phase. Apoptosis analysis is underway.
Conclusions: STEAP1 may influence prostate cell proliferation, and the effect of DHT on prostate cells seems to be dependent of STEAP1 levels. However, more assays are being carried out in order to clarify the role of STEAP1 in cell proliferation and apoptosis.

Keywords: prostate cancer, STEAP1.

TUE-080
Effect of doxorubicin loaded CS MNPs on cell proliferation in sensitive and doxorubicin resistant MCF-7 cell lines

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Targeted drug delivery is a promising alternative in cancer therapy to increase the efficacy of drugs and reduce the destructive side effects of classical chemotherapy. In order to obtain an effective targeted delivery system for Doxorubicin, chitosan coated magnetic nanoparticles (CS MNPs) were synthesized (Unsoy *et al*, 2012). CS MNPs maintain pH dependent drug delivery while providing targeting of drugs to the tumor site under magnetic

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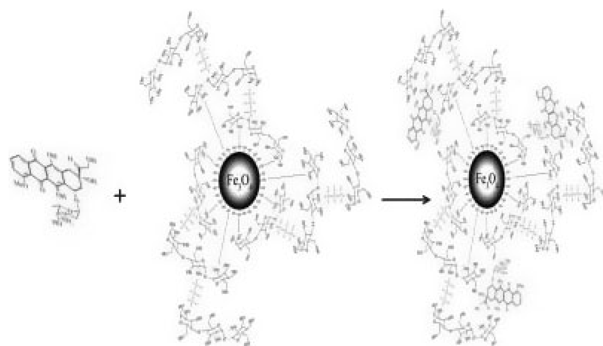

Fig. 1. Schematic representation of Doxorubicin (a), CS MNP (b) and of Doxorubicin loaded CS MNP (c).

field. The anti-cancer agent Doxorubicin, which is an antracycline antibiotic, was loaded onto CS MNPs (Figure 1). As maintained from drug loading, release, and stability characterization studies, CS MNPs have pH responsive release characteristics and are quite stable at physiological pH.

Anti-proliferative effects of Doxorubicin loaded CS MNPs were investigated by XTT cell proliferation assay on MCF-7 and MCF-7/Dox cells at increasing concentrations. IC_{50} values of free Doxorubicin and Doxorubicin loaded CS MNPs indicated that drug loaded nanoparticles not only increase the efficacy of drug but also overcome Doxorubicin resistance in MCF-7/Dox cells. Consequently, CS MNPs can be effectively used for the pH dependent release of Doxorubicin in cancer cells. Results of this study can pave the way for the development of pH responsive targeted drug delivery systems to overcome the limitations of conventional chemotherapy.

Keywords: Doxorubicin, drug resistance, breast cancer, cytotoxicity.

TUE-081
Effect of holothurians triterpene glycosides, cucumariosideA2-2 and frondoside A, on human prostate cancer

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Prostate cancer is the most common malignancy among men and the second leading cause of cancer related deaths. In the beginning the metastasized stage can be controlled by hormone deprivation. However, the vast majority of tumors become castration-resistant in the course of treatment. Although new drugs e.g. Abiraterone, Cabazitaxel and Enzalutamid have been successfully applied in this situation, cancer cells again acquire resistance to these substances with patients facing a dismissal prognosis. Thus, the search for new natural or synthetic compounds with selective anticancer properties remains a high scientific priority.

Holothurians triterpene glycosides cucumarioside A_2-2 and frondoside A were isolated from the holothurian *Cucumaria japonica* and *Cucumaria frondosa* and kindly provided by Drs. Avilov and Silchenko (PIBOC FEB RAS, Vladivostok, Russian Federation). Anticancer properties and mechanism of action were evaluated in the castration-resistant human prostate cancer cell line PC3: Trypane blue staining, MTT test and colony formation assay were applied for cytotoxicity and cell proliferation studies. Influences on cell cycle and apoptosis were evaluated by flow cytometry and western blotting analysis. The effect of triterpene glycosides on protein expression was analysed by 2D-gel electrophoresis followed by MALDI-MS.

EC_{50} for cucumarioside A_2-2 and frondoside A was 3 μM and 2,5 μM determined by trypane blue method and 3,3 and 3,0 μM by MTT assay, respectively. Cucumarioside A_2-2 and frondoside A caused cell cycle arrest in G2/M phase. A relative increase of the cells in the Sub-G_0 phase of the cell cycle was observed indicating cell death. Indeed, both glycosides induced apoptosis in concentrations equalling EC_{50} or less reflected by caspase-3 and caspase-9 activation. Inhibition of tumor cell colony formation of up to 40% was observed after glycoside treatment when compared to controls. In addition, incubation of PC3 cells with triterepene glycosides resulted in a distinct regulation of protein expression: Both substances up-regulated Keratin, type II cuticular Hb1 and Interleukin-1 beta, and down-regulated expression of Cathepsin B and Heterogeneous nuclear ribonucleoprotein A/B, which are involved in metastatic formation, tumor cell invasion and malignant growth.

In conclusion, cucumarioside A_2-2 and frondoside A are promising novel substances showing high efficacy in castration-resistant prostate cancer.

This research was supported by the Eppendorfer Krebs- und Leukämiehilfe.

Keywords: Anticancer activity, Cell cycle, Triterpene glycosides.

TUE-082
Effect of phytoestrogen emodin on doxorubicin-induced cytotoxicity and apoptosis on tumorigenic MCF-7 and non-tumorigenic MCF-10A human breast cells

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Emodin (3-Methyl-1,6,8-trihydroxyanthraquinone), is a phytoestrogenic component of *Rheum and Polygonum* plant extracts which has been used to treat several diseases since ancient times. It has been shown to have anti-microbial, anti-oxidant and anti-cancer effects in nature. The anti-tumor drug doxorubicin, a widely used chemotherapeutic agent, is used for the treatment of many cancer types including lung, gastric, ovarian and breast cancer. In this study, the effects of pre-, co- and alone treatment of doxorubicin and emodin in MCF-7 and MCF-10A cell lines were investigated.

MCF-7 and MCF-10A cells were cultured in the presence of various concentrations of emodin and doxorubicin at 6, 24 and 72 hours. The effect of emodin varies according to the presence of doxorubicin (pre-treatment, co-treatment, post-treatment) on both cell lines. Emodin pre-treatment (0.4 and 4 μM) for 24-hour prior to doxorubicin treatment (0.1, 0.83, 2.5 μM) caused to increase in cell viability of MCF-10A cells, comparing to doxorubicin alone treatment. Whereas no effect was observed in MCF-7 cells. Emodin post- and co-treatment with doxorubicin for 72-

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hour inhibited the survival of MCF-7 and MCF-10A cells in a concentration dependent manner, shown by trypan blue and XTT.

Apoptotic effects of doxorubicin and emodin were investigated by flow cytometer. While emodin (0.4 μM) did not induce apoptosis in both cell lines, doxorubicin alone, pre- and co-treatment (0.83 μM) with emodin (0.4 μM) induced late apoptosis/necrosis in MCF-7 and MCF-10A cells. Mitochondrial membrane potential loss was not observed after doxorubicin and emodin treatment in both cell lines.

Keywords: Apoptosis, Doxorubicin, Emodin.

TUE-083
MAPK inhibitor treatments in human Malignant Mesothelioma cells

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Malignant mesothelioma (MM), is lethal tumor, originates from mesoderm-derived tissues of serosal cavities. The worldwide incidence of MM has risen dramatically as a result of widespread exposure to asbestos together with industrialization. In our country, the disease is a serious public health problem in rural areas of Anatolia particularly, Central and South-eastern parts. The failures of conventional chemotherapeutic regimens have made the search for new approaches and drugs critically important. It is known that disturbed protein kinase activities are associated with human malignancies making these proteins attractive targets for cancer therapy. Therefore, we investigated the effect of stress activated and mitogen activated protein kinase inhibitors including SP600125 and SB203580 (JNK and p38 inhibitors, respectively), and U0126 (ERK inhibitor), in two different MM cells lines. We observed that SPC111 and SPC212 cell proliferation were inhibited at dose and time dependent manner with all inhibitors. SPC111 cells were found more sensitive than SPC212 cells to SP600125 and U0126 compounds. In addition SB203580 inhibitor (<50 μM) reduced both cell number in 72 h. These results suggest involvement of ERK and JNK pathway in cell proliferation. However, decreased cell number with high doses of SB203530 may indicate that proliferation of malign cells also, depend on either highly activated p38 MAP pathway or PKB pathway, which can be blocked by SB203580 approximately 10-fold higher than those required to inhibit p38 pathway. Thus, in turn much more specific p38 and/or PKB pathway inhibitors aimed to be use in order to clarify our result.

Keywords: malignant mesothelioma, MAPK.

TUE-084
Effect of the small GTPase Rheb on the regulation of LKB1 kinase

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The small GTPase Rheb activates the mTORC1 kinase, which promotes cell growth and is implicated in metabolic deregulation in cancer cells. The LKB1-AMPK pathway senses intracellular energy levels, and negatively regulates Rheb-mTORC1 signaling in response to metabolic stress. However, our group recently demonstrated that Rheb activates AMPK through a mTORC1-independent mechanism. This observation suggests that a negative feedback loop between Rheb and AMPK exists, but how Rheb activates AMPK remains elusive. Here, we show that Rheb is unable to induce AMPK activation in LKB1 kinase-null HeLa cells. Since LKB1 kinase is known to induce full activation of AMPK by phosphorylation, we tested whether Rheb regulates LKB1 function. We found that Rheb co-immunoprecipitates with endogenous LKB1 in HEK 293T cells, but GST-pull down assays demonstrated that the interaction between these proteins is not dependent on Rheb's activation state. These results suggested that Rheb forms a complex with LKB1 to affect its function. LKB1 is known to form different complexes in the cell, affecting its stability and catalytic activity. We then analyzed whether Rheb affects LKB1 stability. We transiently co-transfected LKB1 and Rheb in HeLa cells, and the effect of Rheb on LKB1 stabilty was analyzed by pulse-chase and cycloheximide experiments. We found that Rheb increases LKB1 stability. Consistent with this result, shRNA-mediated depletion of Rheb was correlated with a decrease in LKB1 expression in Colo320 cancer cells. Altogether, our results suggest that Rheb induces AMPK activity by interacting with and increasing the stability of LKB1.

Keywords: Rheb, AMPK, LKB1 stability.

TUE-085
Effect of transition metals on sequence-specific binding of p53 family proteins

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The p53 protein family that is coded by TP53, TP63 and TP73 genes play an important role in many cell proesses. For correct function as tumor supresors, sequene specific binding to responsive elements of target genes is required. DNA binding domain (DBD) is responsible for this. It was found, that in all p53 family members oxidation of cysteins in DBD lead to inhibition of sequence specific binding, which is conected with loosing of zinc ions. Zn^{2+} ions are essential for stable conformation of the protein. Another studies showed, that excess of Zn^{2+} ions, simillarly as ions of other heavy metals inhibit sequence specific binding of the p53 protein. It is known, that zinc homeostasis is the result of a coordinated regulation and intracelular zinc concentration is in corelation to cell fate. While absence of zinc ions doesn't allow synthesis of functionable proteins, excess of zinc is cytotoxic.

In our work we focused on effect of two-valent cations of zinc, cobalt, nickel and cadmium on sequence specific binding of p53, p63 and p73 proteins. Ihibition of this binding was detected in micromolar concentrations. Our experiments with DNA fragments and oligonucleotides containing sequences of promotor regions of p53 family target genes (e.g. *p21, MDM2)* confirms inhibition effect of Zn^{2+}. It is interesting, that interaction of p63 and p73 with DNA was inhibited less then p53. Other studied heavy metals inhibited sequence specific binding in similar concentrations as p53.

Keywords: DNA-protein interaction, heavy metals ions, p53 family.

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TUE-086
Effects of plant phenolic compounds tannic acid and resveratrol on prostate cancer metastasis

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Background: Prostate cancer, a heterogeneous cancer, is the third most common cause of male cancer deaths in developed countries, with castration resistance. Current therapeutic approaches for prostate cancer generally have variable efficiency, develop metastasis and drug-resistance, and have high toxicity to normal tissues. Hence, the searching for more effective strategies with moderate or any adverse effects for the chemopreventive intervention of those cancers remains one of the important issues in cancer research. Increasing popularity towards traditional medicine such as herbal remedies or dietary supplements leads the investigators to examine the actions of phytochemicals on xenobiotic metabolism and their antioxidant, antitumor, anti-carcinogenic, and anti-mutagenic effects. For these reasons, this study was aimed to determine the effect of tannic acid and resveratrol on prostate cancer proliferation, metastasis and invasion.
Methods: PC-3 and LnCaP cell lines were grown in Hams' F-12 medium and RPMI-1640, respectively supplemented with 10% fetal bovine serum and 2 mM glutamine. Cytotoxicity of tannic acid and resveratrol on PC-3 and LnCaP cell lines were determined with Alamar blue and IC50 value was calculated. The effects of tannic acid and resveratrol on proliferation, invasive potential and metastasis of cancer cell lines were analyzed by wound healing, colony formation and matrigel chamber assays, respectively.
Results: In this study, we found that tannic acid and resveratrol on has the potential to inhibit the proliferation of PC-3 and LnCaP cell lines in a concentration-dependent manner. Tannic acid and resveratrol inhibited proliferation of PC-3 cell line with IC50 of 35 μM and 78.4 μM while LnCaP cell line's proliferation was determined as 29.1 μM and 76.2 μM, respectively. Both tannic acid and resveratrol treatment of cells led to a significant inhibition in motility and metastasis of PC-3 and LnCaP cell lines ($p < 0.0001$).
Conclusions: The data obtained from this study will supply valuable information about usage of those phenolic compounds in treatment of prostate cancer since tannic acid and resveratrol are found plenty amounts in vegetables and fruits. These results show that tannic acid and resveratrol significantly inhibit the proliferation, invasive potential and metastasis of prostate cancer cell lines, PC-3 and LnCaP.

This work was supported by a grant from TUBITAK, Project No:113Z488, TURKEY.
Keywords: Tannic acid, resveratrol, Prostate Cancer, PC-3, LnCaP, proliferation, metastasis.

TUE-087
Effects of selected growth factors and their receptors inhibitors on invasiveness of two differentially migrating lines of colon cancer cells

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Cell migration plays an important role in metastasis. A better understanding of the molecular mechanisms of cell locomotion is therefore of high clinical relevance.

Epidermal growth factor receptor (EGFR) as well as hepatocyte growth factor receptor (c-Met) are tyrosine kinases which are abnormally overexpressed in numerous tumors of epithelial origin, including colon cancer, where it is connected with high risk of metastasis occurrence. Activated by ligand binding EGFR and c-Met induce activation of two main intracellular pathways - the MAPK pathway and the PI3K/AKT pathway. These pathways lead to the activation of various transcription factors that impact cellular responses such as proliferation, migration, differentiation, and apoptosis. Additionally, some patients with colon cancer have a point mutation in B-Raf, a component of the RAF/MEK/ERK signaling pathway. That's why we decided to use c-Met (crizitinib), EGFR (erlotinib, gefitinib, lapatinib) and B-Raf (vemurafenib) inhibitors to test how two differentially migrating lines of colon cancer cells will react to these treatments. There are also some reports indicating that EGFR inhibitors and c-Met inhibitor as well as B-Raf inhibitor have synergistic effects on viability of some types cancer cells. That's why we decided to test also the influence of these compounds mixes on examined cells.

In this study we used human colon carcinoma cells BE, moving in mesenchymal way and LS174T, moving in ameboid way. At first we showed that EGF as well as HGF influenced positively protrusions formation activity of these cells and their invasion abilities. Then we focused on viability of cells after inhibitors treatment. We observed that for both cells lines lapatinib and crizotinib are the most effective ones in decreasing viability of examined cells. Other EGFR inhibitors as well as vemurafenib didn't influence significantly this parameter. Invasion assay gave similar results – crizotinib and lapatinib were the most potent in inhibiting migration of both colon cancer cells. We noticed also some changes in cytoskeleton of these cells upon inhibitors treatment. Actin cytoskeleton organization and cortactin distribution was observed using confocal microcopy. Cells treated with higher concentrations of lapatinib and crizotinib rounded up and didn't form any protrusions. Usage of lower concentrations of selected inhibitors caused changes in cells morphology and formation of new protrusions. We noticed subtle differences between mesenchymally and ameboidally migrating colon cancer cells after inhibitors treatment.
Keywords: Cancer cells invasion, Cancer signaling, Kinases.

TUE-088
Effects of syntenin expression levels and growth factor stimulation on exosomal protein content

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Exosomes are small secreted vesicles of endosomal origin that have been shown to play a role in various aspects of trans cellular signaling. They are of particular importance during cancer progression because they suppress immunity and stimulate tumor angiogenesis and metastasis. Little is known on the impact of growth factor signaling on exosomal protein content. Also it is unclear whether specific components of the exosomal machineries can differentially affect the content of exosomes.

Here, we compared secreted exosomal proteins from MCF-7 cells upon different growth factor stimulation by proteomic approach. We also tested for the effect of syntenin, a scaffolding protein stimulating exosomal production. For the analysis, we divided our exosomal proteins into 10 families. Interestingly, we observed that upon syntenin gain of function, exosomes contain high amounts of nuclear proteins involved in transcription and

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translation and proteins involved in the metabolism. Upon FGF2 stimulation, exosomes contain mainly proteins involved in trafficking, cytoskeleton remodeling, and polarity proteins as well as junctional and adhesion molecules. These data indicate that cells drastically change their secreted material depending on internal and external stimuli. We are currently optimizing protocols for proteomic analysis of exosomes from MCF-7 cells grown in the presence of various concentrations of serum. By these approaches, we hope to clarify to what extend internal and external stimuli affect exosomal content and to better understand trans cellular signaling.

Keywords: exosomes, syntenin.

TUE-089

Electrical, metabolic and genetic communication between laryngeal squamous cell carcinoma cells via membranous tunneling tubes

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Intercellular membranous tunneling tubes (TT) are recently discovered form of communication between remote cells. In the present study, we demonstrate that human laryngeal squamous cell carcinoma (LSCC) cells in culture form a dense network of non-adherent tunneling tubes that differ in length, thickness and composition. We observed extremely long (up to 1 mm) and thick (up to 5 μm) epithelial bridge-like connections (Zani, 2010), containing F-actin together with α-tubulin, and shorter (up to 100 μm) but extremely thin (less than 0.2 μm) tunneling nanotube-like formations (Rustom, 2004), containing only F-actin. Using fluorescence microscopy and dual whole-cell patch-clamp technique we performed a quantitative evaluation of TT electrical properties permeability to fluorescent dyes of different molecular weight and charge. We found that the thickest and longest intercellular TTs formed during cell dislodgement after division provided open-ended connections able to permit at least 3 kDa molecules, while TTs formed by filopodia or secondary lamelipodia outgrowth mechanism establish intercellular connections through the formation of connexin 43 based gap junctions with consequent voltage gating properties and permeability to smaller molecules < 1 kDa. TTs, containing F-actin together with α-tubulin transported mitochondria, accommodated DAPI stained vesicles and were capable of transmitting double-stranded small interfering RNA between cells. In addition we demonstrated the existence of TTs in the microsections of LSCC tissue samples.

Our findings may contribute to better understanding of intercellular communication between normal and tumor cells providing possible guidelines for the cancer treatment and future drug design.

Keywords: intercellular communication, laryngeal squamous cell carcinoma, tunneling tubes.

TUE-090

Endoplasmic reticulum stress-mediated activation of p38 MAPK, caspase-2 and caspase-8 leads to abrin-induced apoptosis

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Abrin from *Abrus precatorius* plant is a potent protein synthesis inhibitor and induces apoptosis in cells. However, the relationship between inhibition of protein synthesis and apoptosis is not well understood. We initiated studies to examine if abrin can induce endoplasmic reticulum (ER) stress which finally leads to mitochondrial membrane potential (MMP) loss and apoptosis.

Imunoblotting was employed to analyse the activation of ER stress markers such as eIF2α (eukaryotic initiation factor 2α), CHOP (CAAT/enhancer binding protein (C/EBP) homologous protein), p38 mitogen-activated protein kinase (p38 MAPK) and c-Jun N-terminal kinase (JNK) upon abrin treatment. Additionally, caspase-2 and caspase-8 have been reported as key molecules linking ER stress to apoptosis. Therefore, flow cytometry and immunoblotting was done to analyse the activation and involvement of p38 MAPK, JNK, caspase-2 and caspase-8 using specific inhibitors. We also checked for the phosphorylation of H2AX which is a marker for double strand DNA breaks to investigate any direct DNA damage caused by abrin.

Significant phosphorylation of eIF2α, p38 MAPK, JNK and upregulation of CHOP was observed suggesting activation of ER stress signaling in the cells treated with abrin. Further, studies with the help of inhibitors showed that abrin-mediated cleavage of caspase-2 and -8 was found to be dependent on p38 MAPK but not JNK. The cleavage of caspase-2 and caspase-8 triggered Bid cleavage leading to MMP loss clearly delineating the link between ER stress and mitochondrial damage leading to abrin induced apoptosis. We also found significant phosphorylation of H2AX and ATM after abrin treatment which was completely abrogated in the presence of broad spectrum pan-caspase inhibitor suggesting that DNA damage was not a cause but consequence of apoptosis induced by abrin. Therefore present study demonstrates the significance of inhibition of protein synthesis in abrin induced apoptosis.

We acknowledge Council of Scientific and Industrial Research (CSIR) and Department of Biotechnology (DBT) Government of India for the support.

Keywords: DNA damage, p38 mitogen-activated protein kinase, Unfolded Protein Response.

TUE-091

Epigenetic therapy: a promising treatment for chondrosarcoma?

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Chondrosarcomas are rare tumors, potentially fatal since they are chemo- and radioresistant. Current treatment consists to tumor resection which may lead to amputation. The development of new therapeutic approaches is necessary. Among emerging strategies, the targeting of Enhancer of Zeste Homolog 2 (EZH2), the enzyme responsible for the methylation of histone H3 on lysine 27 (H3K27) seems promising. The objective of this work is to validate the concept of epigenetic therapy targeting H3K27 methylation using 3-Deazaneplanocin A (DZNep), an S-adenosyl-L homocysteine hydrolase inhibitor which induces EZH2 protein depletion, to treat chondrosarcomas.

EZH2 expression was determined by immunohistochemistry on high grade chondrosarcomas, enchondromas (benign tumors) and adult articular cartilages from human patients, and western blot on chondrocytes and chondrosarcomas cells lines. SW1353 and CH2879 chondrosarcomas cells lines were cultured in the presence of DZNep, and their growth and survival were evaluated by counting adherent cells periodically. Cell cycle analysis was analysed by flow cytometry. Apoptosis was evaluated by

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Apo2.7 expression using flow cytometry. Cell migration was assessed by wound healing assay. *In vivo* experiments were done with implantation of CH2879 chondrosarcoma cell line in nude mice.

EZH2 is expressed in high grade chondrosarcomas but not in enchondromas or adult articular cartilage. *In vitro*, DZNep reduces EZH2 protein expression and H3K27me3 level. It reduces growth of chondrosarcomas *in vitro* and *in vivo*. DZNep also induces cell death by apoptosis, and reduces migration of chondrosarcomas.

These results confirm the interest of the DZNep for the treatment of chondrosarcomas. Further experiments are required to better understand mechanisms responsible of its effects on tumoral cells, and to determine its putative effects on normal cells.
Keywords: chondrosarcoma, epigenetic therapy, EZH2.

TUE-092
Epithelial transiton of gastric cancer cells by KRC-108

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Investigation of the resistance mechanism in targeted therapy is important because resistance acquired during the treatment can be the cause of relapse or refractoriness to the therapy. The small molecule compound KRC-108 has been developed in an effort to identify an anticancer agent with c-met inhibitory activity in the previous study. Gastric cancer cell lines resistant to KRC-108 were established, and the changes that accompanied resistance were explored. Increased expression of the c-met protein was observed in KRC-108-resistant cells. The phosphorylation of c-met also increased in cell lines resistant to KRC-108. Resistance to the c-met inhibitor resulted in cell morphological changes. Parental cells with a round and poorly differentiated morphology changed to an epithelial cell-like phenotype. Consistent with the transition to an epithelial morphology, the expression of E-cadherin was increased in resistant cells. In an immunoprecipitation study, an interaction between E-cadherin and the c-met protein was observed in the KRC-108-resistant cells. These results suggest that the epithelial transition in KRC-108-resistant cells is mediated by recruiting E-cadherin to c-met protein. This study was supported by grants from the Ministry of Trade, Industry and Energy (MOTIE), Korea Institute for Advancement of Technology (KIAT) through inter-ER cooperation projects (A004500005)
Keywords: c-met, drug resistance, E-cadherin.

TUE-093
ERK-dependent association of HIF-1α with chromatin via a peptide that can inhibit HIF-1 activity when over-expressed in hepatocellular carcinoma cells

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A major aspect of carcinogenesis is the development of hypoxic regions due to increased cell proliferation and limited blood supply. Hypoxia further promotes tumor progression by facilitating angiogenesis and metabolic adaptation of cancer cells. HIF-1α is the regulatory subunit of Hypoxia-Inducible Factor-1, a key transcriptional activator that mediates the cell response to hypoxia. HIF-1α represents an attractive therapeutic target because it is expressed in many cancers and is associated with poor patient prognosis. HIF-1α is regulated by oxygen as well as oxygen-independent mechanisms that involve its phosphorylation. We have previously shown that ERK phosphorylates HIF-1α at Ser641/643 in a domain termed ETD (ERK-Targeted Domain). In the absence of this phosphorylation, HIF-1α is exported from the nucleus in a CRM1-dependent manner while ETD phosphorylation enhances nuclear accumulation and activity of HIF-1. To investigate further the function of ETD, wild-type GFP-tagged HIF-1α or 42-amino acid long ETD forms carrying mutations that either prevent or mimic modification by ERK were analyzed by fluorescence recovery after photobleaching (FRAP) in Huh7 hepatocarcinoma cells. The phosphomimetic mutant full-length HIF-1α or ETD exhibited lower intranuclear mobility than their wild-type counterparts suggesting that phosphorylation of ETD by ERK stimulates its interaction with chromatin components, the identification of which is currently under progress using pull-down assays. Over-expression of the wild-type and phosphomimetic mutant flag-tagged ETD forms, but not the phospho-deficient mutant ETD form, drastically inhibited HIF-1 activity under hypoxia in Huh7 and HepG2 cells, suggesting that exogenous ETD peptides can compete with endogenous HIF-1α for binding to chromatin. We conclude that the ETD mediates ERK-dependent interaction between HIF-1α and chromatin and it can be used as an efficient peptide inhibitor of HIF-1 activity in cancer cells.

Supported by the “ARISTEIA II “ Action of the “OPERATIONAL PROGRAMME EDUCATION AND LIFELONG LEARNING” to G.S. and is co-funded by the European Social Fund (ESF) and National Resources.
Keywords: ERK1/2, HIF-1, Hypoxia.

TUE-094
Ethanol influences MICA/B surface expression and release from the cells

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Expression of proteins MICA and MICB(MIC), the ligands for immunoreceptor NKG2D, has been proposed to play an important role in the detection and elimination of tumor and other damaged cells. The result of the interaction of surface MIC with NKG2D is the increase of cytotoxic activity of NK and T cells and the subsequent killing of affected cells. On the other hand, soluble MIC can depress NK cell functions particularly because of decrease of NKG2D surface expression. We studied in several cell models the influence of ethanol on the expression of MIC and on their release from the cells. MIC expression was analyzed in a model of ethanol-induced cell stress using K562, Jurkat and THP-1 cell lines, as well as human peripheral mononuclear cells. Surface MIC expression was measured by flow cytometry. C1R-MICA transfected line was used as a positive control. Significant levels of spontaneous surface MIC were registered in all lines. Surface expression of MIC increased under the influence of ethanol (0.5-1.5%) and decreased with ethanol dose increasing up to 2% and above. The later process was corresponded to cell death elevation. We did not find spontaneous surface expression of MIC in lymphocytes, but discovered it in CD14-positive cells, increased under the influence of ethanol-induced cellular stress (0.12-1%). Those cells, which have no spontaneous MIC surface expression, have a considerable level of MIC mRNA (detected by RT-PCR). A cause of the increase of MIC expression in cells may be the oxidative stress induced by ethanol resulting in DNA damage. We used ATM/ATR kinase inhibitor for detection DNA damage in cells under the ethanol influence, and we did not detect considerable changes in MIC surface levels at indi-

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cated ethanol doses. With confocal microscopy it was shown, that the inner pool of MIC decreased and the surface pool increased in comparison with the control cells. We can assume that under the influence of ethanol the proteins MIC move from the cytoplasm to the cell surface (detected by ELISA). It has been shown that under the influence of ethanol MIC were released from the cells as soluble proteins and consisting of microparticles and exosomes. The increase of soluble MIC level after cell treatment with methyl-cyclodextrin supports the role of lipid rafts in release MIC from the cells. Thus, ethanol may decrease MIC amount on the cell surface by the elimination of cholesterol and disintegration of lipid rafts in plasma membrane. Thus, we have shown that ethanol can alter MIC expression in some cell lines and normal monocytes and have analysed mechanisms underlying this alteration. Changes in MIC surface and extracellular content induced by ethanol may have amodulating effect on the immune system. This work is supported by RFBR, grant # 14-04-32342.

Keywords: MICA, MICB, NKG2D.

TUE-095

Evaluation of anticancer properties of an endemic geophyte species from Turkey, Muscari muscarimi

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The genus *Muscari* (Hyacinthaceae) is represented by nearly 50 species on the Earth. In Turkey, it is represented with about 30 species. Among them, *M. muscarimi* have been used in traditional medicine as antirheumatic, stomachic, diuretic and expectorant. In addition to this, it has also been used as food for humans and animals, ornamental plants in gardens.

The chemical composition of the *M. muscarimi* is composed of polysaccharides, homoisoflavanons, glycosides and pyrrolizidine alkaloids. Also, many kinds of flavonoids have been identified. Phenolic compounds including flavonoids and phenolic acids are known to be responsible for antioxidant capacities in plants. In our previous studies, total antioxidant activity of ethanolic and methanolic bulb extracts of *M. muscarimi* was determined by β–carotene/linoleic acid assay and we found at 69.43% and 61.55% respectively. The phenolic contents of the both extracts were found 21.8 and 18.3 mg/g GAE respectively. A positive correlation was observed between antioxidant activity and amount of phenolic contents.

Recent studies showed that *Muscari* species have different biological and pharmacological activities such as anti-inflammatory, antimicrobial, antioxidant and antimutagenic. Nevertheless, no reports on the investigaton of anticancer properties. In this respect, the aim of the present study is evaluate the anticancer properties of ethanolic and methanolic bulb extracts of *M. muscarimi* against various human cancer cell lines (MCF-7, H1299, HeLa). Plant samples were collected and their cytotoxic effects on the cancer cell lines were examined at different concentrations (16, 31, 62.5, 125, 250, 500, 1000 μg/mL). Cytotoxicity in plant extract-treated and untreated control groups was measured by the luminometric method using a CytotoxGlo kit. Values for the concentration at which 50% inhibition occurred (IC_{50}) were calculated for all extracts. For the detection of the induction of apoptosis of each extract, cells were treated with IC_{50} values of each plant extract for 24 h. At the end of the incubation period late apoptotic events were analyzed by terminal transferase dUTP nick end-labeling (TUNEL) analysis. Apoptotic cells were counted under the microscope. According to results, both ethanolic and methanolic extracts have the strongest cytotoxic effects on the H1299 cells (IC_{50}: 62.5 μg/mL) in a dose-dependent manner than MCF-7 and HeLa cells. Among the three cell lines, *M. muscarimi* has the biggest effect on induction of apoptosis on MCF-7 cells. Based on the in vitro data, it is suggested that consumption of this plant may impart anticancer effects especially in the breast, lung and the cervix.

Keywords: Apoptosis, MCF-7, H1299 and HeLa cells, Muscari muscarimi.

TUE-096

Evaluation of KRAS gene expression and gene variant in genomic and cell free DNA of Iranian women with endometriosis

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Purpose : Endometriosis is a complex gynecological disease which is defined as the presence of endometrial–like tissue outside the uterus. The pathology of endometriosis remains unknown, although genetic components have been suggested by numerous studies. Among them, KRAS considered as a crucial gene, because its activation results in de novo endometriosis in mice. It has been reported recently that 31% of women with endometriosis as opposed to 5% of control population have KRAS let-7 microRNA binding site variant (rs61764370).

Methods: We screened genomic and cell free DNA of Iranian women with endometriosis for this SNP, whether is associated with the risk of endometriosis. We investigated 58 patients with histopathologically confirmed endometriosis and 60 control group women who were surgically proven to have no endometriosis and 100 randomly selected Iranian people. Quantitative real-time RT–PCR was performed to determine expression of KRAS transcripts in eutopic endometrium tissue samples.

Results: The KRAS variant allele frequency distribution in controls and normal population was in agreement with previous reports. Our results suggest that the variant is not associated with development of advanced endometriosis in Iranian women. We obtained the higher level of KRAS expression in eutopic endometrium of patients with endometriosis compare to healthy women.

Conclusions: The LCS6 let-7 miRNA binding site variant of the KRAS is neither a genetics nor somatic marker for endometriosis risk in Iranian population. However, the higher expression ratio of KRAS mRNA in endometriosis indicates its role in transferring endometrium outside uterine cavity.

Keywords: Endometriosis, KRAS gene, let-7 microRNA, Cell free DNA, Single nucleotide polymorphism.

TUE-097

Evidence for anti-proliferative properties of the anti-diabetic agent 1,1-dimethylbiguanide hydrochloride (metformin) on human ovarian cancer cells

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It is widely supported that the use of metformin (1,1-dimethylbiguanide hydrochloride) in patients with type-2 diabetes mellitus is associated with lower risk of ovarian cancer development and that metformin-treated diabetic individuals with ovarian cancer display enhanced response to chemotherapy and progression-free survival.

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However, the exact molecular pathways underlying this anti-ovarian cancer profile of metformin have not yet been elucidated.

Scope: We sought to investigate the anti-proliferative effect of metformin in human tumor cells of ovarian origin.

Methods: Six human ovarian cancer cell lines (TOV-21G, TOV-112D, ES-2, SK-OV-3, OV-CAR-3, MDAH-2774) were cultured and subjected to metformin treatment. Cell-growth curves and IC50 values were estimated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell-viability assay. Metformin was tested in concentrations ranged from 1μM to 50 mM, in 24 h and 48 h. Induction of apoptosis was estimated by flow-cytometry using annexin-V-FITC/propidium iodide staining, in a FACSCanto™ Flow Cytometer (Becton Dickinson, Germany).

Results: At 24 hr, the IC50 values were found to be: 50 mM for TOV-112D, 30 mM for ES-2, 50 mM for SK-OV-3, and 30 mM for OV-CAR-3, while in the TOV-21G and MDAH-2774 lines reduction of cell viability was not higher than 25% in any of the concentrations tested. The 48 hr-IC50 values were: 30 mM for TOV-21G, 15 mM for TOV-112D, 10 mM for ES-2, 5 mM for SK-OV-3, 10 mM for OV-CAR-3, and 50 mM for MDAH-2774. Moreover, preliminary flow-cytometry experiments marked a significant increase in early-(~8-fold), late-apoptotic (~3-fold) and necrotic cells (~4-fold) in TOV-21G cells after metformin treatment (30 mM, 48 hrs).

Conclusion: Our preliminary results indicate the pro-apoptotic capacity of metformin on ovarian cancer cells. Results from the other cell lines, and further analyses on cell-cycle arrest, pro- and anti-apoptotic gene-expression and immune patterns, as well as possible synergistic effects with standard chemotherapeutics used in ovarian cancer treatment, are needed to elucidate the potentiality of this anti-diabetic drug as an adjuvant anti-ovarian cancer agent.

The project is co-funded by the European Union (ERDF) & Greek national funds: Operational Program *Competitiveness & Entrepreneurship*, National Strategic Reference Framework-Research Funding Program: COOPERATION 2011-11ΣΥΝ-10-1821, and partially (presentation expenses) by the Athens University Special Research Account (#10812).

Keywords: apoptosis, metformin, ovarian cancer.

TUE-098

Exome and transcriptome analysis of cancer cells in chronic myeloid leukemia

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Tyrosine kinases inhibitors (TKI) are highly effective in treatment of chronic myeloid leukemia (CML). However long-term clinical studies demonstrated that about 20% of the CML patients are primary resistant to TKI. Activation of *BCR-ABL*-independent mechanisms could explain this resistance. Here we tried to find exomic variants and expression differences associated with optimal response or failure of the therapy. This approach can both benefit therapy efficiency and understanding of cancer pathogenesis. Exome sequencing was done with Ion PGM technology using Exome Enrichment Kit from LifeTechnologies. Gene expression profiles were analyzed using Illumina HT-12 Expression Bead Chip. According to European Leukemia Net (2013) criteria patients were divided into resistant to TKI therapy – molecular response >10% in 6 months of therapy and optimal responders with molecular response <1% in 6 months of therapy. Exome analysis revealed from 36153 to 38636 single nucleotide variants (SNV) and indels in patients with optimal response. Filtering procedures allowed us to select only 3 homozygous SNVs common for all three examined patients. One of these variants was found to be a frameshift deletion in NCAM1. This gene is an essential marker of T-lymphocytes and its expression is associated with T-NK lymphomas and both acute and chronic myeloid leukemias. It was also found in studies of small-cell carcinomas and lethal median granuloma. Two other variants were registered in SLC4A3 associated with dendrite-cell carcinoma and 28S_rRNA-related gene. Comparative transcriptome analysis revealed 26 differently expressed genes in patients with optimal response and failures. Ten of these genes (*DAZAP2, UBA52, PRR13, ATG7, LOC387820, PAK1, RAB11A, EMP3, GSTM1, GSTM2*) are associated with other cancers and another 4 (*RSAD2, MAP3K11, COMMD1, TNFRSF1A*) are the key members of regulatory pathways including NF-kappa-B, MAPK, JNK and other thought to be disbalanced in many cancers. Enrichment analysis of the differently expressed genes showed the following molecular networks involved ($p < 0.05$): glutathion, drug and xenobiotic methabolism, chemical mutagenesis, glycosaminoglycan degradation and MAPK signaling pathway. According to expression levels we may suggest that these pathways are activated in patients resistant to TKI therapy while. We found two exomic variants and 14 differently expressed genes potentially involved in cancer signaling pathways responsible for differences in patient's response to TKI treatment.

Keywords: CML, Exome analysis, transcriptome.

TUE-099

Exosome mediated Glioma cell-endothelial cell cross talk increase angiogenesis in glioblastoma

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The organ specific heterogeneity of endothelial cells points toward their ability to adapt to the tissue environment and become genetically or phenotypically unique from other endothelial cell types of the body. In glioblastoma, high amount of angiogenesis is a result of steadily proliferating endothelial cell populations via paracrine interactions with glioma cells. Here, we show that human glioblastoma derived endothelial cells (hGECs) are auto-angiogenic, but remain untransformed in nature and that cross-talk of endothelial cells-glioma cells enhance angiogen-

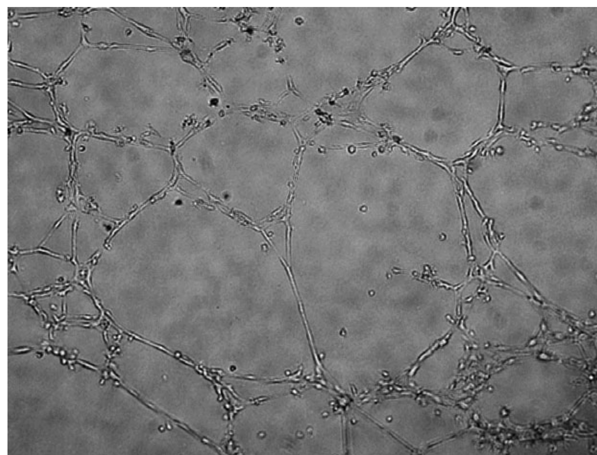

Fig. 1.

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esis via exosome mediated pathway. Although, hGECs showed resistant to glioma chemotherapeutic drug temozolomide induced apoptosis, they remain dependent on glioma cell secreted factors for their survival during in vitro matrigel tube formation assay. Interestingly, when incubated with glioma cell derived exosomes, hGECs showed upregulated angiogenesis as a result of increased initial endothelial cell migration. Also, we show that glioma cells can activate normal brain endothellial cells thus making them proangiogenic in nature.
Keywords: None.

TUE-100
Expression of AID in malignant melanoma with BRAFV600E mutation

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BRAF-activating somatic mutations often exist in malignant melanoma. Malignant melanoma is classified into four subtypes: skin melanomas without chronic sun-induced damage (non-CSD), skin melanomas with chronic sun-induced damage (CSD), acral melanomas, and mucosal melanomas. A previous Japanese study has revealed that *BRAF* mutations were found in 26.7% of Japanese melanoma patients: 71.4% in CSD, 55.2% in non-CSD, 9.1% in acral melanoma, and 5% in mucosal melanoma. The underlying molecular mechanism of somatic *BRAF*-mutation inductions remained to be clear.

Activation-induced cytidine deaminase (AID), a member of a cytidine deaminase family, is essential for somatic hypermutation and class-switch recombination, in immunoglobulin genes. Recently, several studies have shown that abnormally expressed AID acts as a genome mutator that contributes to malignancy such as adult T cell leukemia/lymphoma (ATLL) and chronic active Epstein-Barr virus infection (CAEBV). Another protein APOBEC3B was reported to be an enzymatic source of mutation in breast cancer.

Chronic ultraviolet ray irradiation may play a role in causing *BRAF* mutations in CSD, however, *BRAF* mutations in non-CSD cannot be explained by the ultraviolet ray irradiation.

To explore the correlation between the AID and APOBEC3B expression and *BRAF* mutations, we performed the immunohistochemical study for 16 Japanese melanoma patients. As the previous report from a Japanese group, $BRAF^{V600E}$ mutation was predominant in malignant melanoma patients in our department and any other mutation was not found. Nine out of 10 AID malignant melanoma specimens with high AID expression in our study contained $BRAF^{V600E}$ mutation, while none of the 6 malignant melanomas with low AID expression had $BRAF^{V600E}$ mutation. All 9 melanomas with $BRAF^{V600E}$ mutation in our study took place in the region without chronic ultraviolet irradiation.

In contrast, 5 out of 9 malignant melanoma specimens with high APOBEC3B expression contained $BRAF^{V600E}$ mutation, while 4 of the 7 malignant melanomas with low APOBEC3B expression had $BRAF^{V600E}$ mutation.

We next examined the prognosis of each malignant melanoma patient for one to 4 years. Eight out of 10 malignant melanoma patients with high AID expression develop multi-organ metastases and/or multiple lymph node metastases afterwards during the follow-up, while no metastasis has been observed in melanoma patients with low AID expression during our follow-up until now. However, the sample size was not large enough in this study to perform multivariate analysis to show the relationship between AID expression and the prognosis of malignant melanoma.
Keywords: AID, BRAF, melanoma.

TUE-102
Expression of CRABP1 and CRABP2 in non-small cell lung cancer

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Retinoic acid (RA) is the most active derivative of vitamin A and regulates proliferation, differentiation and apoptosis of normal and transformed cells. Cellular retinoic acid binding proteins (CRABP1 and CRABP2) are important modulators of RA-dependent signaling. Despite the role of RA in carcinogenesis seems to be well defined, participation of CRABPs in tumor development remains poorly investigated. Several studies showed abnormal expression of CRABPs in human tumors and its association with clinicopathological features. Up to date, almost nothing is known about CRABP1 and CRABP2 expression in non-small cell lung cancer (NSCLC).

Here we studied the simultaneous mRNA expression of CRABPs in 48 NSCLC samples using quantitative real-time PCR. Data were analyzed with respect to clinicopathological characteristics. We observed elevated expression of CRABP1 and CRABP2 in 42% and 56% cases, respectively. Decrease of CRABP2 expression was significantly associated with the presence of lymph node metastases ($P < 0.05$). We also revealed significant positive correlation between expression of CRABP1 and CRABP2. The strongest correlation was detected in well and moderately differentiated tumors (Spearman‘s rank correlation coefficient was 0.6, $P < 0.05$).

Overall, we found the significant alterations in CRABP1 and CRABP2 expression in NSCLC samples. Obtained results provide the first evidence of possible implication of CRABPs to lung cancer progression.
Keywords: CRABP1, CRABP2, non-small cell lung cancer.

TUE-103
Fad104, a regulatory factor of adipogenesis, negatively regulates invasion and metastasis of cancer cells.

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Fad104 (Factor for adipocyte differentiation 104) is a regulator of adipogenesis. Previously, we showed that *fad104* regulated cell adhesion, migration and proliferation. Since *fad104* regulated cell migration, it was thought that *fad104* regulated invasion and metastasis of cancer cells. In the present study, we clarified the role of *fad104* in invasion and metastasis of melanoma cells.

Using quantitative PCR and Western blotting, we first analyzed the expression levels of *fad104* in highly metastatic A375SM cells and poorly metastatic A375C6 cells. The expression level of *fad104* in A375SM cells was lower than that of A375C6 cells. Next we assessed the effect of *fad104* on invasion of cancer cells using transwell assays. A375C6 cells transfected with *fad104* siRNA increased in the number of invaded cells compared with control cells transfected with luciferase siRNA. In contrast, *fad104*-expressing adenovirus-infected A375SM cells showed a reduction in invasion compared with b-galactosidase-expressing adenovirus-infected A375SM cells (control cells). Furthermore, the expression of matrix metalloproteinase 2 in *fad104* overexpressing A375SM cells was lower than that of control cells. These results suggest that *fad104* suppresses invasion of cancer cells. We next investigated

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whether *fad104* contributed to metastasis. We first produced murine melanoma cells B16F10 stably expressed *fad104* and we assessed the ability of *fad104* to form colonization in lung *in vivo*. As a result, *fad104* over expressing B16F10 cells showed a reduction in lung colonization. This result suggests that *fad104* negatively regulates metastasis of melanoma cells.

Next, we investigated the molecular mechanism by which *fad104* regulated invasion and metastasis of cancer cells. In melanoma cells, various signaling pathways, such as janus activated kinase (JAK)-signal transducer and activator of transcription (STAT) play essential role for invasion and metastasis. Therefore, we verified the effect of *fad104* on JAK-STAT signaling pathway. Overexpression of *fad104* reduced the phosphorylation level of STAT3. Furthermore, depletion of *fad104* increased phosphorylation level of STAT3. These results suggest that *fad104* inhibits JAK-STAT signaling pathways.

In summary, we demonstrated that *fad104* suppressed activation of STAT3 and controlled invasion and metastasis of melanoma cells.

Keywords: metastasis, signal transducer and activator of transcription 3 (STAT3).

TUE-104

Fragment of mucin MUC1 extracellular domain enhances metastatic potential of cancer cells in vitro

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The speculations about the role of MUC1 which is known to be overexpressed in glandular cancers on metastatic potential of cancer cells originate from the data indicating that cell malignization correlates with the change of apical mucin MUC1 localization to peripheral [1]. But the role of MUC1 in cancer metastasizing is not clear yet [2-4]. The major problem is the absence of adequate cell models. The aim of this study was to create cell models presenting different fragments of human mucin MUC1 extracellular domain on cell surface.

Genetic constructions were generated on the basis of plasmid vector pEGFP-N3. These constructions contain fusion genes coding chimeric proteins composed of different combinations of mucin MUC1 functional domains and identification markers – FLAG-epitope located at the N-terminus and EGFP located at the C-terminus of the chimeric proteins. The constructions were used for stable transformation of human cancer cells HT-29. Transformants obtained were characterized by means of flow cytometry. Low expression level of endogenous mucin MUC1 and high expression level of the recombinant proteins were confirmed by real-time PCR. Microscopic examination of transformed cells confirmed localization of fusion proteins on its plasmatic membrane. Cells obtained are used to be a quite applicable model of MUC1-expressing cancers and might be used for study the role of different functional fragments of mucin MUC1 in metastasizing.

Cells obtained were tested for the ability to penetrate and migrate through the endothelium by using an artificial system. It was shown that the expression of fragments of mucin MUC1 extracellular domain leads to an increase in the proportion of cells capable to overcome the endothelial barrier. Furthermore, a fragment has been identified, the presentation of which on the cell surface leads to the greatest increase in the proportion of cells migrated.

This work is partially supported by: RFBR grant 12-04-31721 and MSERF grant 8800.

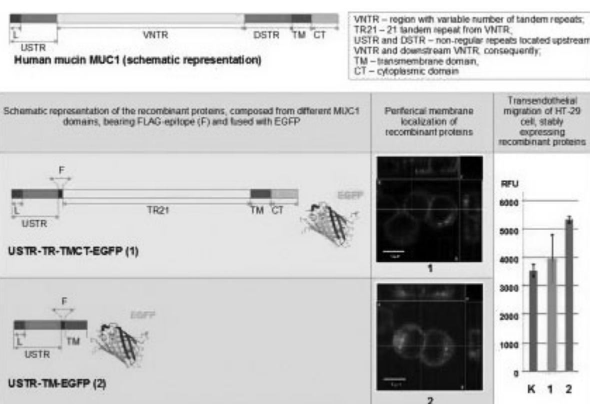

Fig. 1. xx

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Keywords: metastasis, MUC1, mucin.

TUE-105

From ancient formula to modern drug: exploring the Chinese herbal medicine toward cancer therapy

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Chinese herbal medicine (CHM) has been historically used in cancer therapies by skillful Chinese medical doctor for thousands of years. Some clinical used anti-cancer therapeutic agents such as camptothecin, taxol or vinblastine, are derived from CHM that recorded as poisonous herbs. Although the current agents show promising to fight cancers, most of them are with significant side effects including killed the normal cells concomitantly, prompting increased interest in discovering more safety formula or compound toward alternative anti-cancer therapy.

In others and our previous studies, several cancer-associated genes have been identified. CD74, a nonpolymorphic integral membrane protein which has several functions in MHC class II-restricted antigen presentation, is up-regulated in various kinds of cancers thus been suggested to play an important role in oncogenic functions suchas as cancer cell growth and motility. Here we will address the roles of CD74 in oncogenic functions of cancer cells in vitro and in vivo. The regulatory effects and the signaling transduction pathways will be discussed. In addition, we will further show our new findings about CD74-targeted CHM and compound screening: from all selected 400 CHM extracts, more than 40 CHM extracts are identified as high-ranking cell-permeable, low-toxic ones by the two-dimensional high throughput living cell-based screening platforms. Based on the results

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aforementioned, one potential formula, JYCE-102, is selected at the first notch as a potential CD74-targeted agent using cell-based enzyme-linked immunosorbent approaches. The regulatory mechanisms and the signaling transduction pathways of JYCE-102 and its compounds toward oncogenic functions are compared in vitro and in vivo, suggesting its potential as a therapeutic option.
Keywords: Chinese herbal medicine, cancer, CD74.

TUE-106
Functional potential of sulfonamide derivative compounds in the regulation of hypoxia induced carbonic anhydrase 9 (CA9) in human brain cancer

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Genes regulated by Hypoxia induced HIF-1α are mainly responsible for tumour resistance to radiation- and chemo-therapy. Among these genes, carbonic anhydrase isoform IX (CA9) is highly over expressed in many types of cancer especially in high grade brain cancer like Glioblastoma (GBM). Inhibition of the enzymatic activity by application of specific chemical CA9 inhibitor sulphonamides (CAI) like Acetazolamide (Aza.), the new Sulfonamide derivative carbonic anhydrase inhibitor (SU.D2) or indirect inhibitors like the HIF-1a inhibitor Chetomin or molecular inhibitors like CA9-siRNA are leading to an inhibition of the functional role of CA9 during tumorigenesis. Human GBM cells were treated with *in vitro* hypoxia (1, 6, or 24 h at 0.1%, O_2). Aza. application was at a range between 250 and 8000 nM and the HIF-1α inhibitor Chetomin at a concentration range of 150–500 nM. Cell culture plates were incubated for 24 h under hypoxia (0.1% O_2). Further, CA9-siRNA constructs were transiently transfected into GBM cells exposed to extreme hypoxic aeration conditions. CA9 protein expression level was detectable in a cell-type specific manner under normoxic conditions. Whereas U87-MG exhibited a strong aerobic expression,. Aza. as well as SU.D2 displayed inhibitory characteristics to hypoxia induced CA9 expression for 24 h of hypoxia (0.1% O_2) at concentrations between 3500 and 8000 nM, on both the protein and mRNA level. Chetomin application under similar oxygenation conditions led to a sharply reduced expression of both CA IX protein and CA9 mRNA levels, indicating a clear glucose availability involvement. In GBM therapy, either the tumour is eradicated or to convert it into a controlled, quiescent chronic disease. Aza., SU.D2, Chetomin or CA9-siRNA possesses functional CA9 inhibitory characteristics when applied against human cancers with hypoxic regions like GBM. Therefore, they can applied as potential optimal tools for the development of a developed anti cancer therapy in human brain cancer patients.
Keywords: Cancer, Hypoxia, Carbonic Anhydrase 9 (CA9).

TUE-107
FUT11 as a potential biomarker of clear cell renal cell carcinoma progression based on meta-analysis of gene expression data

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Background: Renal cell carcinoma (RCC) is the most common type of kidney cancer that accounts for 2% of the world total of all adult malignancies. The majority of ccRCC cases are detected incidentally and diagnose at the late stage due to asymptomatic course of the disease. As a result it is difficult to treat and rarely cured once spread beyond the kidney.
Observation: We provide a comprehensive summary of available clear cell renal cell carcinoma (ccRCC) microarray data in the form of meta-analysis of genes differentially regulated in tumors as compared to healthy tissue, using effect size to measure the strength of a relationship between the disease and gene expression. Twelve Affymetrix studies of biopsy confirmed, primary ccRCC samples with TNM, F grades, or WHO classifications were included. The data, in the FLEO format, were obtained from ArrayExpress and Gene Expression Omnibus. Arrays were normalized using the Robust Multichip Average method. Eight studies fulfilled inclusion criteria and 222 tumor and 85 control samples were subjected to the analysis. For each array type, the probes were mapped to version 14 Unigene gene identifiers. Moreover, tumors from patients from Western Poland who were diagnosed with urological carcinomas were collected. The tissues were histopathologically verified as ccRCC and screened for VHL mutations, promoter methylation, expression of VHL, HIF1A and EPAS1, and LOH.
Results: We identified 725 differentially regulated genes, with a number of interesting targets, such as TMEM213, SMIM5, or ATPases: ATP6V0A4 and ATP6V1G3, of which limited or no information is available in terms of their function in ccRCC pathology. Downregulated remodeling, blood clotting, vasodilation, and energy metabolism, while upregulated genes were classified into pathways generally deregulated in cancers: immune system response, inflammatory response, angiogenesis, and apoptosis. One hundred fifteen deregulated genes were included in network analysis, with EGLN3, AP-2, NR3C1, HIF1A,and EPAS1 (gene encoding HIF2-α) as points of functional convergence, but, interestingly, 610 genes fail previously identified molecular networks. Furthermore, we validated the expression of 14 top deregulated genes in independent sample set of 32 ccRCC tumors by qPCR and tested if it could serve as a marker of disease progression. We found a correlation of high fucosyltransferase 11 (FUT11) expression with non-symptomatic course of the disease, which suggests that FUT11's expression might be potentially used as a biomarker of disease progression.
Keywords: Biomarker, Cancer, Microarray.

TUE-108
Gain-of-function PDGFRB mutations associated with familial infantile myofibromatosis are sensitive to imatinib

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Infantile myofibromatosis is the most prevalent tumor of soft tissue of childhood. It is characterized by the presence of nodules in the skin, the subcutaneous soft tissues, the bones or the viscera. Recently, germline and somatic heterozygous *PDGFRB* mutations have been associated with familial infantile myofibromatosis (FIM) but have not been functionally characterized. Platelet-derived growth factor receptors (PDGFRA and PDGFRB) are tyrosine kinase receptors that stimulate cell proliferation and motility. Until now, no activating point mutation in *PDGFRB* has been described in cancer. Therefore, the aim of this work was to characterize these new mutations. For this purpose, we transiently expressed three PDGFRB mutants associated with FIM (R561C, P660T and N666K) in HT-1080 cells, a fibrosar-

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coma-derived human cell line. The analysis of the signaling pathways downstream these mutants showed that, in the absence of ligand, the somatic N666K mutant strongly activated mitogen-activated protein kinases (ERK), phospholipase Cγ and signal transducer and activator of transcription 3 (STAT3). The germline R561C mutant was less active than the N666K mutant whereas the P660T mutant showed no difference with the wild-type receptor. Moreover, in foci formation assays with NIH3T3 cells, we observed that the N666K mutant and, to a lesser extent, the R561C mutant were able to transform cells, unlike the P660T mutant. Interestingly, both activated mutants, namely R561C and N666K, were sensitive to imatinib (Gleevec®). Altogether, our results indicate that two of the three reported FIM-associated mutations, R561C and N666K, activate PDGFRB, supporting the hypothesis that these mutations cause the disease. The lower activity of the R561C mutant compared to N666K is consistent with the fact that the first mutation is a germline one whereas the second mutation is somatic. Moreover, imatinib seems to be a promising treatment for patients with visceral tumors carrying these two mutations. To our knowledge, these are the first confirmed gain-of-function point mutations of *PDGFRB* in human cancer.

Keywords: infantile myofibromatosis, PDGF receptor.

TUE-109
Gastrointestinal cancer risk associated with PARP1 (ADPRT) Val762Ala polymorphism

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Poly (ADPribose) polymerase 1 (PARP-1) is a DNA double strand break recognizing protein. and its activation is one of the early responses to DNA damage. PARP-1 gene consists of 23 exons and spans about 47.3 kb which is localized on chromosome 1q41–42. Several SNPs have been found in PARP1 gene, but only for the Val762Ala (rs1136410) a functional analysis has been performed. PARP-1 Val762Ala was well-known to be associated with increased risk of several cancers. Colorectal cancer is one of the most common human malignancies in the world.

A total of 120 paraffin-embedded colorectal cancer and normal specimens were obtained from department of pathology in Cerrahpasa Medical Faculty. These normal tissues were used from same cases as a control group. Archival formalin-fixed, paraffin wax-embedded tissue was used. A single block representative of the tumor was selected in each case and the same block was used for PCR-RFLP. Tissue sections (10 μm) were prepared from formalin fixed, paraffin-embedded colorectal tissues. The sections were stained with hematoxylin and eosin (HE) for histologic examination. Tissue sections were dehydrated in a graded ethanol series, and then dried without a cover glass. Tissue samples from 60 tumors and corresponding 60 normal tissues were cut with sterile needles, and the DNA was extracted with 50 μl extraction buffer (100 mmol/L Tris-HCl, pH 8.0, 2 mmol/L EDTA, 400 μg/mL proteinase K) at 55°C overnight. The tubes were boiled for 15 min to inactivate proteinase K, and 5 μl of each extract was used for polymerase chain reaction (PCR). The primers used in the amplification were: PARP-F -TTTGCTCCTCCAGGCCAACG-3, and PARP-R 5-CATCGATGGGA TCCTTGCTGCT-3, which produce a 110-bp fragment. Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method was used to detection of the polymorphism in PCR products by digestion with 10 units of Acc II restriction endonuclease. Fragmented PCR products, stained with ethidium bromide, were analyzed after agarose gel electrophoresis. All analyses were performed using the Statistical Package for the Social Sciences (SPSS).

Overall, this updated our previous study allowed us to provide a more precise relative risk estimate regarding the association between PARP1 Val762Ala polymorphism and cancer susceptibility. These findings suggested that the PARP1 Val762Ala polymorphism may play a role in gastrointestinal cancer development.

Keywords: Colorectal Cancer, PARP1, Polymorphism.

TUE-110
Genome wide investigation of genes regulated by Beta-catenin in AML

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Acute Myeloid Leukemia (AML) is a disease characterized with impairment of normal hematopoetic proliferation, differentiation and accumulation of abnormal immatured myeloid cells in bone marrow and periferic blood which occurs as a result of genetic changes in early hematopoietic stem cells. Wnt/β-catenin signaling is an evolutionarily conserved signal transduction pathway that governs cell fate decisions during embryogenesis at the stem cell level. Wnt signaling which is known to be activated both in the early embryonic period and various cancer types has been taking serious attention because it is known that lots of genes in this pathway causes neoplastic transformation. β-catenin plays the central role in this pathway. β-catenin is a cytoplasmic protein which translocates to the nucleus via Wnt signal activation. Thus it activates transcription of lots of genes most of which are oncogenes. ChIP-chip is a new technology which combines chromatin immunoprecipitation (ChIP) and microarrays (chip) to determine DNA-protein interactions in cells. It has become an ideal approach to define genome-wide transcription factor targets or chromatin modification regions. In this study, genome-wide promotor binding regions of β-catenin translocated to nucleus was identified by ChIP-chip method. For this purpose, CD34 + hematopoietic stem cells of AML patients with high β-catenin gene expressions were cultured and genome-wide promotor binding sites of β-catenin were determined. It was found that β-catenin binded approximately 16.000 target genes in AML patients and 8000 target genes in the control group. The results showed all probable genome-wide targets of β-catenin protein in AML for the first time.

Keywords: AML, β-catenin, ChIP-chip.

TUE-111
Growth hormone-releasing hormone (GHRH) activates E-cadherin/β-catenin signalling pathway in human prostate cells

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Growth hormone-releasing hormone (GHRH) and its receptors have been implicated in a variety of cellular phenotypes related with tumorigenesis. The E-cadherin/β-catenin complex is involved in the regulation of processes including growth, migration and adhesion in various cancers. The aim of this work was to study

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the involvement of GHRH in E-cadherin/β-catenin signalling pathway. We used three human cell lines: non-tumour prostate epithelial cells (RWPE-1) and prostate cancer cells (LNCaP, androgen-dependent; PC3, androgen-independent). GHRH (0.1 μM) significantly decreased E-cadherin levels, following a time-dependent pattern, in LNCaP and PC3 cells but not in RWPE-1 cells. Conversely, GHRH up-regulated the expression of β-catenin in tumour cells but not in non-malignant cells. In addition, we analysed the subcellular localization of β-catenin in PC3 cells. The results show that the decrease of the cytoplasmic expression of β-catenin is linked to increased protein level into the nucleus after treatment with neuropeptide. The increased level of β-catenin protein into the nuclei of PC3 cells may induce the transcription of target genes involved in the progression of cell cycle. Thus, we assessed the mRNA expression levels of CD44, c-myc, and cyclin D1 in GHRH-treated PC3 cells. Results show that GHRH increased mRNA levels of the three genes evaluated in a time-dependent manner. The present findings shed more light on the involvement of GHRH and E-cadherin/β-catenin signalling pathway in prostate carcinogenesis process.

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Keywords: E-CADHERIN, GHRH, prostate cancer.

TUE-112
Growth hormone-releasing hormone (GHRH) and GHRH antagonists JMR-132 and JV-1-38 modulate metastatic processes in human prostate cells

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Prostate cancer accounts with 29% of all new cancer cases and is the second leading cause of cancer-related deaths among men in the Western world. New treatment options are needed to prevent the progression of prostate cancer toward androgen-independent stage. The aim of this work was to compare the expression of GHRH receptors (pGHRHR) and their splice variants (SVs) as well as the effect of GHRH and its antagonists (JMR-132 and JV-1-38) on viability, proliferation and adhesion in human prostate cells. We used two cell lines: non-tumor human prostate epithelial cells (RWPE-1) and androgen-dependent prostate cancer cells (LNCaP). Higher expression levels of pGHRHR and vice versa for their SVs were detected in LNCaP cells as compared with those in RWPE-1 cells using Western blot analyses and immunocytochemistry. We subjected the cells to a mitochondrial enzymatic (methylthiazoletetrazolium; MTT) assay and a bromodeoxyuridine (BrdU)-incorporation assay in order to assess their viability and proliferation, respectively. After treatment with GHRH (0.1 μM), significant increases of viability and proliferation were observed in both types of cells. Conversely, viability was significantly decreased after treatment with GHRH antagonists (0.1 μM) in RWPE-1 cells as well as after treatment with JMR-132 in LNCaP cells. In addition, GHRH antagonists induced a significant decrease in the proliferation of LNCaP cells. Cell adhesion to collagen decreased after exposure to GHRH, while it was augmented after treatment with GHRH antagonists, JMR-132 and JV-1-38.

The antagonists used block GHRH receptors and, in turn, reduce tumor progression induced by autocrine/paracrine mechanisms of GHRH. The results shed light on the effect of GHRH and its antagonist in non-tumor prostate cells and androgen-dependent prostate cancer cells. These findings support the effectiveness of JMR-132 and JV-1-38 on proliferation and adhesion in androgen-dependent prostate cancer.

This work was supported by the Junta de Comunidades de Castilla-La Mancha [grant number PII10-0189-3222 to A.M.B.] and by the University of Alcalá [FPU/UAH grant to L.M.M.].
Keywords: GHRH, GHRH antagonist, prostate cancer.

TUE-113
GSK-3beta kinase controls Notch3 protein stability and subcellular localization in EMT phenotype acquisition of cancer cells

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Notch receptors participate in a highly conserved signalling pathway that regulates different cellular processes including cell proliferation, differentiation, apoptosis and survival. Deregulated Notch signaling has been implicated in the development of many diseases, including cancer. Moreover, increasing evidence correlates Notch pathway with drug-resistance of many tumors, through different mechanisms including the acquisition of the epithelial-mesenchymal transition (EMT) phenotype, which is critically associated with tumor invasion and metastatis. In EMT, epithelial cells acquire fibroblast-like properties, reduced intercellular adhesion and increased motility. This process is associated with the functional loss of E-cadherin. Down-regulation of Notch signalling leads to partial reversal of the EMT phenotype, through the decreased expression of members of the Snail family of transcription factors, known to repress E-cadherin expression. Recent evidence reports that the specific activation of Notch3 (N3) in ovarian cancer cells causes a spindle and fibroblast-like morphology, induces the expression of smooth muscle α-actin, Slug and Snail and decreases the expression of E-cadherin, indicating that Notch3 activation is able to induce EMT in ovarian cancer cells. However, the general mechanisms that govern the acquisition of the EMT phenotype in cancer cells remain to be elucidated. We have previously demonstrated that Notch3 protein expression and/or stability can be modified by different post-traslational modifications and this is correlated with the regulation of leukemia development and progression. However, the mechanisms that modulate its expression levels, subcellular localization and function remain still elusive, even in solid tumors. Here, we demonstrate that N3 protein is regulated by Glycogen synthase kinase-3β (GSK-3β), a widely expressed and highly conserved serine/threonine protein kinase that plays important roles in several signaling pathways, including Notch signaling. Preliminary results obtained by using insertional mutants of N3 show that GSK-3β is able to interact with and to phosphorylate N3 at specific consensus motifs located inside its intracellular domain ($N3^{IC}$), leading to a regulation of $N3^{IC}$ function in terms of both protein stability and subcellular localization. Our subsequent studies will be focused to understand how the observed Notch3/GSK-3β relationship could be involved in controlling the EMT phenotype associated to tumour invasion and metastasis of breast cancer, in the purpose to improve future strategies for developing effective cancer treatments.

Keywords: Cancer signaling, degradation.

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TUE-114
Haponin promotes cell death in oxidative stress by upregulating p53 transcriptional activity

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The excessive production of "reactive oxygen species" (ROS) in the cell results in nonhomeostatic state referred to as "oxidative" stress. ROS generated in response to endogenous or environmental stimuli have been shown to be implicated in aging, carcinogenesis, and the progression of various pathologies, such as cardiovascular diseases, neurodegenerative disorders, and inflammatory disorders.

Previously we have shown that cellular sensitivity to the oxidative stress directly correlates with the expression level of nuclear protein haponin [1]. Given the involvement of haponin in the cell death under the oxidative stress conditions [2] in conjunction with the previously demonstrated haponin ability to bind GAPDH [3] we have suggested that haponin appears to be involved in GAPDH-mediated p53-dependent apoptosis induced by oxidative stress [4].

Using the panel of cell lines with the altered levels of haponin expression we showed that silencing of haponin expression with siRNA decreased cell death induced by oxidative stress. On the contrary, haponin overexpression promoted cell death under oxidative conditions. Furthermore, we have demonstrated that in oxidative stress nucleus condensation and nucleosomal ladder accumulation are dependent on the hapopnin expression level suggesting that haponin modulates apoptotic cell death. We have found that haponin overexpression stabilizes p53 in the cells and increases its presence in the nucleus. In addition we have shown that haponin siRNA inhibits p53 transcriptional targets activation induced by oxidative stress. Taken together these data indicate that haponin is involved in the regulation of p53 transcriptional activity supposedly through GAPDH-mediated acetylatin of p53.

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Keywords: Haponin, Oxidative stress, p53-mediated apoptosis.

TUE-115
HBP1 is a new target of the PI3-kinase – PKB – FOXO pathway downstream growth factor receptors

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Growth factors, such as the Platelet-derived growth factor (PDGF) or the Fibroblast-growth factor (FGF), induce cell proliferation, migration and differentiation through the activation of different signaling mediators such as the phosphatidylinositol-3 kinase-AKT pathway. Downstream growth factor receptors, AKT inhibits the activity of the Forkhead box O transcription factors (FOXO1, FOXO3, FOXO4 and FOXO6) and represses their expression. The transcription factors FOXO are involved in cell cycle arrest, DNA damage repair, apoptosis and resistance to oxidative stress. They also play an important role in the control of cell proliferation in response to PDGF. Our results showed that FOXO regulated the HMG-box protein 1 (HBP1) expression in different cell types including normal fibroblasts and cancer cells. HBP1 is a transcription factor that plays a role in the control of cell cycle, in premature senescence induced by an oncogene and in cell differentiation.

The analysis of different microarray data led us to the identification of HBP1 as a target gene downstream of the growth factors – PI3K – AKT - FOXO pathway: its expression was down-regulated by PDGF, FGF4 and by two oncogenic forms of the PDGF receptors (Fip1L1-PDGFRα and TEL-PDGFRβ), by constitutively-activated PI3K and AKT, and it was up-regulated by FOXO3 and FOXO4. These results were confirmed by quantitative RT-PCR and Western blot in several cellular models. To further investigate the regulation of HBP1 by FOXO, we analyzed the HBP1 promoter and we identified a potential FOXO-binding site. We cloned the promoter of HBP1 in a luciferase reporter vector and we showed that FOXO1, FOXO3 and FOXO4 induced the activity of HBP1 promoter. We confirmed the localization of the binding site by mutating two residues, which abolished the promoter activity in response to FOXO. The binding of FOXO on HBP1 promoter was then confirmed by chromatin immunoprecipitation (ChIP) and by ChIP-sequencing. Using two HBP1-specific shRNA, we showed that HBP1 played a role in cell proliferation in normal fibroblasts: indeed, HBP1 down-regulation promoted cell proliferation. Finally, HBP1 and FOXO1 expression was decreased in breast cancer. To investigate a possible link between FOXO and HBP1 in these tumors, we analyzed data from The Cancer Genome Atlas (TCGA) and showed that tumors with decreased FOXO1 expression expressed less HBP1, in agreement with our data.

In conclusion, our study identifies HBP1 as a mediator of the PI3K-AKT-FOXO pathway and a regulator of proliferation downstream growth factor receptors in normal and tumor cells.

Keywords: Cancer signaling, PDGF receptor, transcription factor.

TUE-116
Heparan sulfate proteo glycanbiosynthesis enzymesas potential markers for the differential diagnosis of brain tumors

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Glioblastoma, the most common primary brain tumor, is characterized by high invasive potential and short survival. It is known that invasion depends on tumor microenvironment characteristics. Proteoglycans, complex glycosylated molecules, are key components of tumor microenvironment, playing critical role in cell-cell and cell-matrix interactions. Malignant brain tumors have individual proteoglycan profiles, which are closely associated with their differentiation and biological behavior. One of the causes of

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proteoglycans profiles changes may be dysregulation of their biosynthetic system.

In this study we examined the expression pattern of heparan sulfate proteoglycan metabolic enzymes in human brain tumors by RT-PCR and immunohistochemical analysis. Expression level of the most of the enzymes (EXT1/2, NDST1/2, OST1, SULF2) were consistent for different patients. But, interestingly, we showed the significant patient-to-patient variability of GLCE, SULF1 and HPSE expression, enzymes which have been shown to affect proliferation and invasion characteristics of tumors. We showed loss of GLCE expression for 70% of the patients. All of the anaplastic astrocytoma samples were characterized by loss of GLCE and SULF1 expression, but did not change HPSE expression. Analysis of glioblastoma patients showed consistent level of SULF1 expression and heterogeneity of GLCE and HPSE expression. Analysis of expression of these three enzymes revealed discrete groups of patients that can be associated with the clinical prognosis.

From these results, we suggest a new approach for the individualization of brain cancer diagnostics based on the expression pattern of heparan sulfate proteoglycan biosynthesis and degradation enzymes. It will result in more accurate diagnosis and personalized therapeutic strategy for brain cancer.

Keywords: glioblastoma, microenvironment, proteoglycans.

TUE-117
Hepatitis C virus suppresses HNF4α expression and modulates HNF4α-miR feedback circuit

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Hepatitis C Virus (HCV) infection presents with a disturbed lipid profile and can evolve to hepatic steatosis and hepatocellular carcinoma (HCC). Hepatocyte Nuclear Factor 4α (HNF4α) is the most abundant transcription factor in the liver and the master transcription factor in hepatic lipid metabolism. We have previously shown that transient inhibition of HNF4α initiates transformation of immortalized hepatocytes through a feedback loop consisting of miR-24, miR-629, IL6 receptor (IL6R), STAT3, and miR-124, suggesting a central role of HNF4α in HCC. To investigate the effect of HCV replication on HNF4α circuit, Huh7-Lunet cells were electroporated with JFH1 isolate (genotype 2a). During the time course of HCV replication a decrease was observed in both RNA and protein levels of HNF4α. The decrease was also reflected in the downregulation of downstream targets, HNF1α and MTP (Microsomal Triglyceride Transfer Protein). HCV also up-regulated IL6R and activated STAT3 protein phosphorylation. Furthermore, the inverse correlation between HNF4α and miR-24 or miR-629 expression levels in HCV RNA electroporated cells was confirmed. Suppression of HNF4α expression was also validated in Huh7.25/CD81 cells inoculated with JC1 virus strain (genotype 2a) at m.o.i.: 2 (multiplicity of infection). HCV nucleocapsid core protein apart of its role in capsid formation is responsible for many virus-host interactions including usurping the host lipid metabolism mainly by modulating the activity of transcription factors involved in hepatic lipid metabolism. We therefore evaluated the effect of HCV core protein on the expression of HNF4α. Core protein overexpression also reduced HNF4α protein levels implying that HCV might exert its effect through core. Finally, the possible effect of viral proteins, core, non-structural (NS) 5A protein and the whole NS region (NS3-NS5B) on HNF4α promoter activity is being investigated using HNF4α promoter-luciferase constructs. Our findings demonstrated that HNF4α-miR circuit operates in HCV infection and confirm a pivotal role of HNF4α in HCC.

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program “Education and Lifelong Learning” of the National Strategic Reference Framework (NSRF) - Research Funding Program: Thales (MIS 379465). Investing in knowledge society through the European Social Fund.

Keywords: HCV, HNF4α, liver cancer.

TUE-118
HNF4α silencing induced epithelial-to-mesenchymal transition (EMT) in 3D in vitro models of liver cancer

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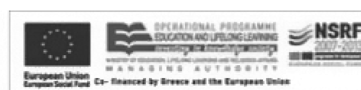
Hepatocellular Carcinoma (HCC) represents the third most common cancer in mortality worldwide. The deregulation of the molecular signaling network of HNF4α-miR24, plays an important role in the mechanism of hepatocarcinogenesis. We aimed to study the process of Epithelial-to-Mesenchymal transition (EMT) in stable monoclonal HNF4α knock down (KD) liver cancer cells. We established monoclonal liver cancer cell lines (HepG2, Huh7) with different expression levels of HNF4α (KD) using lentiviral transduction methods followed by analysis of HNF4α expression levels in RNA (Real-Time qPCR) and protein (Western). HNF4α_KD cells were then cultured as multicellular spheroids, which better recapitulate the 3 Dimensional (3D) tissue microenvironment *in vitro*.

Quantification of EMT markers revealed a correlation of increased HNF4α_KD with increased expression of mesenchymal (N-cadherin, Vimentin), reduced expression of epithelial markers (E-cadherin) and altered expression of EMT master inducers (SNAIL, ZEB1/2, TWIST). Clones were further analyzed for: size of 3D spheroids, cellular context by immunohistochemistry for hepatic protein markers (a-fetoprotein, HEPAR1), viability (Ki67), and apoptosis (Live/Dead Assay) and changes in migratory and metastatic ability (Scratch Assay, Anchorage Independence Growth Assay, Invasion Assay), respectively. Similar gene expression profiles were analyzed in 3D *in vitro* models of HNF4α silencing by transient overexpression of miR24 in liver cancer cells followed by validation of protein expression by immunohistochemistry. Furthermore, we observed for the first time the induction of the Notch and TGFβ signaling pathways in these cellular models by Real Time qPCR Arrays, thus contributing to induction of EMT. The data collectively indicate that reduced expression of HNF4α induces EMT in liver cancer cells.

This research has been co-financed by the European Union (European Social Fund-ESF) and Greek national funds through the Operational Program “Education and Lifelong Learning” of the National Strategic reference Framework (NSRF)- Research



This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Thales (MIS 379465). Investing in knowledge society through

Fig. 1.

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Keywords: EMT, HNF4α, liver cancer.

TUE-119
HNRNPK protein profile in acute promyelocytic leukemia cell lines (NB4 and NB4-R2): role in cell differentiation and survival

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Heterogeneous nuclear ribonucleoprotein K (hnRNPK) participates in several processes such as chromatin remodeling, transcription, RNA splicing and translation. Recently, hnRNPK has been considered a prognostic marker in different types of cancer and implicated in the progression of chronic myeloid leukemia. The role of hnRNPK in leukemogenesis and cell differentiation in acute promyelocytic leukemia (APL) has not been established. APL is mainly caused by t(15,17), which generates an oncoprotein capable of blocking the differentiation of myeloid progenitors at the promyelocyte phase. Treatment with all-trans retinoic acid (ATRA) restores the process of cell differentiation, but fails to produce the same effect in patients with syndrome of resistance to ATRA. The aim of this study was to establish the role of hnRNPK in the APL using NB4 and NB4-R2 cell lines, which are responsive and resistant to the treatment with ATRA, respectively, as well as a possible role of hnRNPK in ATRA-induced cellular differentiation. Analysis by western blotting revealed that hnRNPK level in NB4 cells is lower than in NB4-R2 after ATRA treatment (96 h). In addition, changed the subcellular location of hnRNPK in NB4 cells increasing the isoform b in the cytoplasm in association with cellular differentiation, as evidenced by increase in the Cd11b marker; this suggests a role of hnRNPK in ATRA-induced cellular differentiation. Both cell lines revealed higher level of hnRNPK than control samples (bone marrow, polymorphonuclear cells obtained from donors); this reinforce that the increase of hnRNPK protein is involved in leukemogenesis of APL. The knockdown of hnRNPK (shRNA) in NB4 e NB4-R2 cells promoted massive loss of cell viability due to induction of apoptosis; this suggests that hnRNPK is essential for NB4 and NB4-R2 cell survival and maintenance. NB4 and NB4-R2 cells with knockdown of hnRNPK revealed a decrease in hnRNPK downstream targets such as c-Myc, an oncogene, and Bcl-xL, an anti-apoptotic protein. Our findings appoint hnRNPK protein as up-regulated in APL and suggest its involvement with differentiation and survival.

Financial Support: FAPESP, CEPID-FAPESP, CAPES and CNPq, Brazil.
Keywords: APL lineages, differentiation, hnRNPK.

TUE-120
HOXB5 promotes the proliferation and invasion of breast cancer cell

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HOX transcription factors play an important role in determining body patterning and cell fate during embryogenesis. There has been accumulating evidence that these genes act as positive or negative modulators in many types of cancer, including breast cancer, in a tissue-specific manner. We have reported earlier that HOXB5 is aberrantly overexpressed in certain breast cancer cell lines and breast cancer tissues. Here we investigated the biological role and clinical relevance of HOXB5 in breast cancer. Immunohistochemical analysis of HOXB5 on tissue microarray (TMA) including 34 normal and 62 breast cancer (invasive ductal carcinoma) specimens revealed that HOXB5 was highly expressed in cancer tissues, particularly from ER-positive breast cancer patients. An online survival analysis confirmed the correlation between HOXB5 expression and poor distant metastasis-free survival in ER-positive, but not in ER-negative, breast cancer. In vitro study indicated that HOXB5 silencing in T47D cells significantly decreased cell proliferation and anchorage-independent cell growth. In contrast, MCF7 cells overexpressing HOXB5 displayed EMT characteristics with a greater invasive ability, cell proliferation and colony formation in soft agar. In conclusion, we suggest that HOXB5 acts as a positive modulator most likely by promoting cell proliferative response and invasiveness in ER-positive breast cancer.

Keywords: breast cancer, ER-positive, HOXB5.

TUE-121
Human glioblastoma cells are auxotrophic for arginine and sensitive to recombinant arginase I (Co)-PEG5000 [HuArgI (Co)-PEG5000]-induced arginine depletion

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In this study, we attempt to target arginine auxotrophy in glioblastoma (GBM) cells using a recombinant human Arginase I cobalt [HuArgI(Co)] coupled with polyethylene glycol 5000 [HuArgI (Co)-PEG5000].

HuArgI (Co)-PEG5000 was tested on a panel of 9 human GBM cell lines (U251, U87-MG, T98, U118, Hs683, SF, A172, H4 and SW1088) and on normal human fetal glial cells (SVG p12).

All GBM cell lines tested were sensitive to HuArgI (Co)-PEG5000-induced arginine depletion with IC_{50} values ranging from 128-985 pM and a percent cell kill > 80%. Addition of exogenous L-citrulline failed to rescue cells from arginine depletion-induced cytotoxicity at doses of 1.14, 11.4 and 114 μM and led to the rescue of only 6 GBM cell lines at the highest concentration of 11.4 mM, reflecting the extent of arginine auxotrophy in GBM cells. The ability of excess L-citrulline to rescue cells was dependent on the expression of argininosuccinate synthetase-1 (ASS1) with the 3 cell lines that were not rescued by L-citrulline lacking ASS1 expression. In addition, siRNA Knock down of ASS1 reversed the ability of L-citrulline to rescue GBM cells from argi-

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nine depletion-induced cytotoxicity, further illustrating that the lack of ASS1 expression is the underlying mechanism of complete arginine auxotrophy in these cells. Normal human fetal glial cells, on the other hand, expressed ASS1 and were not sensitive to the HuArgI (Co)-PEG5000-induced arginine depletion, demonstrating the selective toxicity of arginine depletion to GBM cells. Inhibition of autophagy increased GBM cell sensitivity to HuArgI (Co)-PEG5000 indicating that, under arginine deprivation, autophagy plays a protective role in GBM. Analysis of the type of cell death showed a lack of AnnexinV staining and caspase activation, in addition to positive PI staining and a high degree of fragmentation following HuArgI (Co)-PEG5000-induced arginine depletion. This demonstrates that arginine deprivation induces caspase-independent, non-apoptotic cell death in GBM cells.

In this study, we demonstrate that GBM cells are auxotrophic for arginine and are, subsequently, sensitive to HuArgI (Co)-PEG5000-mediated arginine depletion. We also demonstrate that lack of ASS1 expression is the underlying mechanism for complete arginine auxotrophy in GBM cells. Arginine depletion is, therefore, a novel targeted therapy for the potential treatment of GBM.

Keywords: Arginase, Autophagy, GBM.

TUE-122
Human metastatic melanoma cells secrete pro-apoptotic 15d-PGJ2, when stressed with HMG-CoA reductase inhibitor, simvastatin

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Advanced stage melanoma treatment is still a challenge. Autocrine and paracrine signalling plays a crucial role in tumour cell communication, involving small immunogenic molecules like cytokines or prostaglandins. The atypical prostaglandin 15-deoxy-12,14-prostaglandin J_2 (15d-PGJ_2) is an endogenous agonist at the peroxisome proliferator-activated receptor gamma mediating inhibition of the NFkB pathway. Moreover, 15d-PGJ_2 shows anti-tumour activity in melanoma by suppression of metastasis and interaction with the tumour microenvironment.

Here we show that simvastatin evokes apoptosis in human metastatic melanoma cells in a p38 and COX2 dependent manner. Moreover, inhibition of the lipocalin prostaglandin D synthase also prevented activation of the mitochondrial pathway of apoptosis and guided us to the identification of 15d-PGJ_2 as the responsible mediator. Simvastatin treatment resulted in significant concentrations of 15d-PGJ_2 in the medium and cytosol of the melanoma cells, sufficient to activate caspase 8 and 9. Application of 15d-PGJ_2 triggered ROS formation and caspase 9 activation, which were grossly prevented by ROS scavenger N-acetylcysteine. Finally, 15d-PGJ_2 also activated caspase 8 and thereby confirmed 15d-PGJ_2 as the postulated suicide factor triggered by simvastatin in human metastatic melanoma cells.

Given the fact that statins are well tolerated, our results provide novel evidence and supportive rational for usage in adjuvant cancer therapy.

This work was supported by Herzfelder′sche Familienstiftung and the Austrian Science Foundation FWF (P-22385).
Keywords: 15d-PGJ2, apoptosis, melanoma.

TUE-123
Hypoxia impacts on adenosine metabolism and chemoresistance in glioblastoma stem like cells

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Background: Glioblastoma multiforme (GBM) is the most common nervous system primary tumour with one of the worst prognosis of all cancers. Consistent studies have shown glioblastoma stem-like cells (GSCs) to be the tumourigenic population in GBM. Further, their particular properties are required for the extreme chemoresistance of this tumour. One of the most important mechanisms conferring chemoresistance is the overexpression of the ATP-binding cassette (ABC) family transporters, which extrude anticancer drugs from tumour cells, essentially Mrp1 in GBM. We have identified the nucleoside adenosine as a key endogenous modulator of Mrp1 in GBM cells. In addition, it has been recognized that hypoxia promotes therapeutic resistance in GBM. Consequently, our aim was to determine the role of hypoxia on both the adenosine production and the chemoresistant phenotype in GSCs.

Methods: Glioblastoma Stem-like Cells were derived from U87 cell line. Hypoxia was obtained in a chamber at 1% oxygen. The contents of Mrp1, Mrp3, adenosine A_3 receptor and 5′-ectonucleotidase were measured by western blots, flow cytometry and immunofluorescence. Mrp1 activity was estimated measuring the extrusion of the fluorescent substrate CDFA from cells. Adenosine levels were quantified in Tyrode's buffer in regulated oxygen conditions. 2-chloroacetaldehyde derivatives were quantified by HPLC. Cell viability was assayed by the MTT reduction method and flow cytometry. Cells were treated with AOPCP (50 μM, inhibitor of 5′-ectonucleotidase) and MRS1220 (10 μM, selective antagonist of adenosine A_3 receptor).

Observations: We quantified an increased capability for extracellular adenosine production in GSCs compared to differentiated cells. Correspondingly, we determined a higher expression of the 5′-ectonucleotidase, the rate limiting enzyme for adenosine production, and the adenosine A_3 receptor in GSCs under hypoxic conditions (20% vs 1% oxygen). We also found that the expression of the ABC transporters Mrp1 and Mrp3 were influenced by hypoxia. The expression and activity of Mrp1 were reduced under the treatment with a 5′-ectonucleotidase activity inhibitor or an adenosine A_3 receptor selective antagonist. Importantly, the viability of GSCs decreases by 50% under the vincristine treatment, an antitumour drug substrate of Mrp1, when combined with the antagonist of the A_3 receptor.

Conclusions: We have demonstrated the role of the purinergic signaling on the chemoresistance of GSCs. Furthermore, we validated *in vitro* the efficacy of targeting adenosine A_3 receptor to sensitize GSCs to antitumour drugs substrates of Mrp1. We propose a new therapeutic alternative to the treatment of this tumor targeting the GSCs chemoresistance.

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Keywords: hypoxia, adenosine, glioblastoma stem like cells.

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TUE-124
Ibulocydine sensitizes human cancers to radiotherapy by induction of mitochondria-mediated apoptosis

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Background and Purpose: Ibulocydine (IB), a novel prodrug of CDK inhibitor, has been reported to have anti-cancer effect in human hepatoma cells. In order to address its feasibility as a radiosensitizer to improve radiotherapeutic efficacy for human cancers, this study was designed.
Material and Methods: Human cancer cells of lung and colon were treated with IB and/or radiotherapy (RT). The cellular effects were assessed by CCK-8, clonogenic, flow cytometric, and western blotting assays. *In vivo* radiotherapeutic efficacy was evaluated using xenograft mouse model.
Results: Combined treatment of IB and RT significantly reduced viability and survival fraction of the cells. Apoptotic cell death accompanied with activation of caspases, decrease of Bcl-2/Bax expression, loss of mitochondrial membrane potential (MMP) leading to release of cytochrome *c* into cytosol was observed. Recovery of Bcl-2 expression level by introducing Bcl-2 expressing plasmid DNA compromised the loss of MMP and apoptosis induced by IB and RT. *In vivo* therapeutic efficacy of combined treatment was verified in the xenograft mouse model, in which tumor growth was markedly delayed by RT with IB.
Conclusions: IB demonstrated the property sensitizing human cancer cells to RT by induction of mitochondria-mediated apoptosis, suggesting that IB deserves to be applied for chemoradiotherapy.
Keywords: Ibulocydine, radiotherapy, apoptosis.

TUE-126
Identification of genes in Wnt signaling pathway with DNA microarray analysis in acute myeloid leukemia

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Introduction: Acute myeloid leukemia(AML)is a clonal disease resulting from a malignant transformation of a hematopoetic stem or progenitor cell.Recently,many studies confirmed that dysregulations in the Wnt/b-catenin signaling have been identified in AML cases.About gene expression levels of the b-catenin,which is the key element of Wnt pathway,carried out many studies in the literature and variations in b-catenin gene expression levels have been identified among AML patients.It is aimed to identify the related genes with b-catenin and the downstream pathways in AML patients through determining the differences between the AML patients classified according to gene expression levels of b-catenin and healthy control groups.
Materials and Methods: This study has been performed on 19 AML patients and 3 healthy individuals.RNA was extracted from CD34 + hematopoietic cells using Trizol reagent.QRT-PCRassays were used to determine the expression of β-catenin.RNA was used to generate cRNA,subsequently hybridized to HGU133Aplus2.0arrays according to the Affymetrix Genechip Expression Analysis Technical manual.
Results: We found that β-catenin was overexpressed in 8 out of 19 AML patients in compared with controls($p < 0.05$).β-catenin levels in AML samples were determined to be varied.According to these results in patients with high and low β-catenin compared with controls of gene profiles,it has been reached different conclusions.
Discussion: It is the first study to identified the transcriptome profile in the AML cases which is divided into two groups depending on the b-catenin levels.In this study,detection of b-catenin related genes and pathways will put forward an important data about the molecular biology of AML as well as providing diagnostic,prognostic and therapeutic new candidate molecules.
Keywords: AML, beta catenin, microarray.

TUE-127
Identifying modifiers of Wnt and EGFR/RAS/MAPK signaling through quantitative *C. elegans* genetics

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A number of studies aim at investigating the origin and potential treatment of monogenic diseases. However, polygenic diseases arising from an interplay between several malfunctioning signaling pathways are the predominant cause of up to 60% of deaths in the human population. Quantitative genetic approaches provide a powerful tool to elucidate genetic risk factors underlying such complex diseases.

We use two isolates of *C. elegans*, N2 Bristol and CB4856 Hawaii to study the influence of naturally occurring polymorphisms on mutant genetic backgrounds well-known to promote diseases such as cancer. We chose the development of the *C. elegans* vulva as the phenotypic readout of Wnt and EGFR/RAS/MAPK signaling activity, where changes in signaling result in either a multivulva or vulvaless phenotype that can be quantified at single-cell resolution.

Previous work has established comprehensive QTL maps spanning the entire genome. We generated these maps by comparing an N2 strain carrying a mutation in *ras/let-60* or β-*catenin/bar-1* to animals carrying the *let-60* or *bar-1* mutation in a mixed N2/CB4856 background (so called mutation included recombinant inbred lines, miRILs). Interestingly, one QTL on the first chromosome (LGI) is shared between the two mutant backgrounds suggesting a link between Wnt and Ras signaling. We are currently identifying polymorphic modifier genes of the two pathways. To this aim, we narrow down a genomic region containing the QTL by generating N2 strains carrying well-defined CB4856 introgressions and by performing RNAi knockdown of candidate genes followed by mutant analysis.
Keywords: EGFR/RAS/MAPK, quantitative genetics, Wnt.

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TUE-128
IDH1 mutations in a Turkey series of primary glioblastoma

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Purpose: To establish the frequency of IDH1 mutations in primary glioblastomas at a population level.
Experimental Design: We screened primary glioblastomas in a population-based study for IDH1 mutations and correlated them with clinical data.
Results: IDH1 mutations were detected in 5 of 40 primary glioblastomas (12,5%). Primary GBM patients carrying IDH1 mutations were significantly younger, mean age of 41 ± 5.06 years, than patients with wild-type IDH1, mean age of 57 ± 2.29 years, p = 0.011. The mean survival time of all GBM patients with and without IDH1 mutations was 19 months (5 cases) and 16 months (35 cases), respectively (P > 0.05).
Conclusion: In summary, our study is the first study to investigate the IDH1 status in primary GBMs in Turkish patients and confirmed IDH1 mutation as a genetic marker for GBM, and therefore as complementary information to help predict to the outcome of patients with GBM. We have shown consistent IDH1 status and younger age and lack of association between IDH1 mutation and survival time.
Keywords: Glioblastoma Multiforme, IDH1 mutation, Turkey.

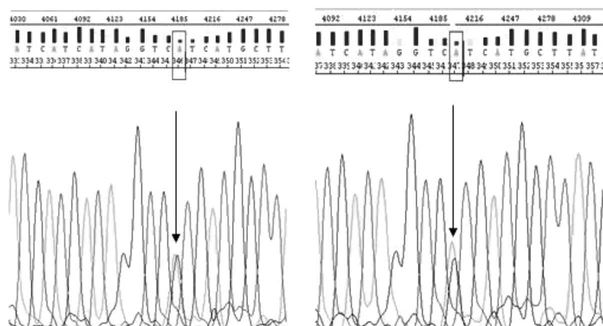

Fig. 1.

TUE-129
IDH2 mutations in primary glioblastoma

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Purpose: To establish the frequency of IDH2 mutations in primary glioblastomas at Turkısh population level. In this study GBM patients studied with IDH1 gene mutation analysis was performed samples. Evaluated IDH2 gene mutations, compared with clinicopathological features.
Experimental Design: We screened primary glioblastomas in a population-based study for IDH2 mutations and correlated them with clinical data. We studied the frequency of IDH2 mutations in a series of 40 primary GBM patients from the Turkish population according to patient age, gender, GBM type and survival time. Tumor samples were collected during surgical procedures by the Department of Neurosurgery of Eskisehir Osmangazi Uni-

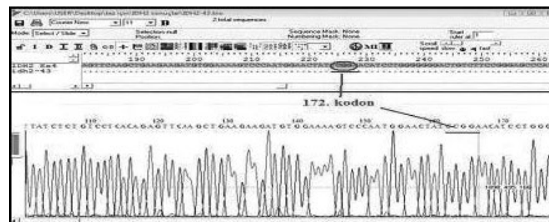

Fig. 1. Fluorescent in situ hybridization (FISH) reveals a split of red and green probe in an *ALK*-positive tumor.

versity. The samples included frozen tissues, collected upon surgical removal. DNA was extracted from the frozen tissues by Magna Pure Compact DNA isolation kit. Polymerase chain reaction (PCR) followed by DNA sequencing was applied to detect IDH2 mutation. The mean age of 40 GBM patients was 54,89 ± 3.34 years, with 51,71 ± 3,98 females and 58,07 ± 2,71 males. A total of 40 cases were primary GBMs. Calculations were performed using SPSS 15.0 software (SPSS, Chicago, IL, USA), with statistical significance of $p < 0.05$.
Results: No IDH2 gene mutation was detected in tumor samples. Since the analysed tumors were the primary GBMs, the data of undetected IDH2 gene mutation was in agreement with the literature. All of our cases, the average survival time of 17 months, respectively.
Conclusion: In summary, our study is the first study to investigate the IDH2 status in primary GBMs in Turkish patients. Anaplastic astrocytoma or glioblastoma patients with IDH1/IDH2 mutations, which has been reported to be younger age than carrying wild-type allele. According to result IDH1/IDH2 mutations observed more frequently in patients at an early age. In our study, mutations were not detected in our study group for this reason could not be made to such a statistical ratios. But all of our samples were wild-type allele therefore the average age was 54.5 ± 2, respectively.
Keywords: Glioblastoma Multiforme, IDH2, sequencing.

TUE-131
Impact of CYP1A2 gene polymorphisms in adenocarcinoma lung cancer risk among tunisian population

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Four single nucleotide polymorphisms (SNPs) of CYP1A2 gene were analysed in 109 healthy smokers and in 101 lung cancer cases, including 63 with squamous cell carcinoma (SCC) and 41 with adenocarcinoma (AD). The genotyping for the SNPs -3860 G>A, -2467delT, -739T>G and -163C>A was performed by polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis.

The results showed that smokers with CYP1A2 gene polymorphisms were associated with an increased risk for the development of lung AD. There was however no significant increased risk of developing lung SCC in smokers having CYP1A2 gene polymorphisms. An increased risk of developing AD was observed in smokers who are carriers of at least one copy of -3680A or -739G giving a significant odds ratio (OR) of 6.02 (CI = 2.91–12.9) and 3.01 (CI = 1.54–5.98), respectively.

These genotyping data are consistent with the hypothesis that tobacco-specific-N-nitrosamines (TSN) such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are major contributors to the development of lung AD and that CYP1A2 gene product

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plays an important role in the metabolic activation of NNK. This study suggests that SNPs of CYP1A2 could be considered as promising biomarkers in the aetiology of lung AD in smokers.
Keywords: CYP1A2 gene, Polymorphisms, Lung cancer.

TUE-132
In vitro and in vivo analysis of the oncogenic role of Otx2 in medulloblastoma

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Medulloblastoma (MB) is a malignant and invasive tumor of the *cerebellum*. It comprises four distinct molecular variants: Wnt and Sonic hedgehog (Shh) groups, in which Wnt and Shh signalization are deregulated, respectively, Group 3 (also known as Myc-medulloblastoma) characterized, among other genomic deregulations, by the overexpression and/or amplification of the Myc gene and of the transcription factor Orthodenticle homeobox 2 (Otx2) gene, and Group 4, which is not well characterized. Recently, Kawauchi et al. generated an animal model of Myc-medulloblastoma using orthotopic transplantation of granule cell precursors (GCPs) overexpressing Myc and a dominant negative form of the tumor suppressor protein P53 into the cerebellar cortex of the mouse.

Otx2 is overexpressed in 74% of medulloblastomas and it is also frequently amplified in type 3 MB. In the adult cerebellum, this transcription factor is expressed posteriorly by granule cells, which represent the most abundant neuronal cell type in the CNS. During cerebellum development, Otx2 is expressed by GCPs, which have a high proliferation rate. Deregulation of GCPs proliferation may favor oncogenic processes, as seems to occur in the Shh group of MB.

Our objective is to study the role of Otx2 in normal and oncogenic development of the cerebellum. We are investigating the role of Otx2 in the control of GCPs proliferation and differentiation using a mouse genetic model where Otx2 is co-expressed with the green fluorescent protein GFP, allowing identification and purification of Otx2-expressing (Otx2+) GCPs from developing cerebellum. Purified cell populations are subjected to different tests of proliferation (Ki67 expression, Edu incorporation) to assess the proliferation rate of Otx2+ versus Otx2- GCPs. Preliminary results show that Otx2+ GCPs have an increased proliferation rate compared to Otx2- GCPs, suggesting that Otx2 may have an oncogenic role in the establishment of medulloblastoma through the regulation of GCPs proliferation. We are now studying the effect of Otx2 overexpression or silencing on the proliferation rate of Otx2+ GCPs in *in vitro* studies. Finally, we will test the oncogenic potential of Otx2 in Myc-medulloblastoma using the transplantation model described above.
Keywords: granule cell precursors, medulloblastoma, Otx2.

TUE-133
In vitro modulation of enzymatic processes using biologic active substances with relevance for cancer therapy

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The therapeutic possibilities offered by plants and insects have been known to mankind for centuries; the pharmaceutical companies continue to invest enormous resource in identifying agents, to define and understand their most appropriate therapeutic uses.

Using an original patented technology, we have obtained a bioactive complex (Etno P) from entomological source which can be used in drugs and cosmetics industry. The complex is rich in proteins, essential amino acids, essential fatty acids and mineral salts (Ca, Na, K, Fe, Mg, Se, Ni, Cu, Si).

Determination of the relation between antioxidant effect and enzymatic activity of MMP-2, MMP-9 and Hyaluronidase, under the action of Etno P, is important for in vitro evaluation of potentially chemotherapeutical agents. Matrix metalloproteinases (MMPs) and hyaluronidase are involved in the degradation of the extra-cellular matrix under normal physiological conditions and during the metastatic process. Is well known that MMP-9 is a key effector molecule that promotes tumor cell invasion through type-IV collagen degradation and its expression has been observed in tumors of various organs, including the prostate. Hyaluronidase (HYAL-1) degrades hyaluronic acid into proangiogenic fragments that support tumor progression. If free radicals are not inactivated, they can accumulate in time and chemical reactivity of this species can damage cellular macromolecules including proteins, carbohydrates, lipids and nucleic acids. Because reactivity of free radical species are associated with several forms of tissue damage and disease, people had been trying to evolve a highly sophisticated and complex antioxidant protection system, that functions interactive and synergistic to neutralize free radicals before they attack the cells.

In order to emphasize the potential preventing antitumoral ability of the biologic active substances isolated and purified in ours laboratories from entomological source, enzymatic studies were done on the cell line DU 145. The biochemical parameters monitored was the enzymatic activity of catalase, SOD, MMP-9, MMP-2 and Hyaluronidase.

The experimental data demonstrated that bioactive substances are favourable to modifying enzymatic activity (MMP-2, MMP-9 and Hya) and presenting antioxidant activity. This study indicated that our bioactive complex can be included in pharmaceutical preparations, used individually or synergistically with other bio products in order to obtain a convenient efficiency/toxicity report.

This study was funded by Romanian National Authority for SCIENTIFIC RESEARCH, INNOVATION RESEARCH PROGRAMME, CONTRACT 26 DPST / 2013
Keywords: DU145, Hyaluronidase enzyme, MMP-9.

TUE-134
In vivo tumour-suppressive effects of alpha-tocopheryl succinate in Ehrlich ascites carcinoma

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Introduction: Tumour-specific targeting of distinct molecular pathways involved in apoptotic process of malignant cell represents the highly beneficial concept in development of novel anticancer therapeutics. Alpha-tocopheryl succinate (α-TOS) has been shown to selectively inhibit cell proliferation and to induce cell death in variety of transformed cell lines while shielding normal cells. The aim of this study was to evaluate the effects of the administration of α-TOS on the vitality of Ehrlich ascites carcinoma cells (EAC) as well as its influence on the activity of glutathione-dependent antioxidative enzymes in EAC.

Material and methods: Two days after the transplantation of EAC cells in the abdominal cavity, Swiss mice were intraperito-

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neally treated with 50 μl or 100 μl α-TOS (0.2 M) each third day, four days in total. Aseptic evacuation of peritoneal content on tenth day post-transplantation, enabled the collection of EAC, and cell viability and enzyme activity were assessed. All experimental procedures were approved by the Ethical Committee of the University of Novi Sad.

Results: We observed the decreased viability of EAC cells in treated animals, indicated by the leakage of cytosolic lactate dehydrogenase (LDH) into the extracellular compartment that was significantly increased in the groups treated with 50 μl ($p = 0.002$) and 100 μl ($p = 0.01$) of α-TOS compared to control. The specific activities of glutathione-dependent antioxidative enzymes showed the dose-dependent decrease in both experimental groups, however, only the decrease in specific activity of glutathione peroxidase (GPX) was statistically significant ($p = 0.003$ for 50 μl, and $p = 0.0005$ for 100 μl of α-TOS). The concentration of total intracellular proteins has also been significantly decreased in treated groups ($p = 0.053$ for 50 μl, and $p = 0.045$ for 100 μl of α-TOS) compared to control, which is in accordance with results that show the decreased activity of antioxidant enzymes as well as increased leakage of LDH.

Conclusion: The results of pro-oxidant activity of α-TOS in EAC cells using the *in vivo* model followed by decreased viability of malignant cells, may indicate the potential anti-tumour properties of α-TOS, which could be considered as promising adjuvant agent in developing novel therapeutic strategies.

Acknowledgment: This work is supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, grant No. III41012.
Keywords: None.

TUE-135
Induction of apoptosis in HeLa cancer cells by Gracillaria gracilis M. Steentoft, L.M. Irvine & W.F. Farnham hexane extracts

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Background/Aim: The extracts from red seaweeds possess broad spectrum therapeutic properties such as anticancer and antigenotoxic effects. Recently, much attention has been paid to the anticancer activity of seaweeds. Hence, the aim of the study was to evaluate the *in vitro* genotoxic and cytotoxic/pro-apoptotic potency of the hexane extracts of *Gracillaria gracilis* (Rhodophyta) from the Coast of Urla in Aegean Sea.

Materials and Methods: Field collections of algae were made from several reefs (depths of 1–2 m) along the Izmir (Urla) coast during April 2013. Algae samples were dried at 45°C. Powdered material was extracted with n-hexane at room temperature and stored at 40°C. Cytotoxic effects of extracts have been tested in 5 different human cancer cell lines (CaCo-2, HeLa, MCF-7, A549, U87MG) and a healthy cell line (HEK 293) by MTT assay. The most effective cancer cell line was selected for apoptosis panel according to the IC_{50} results. To exhibit the expression profile of 84 genes involved in apoptosis, derived cDNAs from treated each cell line with the extract applied to RealTime ready Human Apoptosis Panel 96 (Roche, Germany). The genotoxicity of the extract was evaluated by Ames test that was conducted by the pre-incubation method using *Salmonella typhimurium* strain TA 98 and TA 1537 for the detection of frameshift mutations and TA 100 and TA 1535 for pair substitution.

Results: MTT results indicated that while hexane extract have significant cytotoxic activity against HeLa cells with IC_{50} values of 22.4 μg/mL, it showed cytotoxicity against other cancer cell line with IC_{50} values of >50 μg/mL. We found that comparing changes in expression of apoptosis related genes as fold change the hexane extract negatively modulated HeLa cancer survival, according to untreated control cells which might be mediated through up-regulation of BAK1 and down-regulation of NF-kB. Furthermore, this compound can extrinsic or intrinsic pathway induce apoptosis on HeLa cells, which might be mediated through both pro-apoptotic and anti-apoptotic Bcl-2 family and caspase family. When the chemical concentrations and resulting his^+ revertants produced by the extracts were compared with those produced by controls, even 5 mg/mL dose of the all extracts demonstrated no mutagenicity in the Ames test.

Conclusion: Cytotoxicity and genotoxicity results and gene expression assays demostrating that this extract may have potential in servics cancer treatment.

Keywords: None.

TUE-136
Influence allogeneic mesenchymal stem cells on the tumour cells parameters

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Nowadays stem cells participation in tumor growth has been extensively discussed in anticancer therapy. In vitro was investigated, mesenchymal stem cells (MSCs) produced the transient arrest of tumor cells in the G_1 phase of cell cycle; this was accompanied by a reduction in the apoptotic rate. However, previous studies have produced controversial results regarding whether MSCs promote or inhibit tumor growth and progression. In view of these controversial data, we conducted a study on the impact of allogeneic MSCs on biological properties of the primary tumor cells of Lewis lung carcinoma.

The investigation was carried out in C57Bl/6 male mice weighing 20-22 g aged 2 to 3 months. Mice of experimental group received the course of inoculation of MSCs (in concentration $1{,}25x10^4$ cells). The primary culture was obtained from transplantable Lewis lung carcinoma. Cell cultivation was conducted under standard conditions. The number of living cells was determined using MTT-colorymetric test. Apoptotic level and distribution of cells primary culture in phases of cell cycle were assessed by cytofluorimetry.

In order to determine MSCs influence on biological characteristics of tumor cells they were isolated from primary tumor, incubated 24 hours and then apoptotic level was assessed in control (20 days after tumor transplantation) and test samples (intravenous administration of allogeneic MSCs). The number aneuploid cells increased in 1.3 times upon MSCs influence on transplantable lung Lewis carcinoma. According to morphological characteristics, growth of subpopulation of cells of non-adhesion fraction with increase of nucleocytoplasmic ratio was detected in primary culture. Among aneuploid cells population, increase of cells of proliferative pool were detected after MSCs administration in comparison with control (G_2/M+S). Decrease of per cent of diploid cells subpopulation in 1,7 times in primary culture after MSCs administration and their more than 90% synchronization G_0/G_1 phase of cells cycle was shown. Regarding aneuploid cells

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after MSCs influence, increase of this population to 77.02 + 3.83% versus 59.20 + 1.71% in control was accompanied by growth of proliferative pool subpopulation G_2/M+S.

Thus, biological characteristics of tumor cells under the influence of MSCs are primarily associated with the increase of aneuploidy.

Keywords: aneuploidy, mesenchymal stem cells (MSCs), tumor cells.

TUE-137
Influence of lumican on biological properties of selected cell lines

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Lumican is the major leucine-rich keratan sulfate proteoglycan of the extracellular matrix in corneal stroma, skin, muscle and cartilage. As proved by knocking-out of its gene in mouse, lumican plays a critical role in collagen fibrillogenesis. Due to direct interactions with cell membrane proteins, under pathological conditions lumican influences key biological processes such as cell growth, proliferation, adhesion, migration and apoptosis. It is known that lumican by binding to α2 integrin I domain and interaction with β1 integrin subunit inhibits melanoma cell migration but increases their adhesion. Previously, we shown that lumican is an efficient protein to block MMP-9 and MMP-14 expression and pro-angiogenic activity of endothelial cells. In present study we compare its influence on biological properties of human colon adenocarcinoma cells (HT29) and human prostate cancer cells (PC-3). We demonstrate that in both cell lines the presence of lumican caused upregulation of ß1 integrin subunit level. We also provide an evidence that changes in expression of ß1 integrins cause increasing of invasion through Matrigel™ and enhance their ability to form microcapillary tubes. However, statistically significant changes in invasion of HT29 cells were not observed. Summarising, lumican causes changes in the invasive properties of human prostate cancer cells (PC-3), but not in human colon adenocarcinoma cells (HT29).

Financial Support: MUL (502-03/6-098-01/502-64-006), Pol-Nor/202952/5/2013, HARC FP7-REG-POT-2012-2013-1
Keywords: integrin beta1, lumican.

TUE-138
Inhibition of the Rheb/mTOR pathway in TSC2-deficient AML cells results in down-regulation of IRF7

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Lymphangioleiomyomatosis (LAM) is a rare disorder that causes obstruction of lung tissue. It occurs predominantly in young women, and is caused by mutations in one of the tuberous sclerosis (*TSC*) genes, that serve as a GTPase-activating protein for the small GTPase Rheb. As a result, Rheb and mTORC1 are chronically activated in TSC1- or TSC2-deficient cells, and this activation can be diminished using the appropriate inhibitors. Rapamycin (sirolimus) is a known specific inhibitor of mTORC1, while S-trans,trans-farnesylthiosalicylic acid (FTS; salirasib) has been shown to inhibit Rheb. To examine the effect of the Rheb/mTOR inhibition pathway and further establish FTS as a potential treatment for LAM, we used human TSC2-deficient angiomyolipoma cells. FTS indeed inhibited Rheb in these cells and attenuated their proliferation. After comparative treatments with FTS or rapamycin or by re-expression of TSC2 we carried out a gene array analysis. This yielded a substantial number of commonly altered genes, many of which we identified as downstream targets of the interferon regulatory factor 7 (IRF7) transcription factor, a central activator of the interferon (IFN) type 1 immune response. Furthermore, nuclear localization of IRF7 was impaired by each of the three treatments. Interestingly, the phenomena seen on FTS or rapamycin treatment were selective for TSC2-deficient cells. Moreover, knockdown of IRF7 by siRNA mimicked the decrease in number of the abovementioned genes and also inhibited angiomyolipoma (AML) cell proliferation. Altogether, these findings support FTS as a potential treatment for LAM and IRF7 as a novel target for treatment.

Keywords: mTORC1, Rheb, TSC2.

TUE-139
Inhibitory effect of liposomal bovine lactoferrin (LbLF) on DSS-DMH-induced rat colorectal tumors

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The Ulcerative Colitis (UC) increases year by year, and colorectal cancer cancer occurs in 7–8 years. Bovine lactoferrin (bLF), a member of the transferrin family, is an 80-kDa ironbindeng glycoprotein abundant in exocrine secretion of mammals, including milk, saliva, and neutrophils. Because bLF is present at high concentrations in cow's milk(1.9 mg/ml), it is regarded as a safe food-derived substance. Moreover, bLF is a multifunctional protein with anti-inflammatory, anti-bacterial, anti-viral,anti-tumor, and immunoregulatory effects. In this study, we used LbLF which encoded in soybean lecithin raising rate of absorption of LF, and investigated the anti-infammatory and anti-tumor effect of LbLF on DMH-induced rat colon cancer after the dosage with Dextran Sulfate Sodium (DSS) and on cultured colon cancer cells.

Method: 36 male F344 rats were divided into 3 groups (Control, 500 mg/kg/day LbLF and 1000 mg/kg/day LbLF group). After all the experimental rats drank 1% of DSS solution for one week, DMH(20 mg/kg) was injected to them for 8 weeks. All the rats were sacrificed 26 weeks after commencement of DMH administration, and aberrant crypt foci (ACF) and the colorectal tumors were examined. RKO 5000 cells were plated on 24 well plates and were preincubated with 0, 1, 10 and 100 ug/ml bLF and 100ug/ml LbLF, and trypsinized cells counted at 0, 1,2 and 3 day by Cell Counter. RKO cells were preincubated with 100ug/ml bLF for 4 h before LPS(100 ng/ml) stimulation, and cells were carefully washed twice with PBS. Cells were then cultured in medium with or without LPS, TNF-α mRNA expression was analyzed LPS stimulation.

Results and Conclusion: 500 or 1000 mg/kg/day LbLF group compared with control group showed statistically lower number of ACF, adenoma and adenocarcinoma of the colon. 1000 mg/kg/day LbLF group compared with 500 mg/kg/day LbLF group showed statistically lower number of adenoma. LbLF inhibits the development of colon tumor in DSS induced UC, and may be suggested to be useful for prevention of colitis cancer.

Keywords: cancer, colon, lactoferrin.

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TUE-140
Insight into the comprehension of prolactin receptor mechanistic activation by a structural approach

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Based on accumulating evidence suggesting that prolactin receptor (PRLR) signalling may be involved in breast and prostate tumorigenesis, there is a real need to develop new therapeutic strategies to block the activation of this receptor. The active complex between prolactin (PRL) and its receptor is a heterotrimer involving two receptor moieties and one hormone molecule, interacting at binding sites 1 and 2. The classical approach that proved to be efficient to inhibit activation of the homologous growth hormone receptor consists in the development of competitive receptor antagonists engineered by substituting an Arginine for the natural Glycine residue lying at the bottom of the site 2 cavity in the ligand. For the PRL/PRLR system, however, this strategy generated a mild (partial) PRLR antagonist (G129R-hPRL) displaying residual agonism. An additional modification, i.e. the deletion of the 9 N-terminal residues, was necessary in order to obtain a pure PRLR antagonist (Jomain, JBC 2007). We also investigated an alternative strategy, consisting in the mutation of Glycine 129 into various types of amino acids while maintaining the N-terminus intact. Interestingly, this resulted in PRL analogs exhibiting very different antagonistic potency. G129P-hPRL was even less potent than G129R-hPRL while G129V-hPRL appeared as potent as Del1-9-G129R-hPRL, indicating that deletion of the N-terminus is not mandatory to achieve pure antagonism. Although these variants were shown to exhibit different stabilities, their specific biological properties remained poorly understood.

In order to give some light on the activation mechanism of the receptor and to explain the versatile antagonistic properties of the various G129 analogs, we undertook a systematic crystallographic study of the different prolactin mutants and of PRL/PRLR complexes (van Agthoven JBC 2010), and submitted them to a normal mode analysis. We did performed an in-depth comparative structural analysis of all the obtained structures, showing that binding of the hormone at site 1 leads to conformational changes within the PRL which may facilitate (or allow) the interaction at binding site 2. We also highlighted the importance of flexibility and allosteric movements for the activation of the receptor.

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Keywords: allostery, Cancer signaling, Cytokines.

TUE-142
Intracellular CHI3L1 production promotes malignant transformation of 293 cells

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High levels of chitinase-3-like-1 oncoprotein (CHI3L1) were found to accompany development of glioblastoma and some other malignant tumors. In present study it was established that transfection of plasmid with *CHI3L1* cDNA insert led to appearance of several new morphological and functional properties typical for malignantly transformed cells. By means of atomic force microscopy and fluorescent confocal microscopy the enlargement of cell volume and cell surface square, modification of cells shape, increased amount of filopodia, and decreased amount of long outgrowths in 293_*CHI3L1* cells, stably producing CHI3L1 protein, compared to 293 cells transfected with "empty" vector (293_pcDNA3.1) were established. In 293_*CHI3L1* cells distribution of filamentous actin (F-actin) was revealed to be diffusive and uniform while in 293_pcDNA3.1 cells it was localized predominantly nearby plasma membrane with actin filaments being organized in dorsal stress fibers. Size, modification of distribution, increased number and membrane potential of mitochondria in 293_*CHI3L1* cells were likely to be associated with expression of *CHI3L1*. Increased number and modification of spatial distribution of lysosomes in cells 293_*CHI3L1* being probably associated with F-actin cytoskeleton reorganization involved in exocytosis of CHI3L1 protein were observed. Constant depolarization of 293_*CHI3L1* cell plasma membrane and acidification of extracellular medium due to activation of Na^+/H^+ exchangers in these cells was revealed. Extracellular acidification was shown to be involved in filopodia formation in 293_*CHI3L1* cells. It was also ascertained that transfection of plasmid with *CHI3L1* cDNA insert into 293 cells promoted cellular redox state modification. 293_*CHI3L1* cells in comparison to 293_pcDNA3.1 cells were characterized by decreased endogenous hydrogen peroxide and mitochondrial superoxide anion radical production, by elevated level of reduced glutathione, increased constants of H_2O_2 utilization, enhanced resistance to oxidative damage induced by H_2O_2 and menadione. Role of ERK1/2 in mechanisms of intracellular redox state regulation was established. Revealed cells functions modifications associated with CHI3L1 production are closely linked to malignant cell transformation, thus allowing consideration of CHI3L1 as one of the perspective targets for anticancer therapy.

Keywords: CHI3L1, Malignant Transformation.

TUE-143
Intracellular proteases promoting endometrial hyperplasia and carcinoma development

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Endometrial disorders in fertile and menopausal women are related to estrogen stimulation resulted in hyperplasia, adenomatous hyperplasia, atypia, and even malignancy (carcinoma). In order to evaluate possible cellular biomarkers and molecular targets for rational therapy of disease progression the intracellular proteases and their inhibitors were studied. Proteolytic machinery is tightly implicated with the disorder progression and might have prognostic value as it was shown for many cancers. High-

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throughput approach for assaying gene expression has shown that a variety of proteolysis-related processes are overactivated in endometrial cancer patients. Despite extracellular processes of physiological importance (inflammation, coagulation, etc.) a wide range of inter- and intracellular proteolytic pathways such as extracellular matrix remodeling, aberrant cell-cell interaction, proteasome-dependent protein quality control, autophagic protein degradation, apoptosis, known to be affected in disease. Carcinoma invasion requires the close interaction of tumor cells with components of the ECM. Matrix components might by degraded through a cascade of matrix metalloproteases initiated by plasmin. Plasminogen activation depends on turn on uPA and tPA activity promoting by other proteases (for example lysosomal cathepsin B). To evaluate intracellular players in damaged tissue the activity, content and mRNA expression were measured for proteases of different catalytic type and subcellular localization such as cathepsins B and D, Ca^{2+} -dependent m- and μ-calpains, caspases-3, -8, and -9. Substantial changes in protease synthesis and activity were observed in adenocarcinoma lesions when compared to normal endometrial cells. Regarding calpain function in cell motility it might reflect high metastatic potential of the tumor. Cathepsin B was upregulated both in adenomatosis and adenocarcinoma with maximal activity on lesion periphery. In this study, we found that activity of initiator and effector caspases (caspase-8, -9, and -3) were decreased in advanced endometrial tumors compared with the normal endometrium, while there was no correlation between caspase activity in patients with benign adenoma and healthy women. Due to conventional roles of proteases in cell migration and apoptotic cascade it might indicate apoptotic inefficiency in tumors due to insusceptibility of tumor cells to apoptotic signals. Further studies involving both in vivo and in vitro models will be needed to further clarify the significance of intracellular proteases in endometrial pathology and their biomarker relevancy. This work was supported by RFBR grant 12-04-01597 and “Leader Scientific Schools” grant 1410.2014.4.
Keywords: proteases endometrium estrogen.

TUE-144
Intracellular proteins controlling the activation of β1 integrins in HT29 cells overexpressing Snail

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Snail is a key transcription factor involved in the epithelial to mesenchymal transition process (EMT) and it is known to promote proliferation, cell adhesion and migration of human cancer cells, including colorectal cancer cells (CRC). It has been shown that overexpression of Snail induces EMT but the molecular basis of how Snail regulates that process has not been fully clarified. We have focused on early changes in CRC cells - how Snail regulates adhesive properties of colon cancer cells (HT29) and how its expression influences integrin signaling since integrins, heterodimeric, transmembrane adhesion and signaling proteins, are involved in migratory and adhesive properties of cells. We reasoned that intergrins may interact with miscellaneous proteins that cause its switch from an inactive, low affinity to active, high affinity state.

We performed an analysis of the expression levels of several proteins that are known to inhibit or activate β1 integrins: talin, kindlin, migfilin, ILK, DAB-2 and filamin, in CRC cells with overexpressed Snail* *in vitro* using Western Blotting technique. We found that levels of filamin-1 (FLNA), but not filamin-3, are positively correlated with Snail overexpression, invasive phenotype and capillary structures formation. Subsequently we silenced filamin-1 expression, using specific anti-FLNA siRNA, to investigate the impact of filamin-1 on migration and adhesion processes in HT-29-Snail cell clones.

Our results show that filamin-1, apart from its numerous cell functions, may interact with Snail in cancer cells and that can lead to changes in migratory properties of the cells and their invasiveness. We believe that this study may change our understanding of the major pathophysiological processes underlying development of colon cancer metastases and possibly will help to develop future strategies to block metastasis.

HT-29-Snail clones were obtained by Joanna Boncela, PhD, Institute of Medical Biology, Polish Academy of Sciences.

This research is supported by the National Science Centre grant - DEC-2011/02/A/NZ3/00068 and HARC FP7-REGPOT-2012-2013-1.
Keywords: colon cancer, integrins, snail.

TUE-145
Investigation of the Taspine's antiproliferative, antiangiogenic and apoptotic effects on U118 and U87 glioblastoma multiforme cell lines

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Brain tumors are responsible for 1.5% of all cancers and 2% of cancer deaths. In adults, glioblastoma multiforme (GBM) is the most common and most malignant brain tumors. There is no curative treatment of the disease. In addition to the standard treatment protocols, herbal therapies as an alternative methods of treatment can be used in cancer therapy. Taspine as an alkaloid, not only can pass the blood-brain barrier, but also has effect on apoptosis and angiogenesis in various cancer. In our study antiproliferative, apoptotic, anti-angiogenic effect of Taspine was investigated on GBM cell lines for the first time.

In this study the effects of Taspine were investigated on two different cell lines p53 mutant U118 and p53 normal U87 GBM cell lines which were close to the *in vivo* characteristics of tumors. The sensitivity of GBM cell lines to Taspine was determined by MTT. Also the proportion of DNA fragmentation after Taspine application was analyzed by COMET assay. Expression levels of angiogenic genes, VEGF, EGFR; apoptotic genes, p53, Bim, Bad, Bax, Bcl2 and autophagic gene Beclin 1 were investigated after Taspine added to each cell lines.

Taspine addition did not change VEGF and EGFR expression in p53 normal U87 cell line however, it increased the expression level of Bcl2, VGEF, EGFR significantly in p53 mutant U118 cell line. The reason of the different sensitivity to Taspine was thought to be because of the presence and absence of p53 mutation.

It was concluded that, Taspine has antiproliferative effect on GBM cell lines. The antiproliferative effect of Taspine on p53 normal cell line was due to apoptosis, but on p53 mutant cell line, it was due to another programmed cell death, other than apoptosis and autophagy. We also showed that p53 mutation was one of the important factor in response to the treatment of GBM.
Keywords: Taspine, Glioblastoma Multiforme, Apoptosis, Antiproliferative effect.

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TUE-146
Investigation of potential biomarkers of biochemically recurrent prostate cancer

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Improved diagnostic biomarkers of prostate cancer (PCa) are needed to prevent overtreatment of indolent tumors, and to ensure early detection of aggressive PCa that requires treatment. Human Gene Expression Microarrays (Agilent) have been widely used for discovery of new candidate biomarkers in cancer. This study investigates genome-wide profiling of gene expression for identification of biomarkers of biochemically recurrent (PSA >0.2 ng/ml) PCa showing the tendency to progress.

Gene expression profiling was analyzed using Agilent microarrays on cancerous prostate tissues from PCa patients with and without biochemical recurrence (BCR) after radical prostatectomy. Change in expression of 455 genes (FC>2, $p < 0.05$) was identified. Out of 34 genes showed up-regulated expression in relation to BCR. Later on, 11 genes and 5 reference genes were selected for further validation with TaqMan Low-Density Arrays (TLDA) in expanded group of PCa cases. These arrays enabled to identify changes in 5 gene expression in cancerous tissues to compare with non-cancerous prostate tissues (NCPT) from another PCa patients set. Moreover, significant ($p < 0.01$) down-regulation of 2 genes marked the PCa cases with BCR. Last step of our study was to perform QPCR with 12 TagMan Gene Expression Assays, including 2 reference genes, in new PCa patient cohort. Screening was done only in cancerous prostate tissues (≥ 60% cancerous cells per sample).

The results of our study indicate that the down-regulation of several gene expression may be important for relapse of prostate cancer and therefore, identification of these genetic biomarkers may assist for early detection of aggressive PCa cases.

Keywords: Biomarker, gene expression, prostate cancer.

TUE-147
Investigation of the role of CYLD in mammary gland development and tumorigenesis

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Breast cancer is a leading cause of cancer-associated mortality in women. CYLD is a tumor suppressor protein that has been associated with breast cancer development. More specifically, it has been shown previously that CYLD mediates the growth suppression of the aggressive human breast cancer cell line BT549 by inducing apoptosis. Furthermore, it was recently shown that CYLD expression is downregulated in human breast cancer tissues in comparison to normal breast tissues. In order to characterize in vivo the functional role of CYLD in mammary gland development and homeostasis we have generated mice with targeted inactivation of CYLD in the mammary gland using a conditional approach. For this purpose, mice in which *Cyld* exon 9 has been flanked with loxP sites (*Cyldflx*^{9}) were crossed with mice expressing the Cre recombinase under the control of the mouse mammary tumor virus (MMTV) LTR (*MMTVCre*) in order to achieve targeted CYLD inactivation in the mammary epithelium. Exon 9 codes for an essential catalytic motif of the deubiquitination domain of CYLD and its removal places exon 10 and downstream exons out of frame after splicing to exon 8. Pathological and immunohistochemical analysis of nulliparous, female mice indicates that inactivation of CYLD in the mammary epithelium leads to marked alterations in the cellular proliferation and apoptosis as well as the gross tissue histology. *MMTVCre-Cyld*^{flx9} mice display hyperplastic luminal mammary epithelia with increased proliferation rate and low level apoptosis as opposed to control mice in which the luminal epithelium retains its monolayer architecture low level cell proliferation and extremely limited apoptosis. The increased proliferation rate in *MMTVCre-Cyld*^{flx9} mice is consistent with the observed augmented Cyclin D1 levels in the hyperplastic epithelia. Our experiments indicate that CYLD plays an important role in mammary epithelium homeostasis possibly by affecting Cyclin D1 expression.

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program “Education and Lifelong Learning” of the National Strategic Reference Framework (NSRF) - Research Funding Program: ARISTEIA-1921-EMBRACE. Investing in knowledge society through the European Social Fund.
Keywords: Animal Model, Cyld, Mammmary Gland Tumorigenesis.

TUE-148
Investigation of the Taspine's antiproliferative, antiangiogenic and apoptotic effects on U118 and U87 glioblastoma multiforme cell lines

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Brain tumors are responsible for 1.5% of all cancers and 2% of cancer deaths. In adults, glioblastoma multiforme (GBM) is the most common and most malignant brain tumors. There is no curative treatment of the disease. In addition to the standard treatment protocols, herbal therapies as an alternative methods of treatment can be used in cancer therapy. Taspine as an alkaloid, not only can pass the blood-brain barrier, but also has effect on apoptosis and angiogenesis in various cancer. In our study antiproliferative, apoptotic, anti-angiogenic effect of Taspine was investigated on GBM cell lines for the first time.

In this study the effects of Taspine were investigated on two different cell lines p53 mutant U118 and p53 normal U87 GBM cell lines which were close to the *in vivo* characteristics of tumors. The sensitivity of GBM cell lines to Taspine was determined by MTT. Also the proportion of DNA fragmentation after Taspine application was analyzed by COMET assay. Expression levels of angiogenic genes, VEGF, EGFR; apoptotic genes, p53, Bim, Bad, Bax, Bcl2 and autophagic gene Beclin 1 were investigated after Taspine added to each cell lines.

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Keywords: Taspine, Glioblastoma Multiforme, Apoptosis, Antiproliferative effect.

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TUE-149
In-vivo analysis of formation and endocytosis of the Wnt/β-catenin signaling complex in zebrafish embryos

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Wnt/β-Catenin signaling is one of the most important paracrine pathways during development, tissue regeneration and stem cell regulation. After activation by Wnt ligands, a multi-protein complex assembles at the plasma membrane clustering membrane-bound active receptors and intracellular signal transducers into the so-called signalosome. However, the dynamics of signalosome formation and dissolution are unclear and how these processes are related to Wnt/b-Catenin signaling remains to be determined. Our imaging studies in live zebrafish embryos show that the signalosome is a highly dynamic structure, which is continuously assembled by Dvl2-mediated recruitment of the destruction complex to the receptors and partially disassembled after clathrin-mediated endocytosis. We suggest that the μ-subunit of the endocytic Clathrin adaptor Ap2 interacts with Dvl2 in zebrafish and that this interaction is required for signalosome formation and activation of Wnt signaling. We find that after internalization, the ligand-receptor complex and the signal-transducer complex take separate routes. While Wnt ligand follows an endocytic path, the destruction complex still bound to Dvl2 is temporarily kept inactive. Thus, blockage of Ap2μ2 function at the plasma membrane inhibits the formation of an active ligand-receptor complex and its subsequent internalization and thereby reduces Wnt signaling.

Keywords: AP2, WNT signalling, zebrafish.

TUE-150
Ion interactions and thrombospondins, a multiscale molecular modelling study

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In this presentation we show our study around the Thrombospondin (TSP) Signature Domaine (SD). This domain is implicated in various important biological processes. One of them is nitric oxigen introduced angionesis, which highlights the molecules importance in cancer research. Mutations in the SD are further the cause for heriditary diseases that cause bone malformation such as pseudoachondroplasia (PSACH). The signature domain features around 30 calcium ions in its resolved structures. In the "in silico" study that we present, we eludicate which and how these calcium ions dissasociate from the SD. Differences in calcium concentrations have been shown to be important for various protein-protein interactions such as those with Integrin, Cluster of Differentiation (CD-47) and Collagen. Besides pointing out these interesting results of major biological relevance, we highlight developments in charge parameterization from ab-intio quantum mechanics, and cinematic visulization that we have made as a part of this mulitscale molecular modeling study.

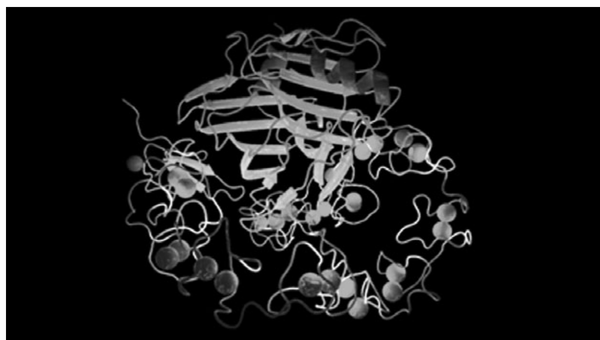

Fig. 1.

Keywords: computational modeling, Ion interactions, molecular dynamics.

TUE-151
Ionomycin/PMA treatment modifies the expression of genes involved in apoptosis, proliferation and endoplasmic reticulum stress in T cell leukemia cell line

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The cytokine milieu during T cell activation is one of the most important factors in determining T cell fate. Activation of T cells by direct stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin (Io) results in numerous downstream signals that activate pathways enabling T cells to proliferate and produce cytokines. Inducible T cell activation is regulated predominantly at the transcriptional level. Therefore, we performed the analysis of the transcriptional activity of 19 genes involved in the regulation of several important cellular processes in Jurkat T cell leukemia cell line upon Io/PMA treatment by quantitative real-time (RT) PCR method using mRNA-specific primers and SybrGreen.

Our results showed c-kit expression in Jurkat cells, further confirmed by sequencing of c-kit mRNA specific PCR product. The expected increase in interleukin (IL)-2 mRNA expression, together with moderate Ki-67 upregulation, suggests the induced proliferation of PMA/Io-treated Jurkat cells. Significant upregulation of nuclear factor (NF)-κB, JNK and the prosurvival Bcl-2 was followed by activation of only one (protein kinase RNA-like endoplasmic reticulum kinase (PERK)) out of 3 main endoplasmic reticulum (ER) stress subpathways (ATF6 and spliced XBP were downregulated). NF-κB and JNK activation, as well as ERK downregulation were reactive oxygen species (ROS)-independent, shown by the lack of activation of antioxidative enzymes (SOD, NOS, GSTP1, gGCS and GR). C-kit was downregulated in the absence of exogenous SCF (c-kit ligand).

Based on our data set it is concluded that the PMA/Io treatment of Jurkat cells induced the increased expression of IL-2, followed by upregulation of prosurvival genes belonging to the Bcl-2 family. Neither c-kit nor the antioxidative system were activated, excluding their role in Jurkat T-cell activation in the absence of exogenous c-kit ligand SCF. Based on our dataset we may suggest that the upregulation of JNK and (NF)-κB upon PMA/Io treatment in Jurkat cells was the antiapoptotic, prosurvival and proliferative signal, resulting in IL-2 overexpression followed by the transcriptional block of numerous genes involved in apoptosis induction and antioxidative ROS scavenging.

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Keywords: Apoptosis, Endoplasmic reticulum stress, Oxidative stress.

TUE-152
Ions and side chains: probing functional hotspots in histone deacetylase 8

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Histone deacetylases (HDACs) play a key role in a variety of cellular events including transcription, protein degradation and apoptosis. By deacetylating lysine side chains of their target proteins – histones and non-histone proteins - they constitute the counterparts of histone acetyltransferases (HATs). In this project we study human HDAC8 (42 kDa) as a model system to investigate the mechanism, activity and regulation of histone deacetylases from a structural and dynamical perspective using nuclear magnetic resonance (NMR) experiments. The challenges we are confronted with by studying HDAC8 encourage us to extend the methodological repertoire of existing NMR experiments. For example, we developed a set of new, carbon-detected NMR experiments to study arginine side chains and – via Ne-relaxation experiments - their dynamics at physiological pH, which was previously impossible for proteins of the size of HDAC8. Further, we developed a strategy for mapping and characterising potassium binding sites in potassium-binding enzymes such as HDAC8 using 15N labelled ammonium. These experiments in combination with established methods such as methyl-TROSY based approaches are used in this project to probe side chains and ions in functionally relevant parts of HDAC8.

Keywords: histone deacetylase, NMR Spectroscopy, Protein dynamics.

TUE-153
Leptin induces Src/ERK1/2-mediated, upregulation of sphingosine kinase 1 in ER-negative breast cancer: a potential mechanism for obesity driven cancer progression

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Background: Obesity is a known risk factor for breast cancer. Sphingosine kinase 1 (SK1) is an oncogenic lipid kinase that is overexpressed in breast tumours and linked with poor prognosis, however its role in obesity-driven breast cancer was never elucidated.

Methods and Results: Our findings show for the first time that human primary breast tumours and associated lymph node metastases exhibit a strong correlation between SK1 and leptin receptor expression (Pearson $R = 0.78$ and $R = 0.77$, respectively, $p < 0.001$). Both these genes are elevated in metastases of ER-negative patients and show a significant increase in patients with higher BMI. Leptin induces SK1 expression and activation in ER-negative breast cancer cell lines MDAMB-231 and BT-549, but not in ER-positive cell lines. Pharmacological inhibition and gene knockdown showed that leptin-induced SK1 activity and expression are mediated by activation of extracellular signal-regulated kinases 1/2 (ERK1/2) and Src family kinases (SFKs) pathways, but not by the major pathways downstream of leptin receptor (LEPR) - janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3). Src-homology 2 domain-containing phosphatase 2 (SHP2) appeared to be key to SK1 activation, and may function as an adaptor protein between SFKs and LEPR. Importantly, leptin-induced breast cancer cell proliferation was abrogated by SK1 specific siRNA.

Conclusions: Overall, our findings demonstrate a novel SFK/ERK1/2-mediated pathway that links, leptin signalling and expression of oncogenic enzyme SK1 in breast tumours. Our data indicate the potential significance of this pathway in ER-negative breast cancer and identifies this as a new possible mechanism for obesity driven breast cancer progression.

Keywords: Cancer, Obesity, Leptin, sphingolipid, Src.

TUE-154
Loss of heterozygosity – plausible mechanism underlying betaglycan down-regulation in primary endometrial adenocarcinomas

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TGFβ signaling pathway regulates plethora of cellular processes including proliferation, differentiation, apoptosis, angiogenesis and immunological response. Recent findings indicated the particular role of TGFβ accessory receptors in cancer development. Betaglycan is a transmembrane proteoglycan responsible for TGFβ ligands presentation to the canonical receptors TGFβRI and TGFβRII. It is encoded by *TGFBR3* gene, which is located on chromosome 1p33-p32. Our previous findings indicated significant down-regulation of *TGFBR3* gene in sporadic endometrial adenocarcinomas. Moreover, observed deregulation was associated with increased cancer malignancy characterized by cancer cell grading (WHO classification).

The aim of the study was the evaluation of allelic imbalance in *TGFBR3* locus (1p33-p32), as a potential mechanism determining betaglycan deregulation in primary endometrial adenocarcinomas. Loss of heterozygosity in *TGFBR3* locus was analyzed using D1S188, D1S435 and D1S1588 microsatellite markers. The study group comprised 48 cases of sporadic endometrial adenocarcinomas obtained from women aged from 36 to 81.

It was stated that 54% (15/28), 36% (8/22) and 35% (7/20) of informative cases displayed LOH for D1S188, D1S435 and D1S1588 microsatellite markers, respectively. Analysing summarized allelic imbalance in *TGFBR3* locus, 25/39 (64%) of informative cases (25/48 of studied cancer specimens) revealed LOH in at least one microsatellite marker. Microsatellite instability (MSI) was observed only in the case of D1S188 and D1S1588 marker, but at low extent, i.e., in two and one cases, respectively.

Loss of heterozygosity in betaglycan gene *locus* may be implicated in disruption of TGFβ signaling in endometrial cancer. However, the presence of allelic imbalance in *TGFBR3* locus seemed not to predetermine aggressiveness of the sporadic endometrial carcinomas. Correlation between LOH in *TGFBR3* locus and clinical and pathological parameters of endometrial cancer samples was not found to be statistically significant.

This work was founded by grant UMO-2011/01/N/NZ4/01723 from National Science Centre (NCN).
Keywords: Cancer, LOH, TGF-beta signalling.

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TUE-155

Low resolution structural characterization of the complex between protein kinase ASK1 and 14-3-3 protein

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ASK1 (Apoptosis signal-regulating kinase, MAP3K5) acts as a critical initiator of the apoptosis triggered by reactive oxygen species (ROS), immune response or various anticancer agents. So far it has been connected with the development of several neurodegenerative or cardiovascular diseases, diabetes and cancer. ASK1 is a homodimeric serine/threonine kinase from MAP3K family therefore it activates MAP2K kinases which in turn activate JNK or p38 kinases promoting cellular apoptosis via caspase 3/9 dependent manner. Enzymatic activity of ASK1 is tightly regulated by phosphorylation, oligomerization and protein-protein interactions. Formation of high molecular complexes, ASK1 signalosomes, was also observed as an essential element for oxidative stress-induced cell death althought its exact composition is still unclear. The 14-3-3 protein was identified as one of the most important physiological regulator of ASK1. It binds to the phosphorylated serine 966 at the C-terminus of the kinase domain ASK1 and maintains its inactive state preventing the signaling iniciation. It has been shown previously that ASK1 is activated after dephosphorylation of serine 966 and dissociation of 14-3-3 in the presence of ROS(1). However, the structural mechanism of ASK1 activation through this interaction remains unknown. Here we present first biophysical and low resolution structural characterization of interactions between the 14-3-3 protein and catalytical domain of ASK1 using AUC and SAXS.

Reference

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Acknowledgments

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Keywords: 14-3-3, ASK1, SAXS.

TUE-156

Mass spectrometry – based analysis of proteome components related with tumor presence and associated with risk of metastasis of breast cancer

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Breast cancer diagnosed at early clinical stages is relatively well cured, yet even in this group some patients are at high risk of metastasis and failure of the treatment. Optimal selection of adjuvant treatment for these patients would be facilitated if reliable prognostic markers of risk of metastasis were available in clinical practice. Serum proteomics allows characterizing processes related to progression of cancer and its influence on patient's organism. Hence, serum proteome might be a source of knowledge on factors reflecting or enhancing spread of cancer cells.

Here we aimed to identify components of serum proteome specific for early stage breast cancer, and to identify components which abundance is associated with risk of metastasis. Blood samples were collected before the start of therapy, after the surgical resection of tumors and one year after the end of therapy in a group of 92 patients with low advanced breast cancer and in a group of 100 healthy women. The low-molecular-weight fraction of serum proteome was analyzed using MALDI-ToF mass spectrometry. We found several serum proteome features specific for patients with low advanced cancer and detected changes characteristic for toxicity of adjuvant treatment that could be observed one year after the end of therapy [Pietrowska et. al. 2009; 2010]. After five years of the follow-up 15 patients from the initial group suffered metastasis or cancer relapse. Blood collected from this group of patients was analyzed together with material collected from 45 patients who benefited from successful treatment. This allowed identification of serum proteome components putatively associated with increased risk of cancer metastasis or relapse.

This work was supported by Polish National Science Center, Grant UMO-2012/05/N/NZ4/02307 and Grant NN 402 685640.
Keywords: breast cancer, mass spectrometry, Proteomics.

TUE-157

Mechanism of action of 9-norbornyl-6-chloropurine – a novel carbocyclic nucleoside analogue

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6-Chloropurines substituted at position 9 with variously modified bicyclic skeletons have recently been reported as anti-enterovirus agents [1]. Importantly, these compounds also showed considerable cytotoxicity and could be considered as candidates for the development of new anticancer drugs. 9-[(1*R**,2*R**,4*S**)-Bicyclo [2.2.1]hept-2-yl]-6-chloro-9*H*-purine (9-norbornyl-6-chloropurine, NCP) represents the most active compound from the series and thus served as a model compound for biochemical studies. In this work we explored the mechanism of action of NCP.

As described previously, NCP was shown to be a substrate for glutathione-*S*-transferase, forming glutathione conjugate (NCP-GS) as a major metabolite, with rapid membrane permeation into cells [2, 3]. Reduced glutathione (GSH) is the most abundant low molecular weight thiol in animal cells and is involved in many cellular processes [4]. It was shown that GSH level is rapidly decreased in treated cells depending on the time of incubation. GSH depletion led to increase in oxidative stress and the induction of apoptosis. Furthermore, NCP activates Nrf2-Keap1 pathway and its downstream targets, such as NAD(P)H:quinone oxidoreductase (NQO-1) or γ-glutamylcysteine synthetase, modifier subunit (GCLm).

Altogether, the novel nucleoside analog NCP represents a promising orally available antileukemic agent, acting through lowering GSH levels in tumor cells.

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Keywords: None.

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TUE-158
Metal phthalocyanines in experimental photodynamic therapy – a proteomic evaluation of dysplastic keratinocytes apoptosis

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Photodynamic therapy (PDT) is a therapeutical approach directed to selective destruction of tumoral cells widely used in dermatology, for treatment of several muco-cutaneous tumors such as basal and squamous cell carcinoma, Bowen disease and oral dysplasia [1]. As an alternative treatment of muco-cutaneous tumors PDT uses a light source able to photoactivate a photosensitizer destroying tumoral cells mainly by induction of apoptosis. Among metal phthalocyanines, the aluminium-substituted bi-sulphonated phthalocyanines [Al(III)S2Pc] exhibit a good photosensitizing potential, however the intimate molecular apoptotic mechanisms activated by PDT with this type of phthalocyanine in dysplastic human oral keratinocytes are not completely elucidated so far.

Protein microarray technology could be an excellent proteomic tool for apoptosis quantification post-PDT procedure. We evaluate apoptotic cell death following experimental PDT in dysplastic oral keratinocytes cell line (DOK) treated with Al(III)S2Pc, using a semi-quantitative protein microarray platform for simultaneously detection of 155 proteins involved in apoptosis course. Alteration of the most important proteins in cells subjected to PDT studied with the chosen array platform, affects mainly Bcl-2, P70S6K, Raf-1 and Bad family proteins expression. Using this proteomic approach a better understanding of apoptotic tumor cell death upon PDT could be achieved, revealing also new insights and molecules as potential new targets for anti-tumoral therapies in the dermato-oncology field.

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Keywords: oral dysplastic keratinocytes, photodynamic therapy, protein microarray.

TUE-159
Metastasis-promoting glycerophosphodiesterase EDI3 influences tumour cell migration, integrin-mediated adhesion and spreading

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Metastasis from primary tumours is the leading cause of death in cancer patients and remains a major therapeutic challenge. Recently, we have identified the glycerophosphodiesterase EDI3 (Endometrial carcinoma differential 3) as a novel factor involved in metastasis (Stewart JD, Marchan R, Lesjak MS *et al.*, *PNAS*, 2012). High expression of EDI3 is associated with metastases in both primary endometrial and ovarian carcinomas and significantly reduces tumour-free survival time in patients. EDI3 hydrolyses glycerophosphocholine to generate glycerol-3-phosphate and choline, thereby influencing both cellular choline and lipid metabolism. Investigations using both classic scratch assays and transwell migration assays show that EDI3 has a major impact on cellular migration in different cancer cell lines, most likely via its direct influence on PKCα signalling. Further experiments using MCF-7 and OVCAR-3 cells reveal that EDI3 can also have an effect on both integrin-mediated adhesion and spreading, two processes which are closely related to cellular migration. Reduced expression of the key integrin subunit β1 upon EDI3 knockdown impairs cell attachment and spreading on a fibronectin matrix, which is accompanied by delayed formation of membrane protrusions. Accordingly, stable overexpression of EDI3 in MCF-7 cells increases integrin $\alpha5\beta1$ expression and is associated with enhanced cell attachment and spreading. The underlying mechanisms remain to be elucidated; however, initial results suggest that EDI3 influences the reorganisation of the actin cytoskeleton and the formation of focal adhesions independent from the FAK-Src signalling cascade. Overall, our data provide some insight into the biological function of the poorly characterised enzyme EDI3 emphasising its importance for the metastatic process.

Keywords: None.

TUE-160
Mitochondriotropic carbon nanotubes for efficient tumour targeting

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Cancer is the uncontrollable growth of cells which are devoid of apoptosis. It has a very large impact around the world as there are around 10 00 000 new cases diagnosed annually & around 3,35,000 deaths. We tried to develop a novel strategy by developing a Ligand mediated tumor targeting via carrier systems. So for the study I selected multiwalled carbon nanotubes for mitochondrial targeting as it has Central role in Apoptosis, there are Multiple activation pathways, the tumour growth depends on energy & the availability of mitochondriotropics.

Rhodamine-123 is shown to be rapidly taken up by the mitochondria in living cells and serves a triple purpose as a stain, ligand and therapeutic agent. Multiwalled Carbon nanotubes (MWCNTs) are the choice of delivery system as it directly enters into the cell without passing through endo-lysosomes, have large inner volume, have distinct inner and outer surfaces & have ability to enter the cell by spontaneous mechanism. Thus, the proposed work envisages Rhodamine-123 conjugated Paclitaxel loaded *functionalized*-CNTs to provide an enhanced cell permeation and mitochondrial localization in order to enhance mitochondrial availability of Paclitaxel.

Methods: The raw MWCNT were procured and were purified, oxidized & then conjugated with rhodamine-123 by carbodimide method. The MWCNT's were characterized *in-vitro* for shape & size by Scanning (SEM) & Transmission Electron Microscopy (TEM), FTIR analysis, X-ray diffraction and zeta potential determined. Stability studies were performed at exaggerated conditions along with Hemolytic Toxicity Study. The Cell Cytotoxicity Study- MTT Assay was done using Hela cell lines. Mitochondrial localization was determined by CLSM

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study. The *in-vivo* part of the study comprised of determining the distribution of drug in various organs by fluorescence microscopy.

Results: The Rhodamine-123 conjugated MWCNTs were prepared and characterized. The CNTs showed high paclitaxel loading, sustained release, and excellent biocompatibility as evident by *in-vitro* drug release and low hemolytic toxicity. MTT assay against HeLa cell lines suggested the potential anticancer activity of the developed system. Confocal microscopic study suggested that mitochondrial specific localization of Rhodamine-123 conjugated MWCNTs in HeLa cells.

Conclusion: Thus, we concluded that Rhodamine-123 conjugated Paclitaxel loaded *f*-CNTs system have potential to provide an enhanced cell permeation and mitochondrial localization for effective tumour chemotherapy.

Keywords: Cancer, nanotubes.

TUE-161
Modulation of cell motility and signaling pathways of ErbB proteins in tumor cell lines by epigallocatechin-3-O-gallate

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Multiple lines of evidence support the concept that epigallocatechin-3-*O*-gallate (EGCG) displays anti-cancer activity [1, 2]. However, the complete molecular mechanisms of action of EGCG in tumor cells still remain to be clarified. We aimed to study the effect of EGCG in cancer cell lines overexpressing ErbB proteins.

We continued our previous experiments [3] and we studied the influence of EGCG on tumor cell motility and on the signaling pathway of ErbB proteins including phosphorylated focal adhesion kinase (pFAK) at S910 site, phosphorylated protein kinase B (pAkt) at S473 site, extracellular-signal regulated kinases (ERK), phosphorylated ERK (pERK) at Y204 site and a nuclear transcription factor, c-fos which is known to be overexpressed in cancers. The study of the EGCG effects on tumor cell migration demonstrated the ability of the flavonoid to inhibit the motility of the A-431 epidermoid adenocarcioma and SK-BR-3 mammary adenocarcinoma cells. The effect was further enhanced by serum starvation.

Investigations on the signaling proteins using flow cytometry method showed that 50 μM EGCG at 48 h of treatment induced partial inhibition (20 – 50%) of pFAK, pAkt and pERK in A-431, but not in SK-BR-3 cell line. Dose-response curves and clonogenic assay experiments are in progress. Our results further support the anti-cancer activity of small natural flavonoids, as EGCG.

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Keywords: cancer, cell signaling, EGCG.

TUE-162
Modulation of VCAM-1 and ICAM-3 in metastatic osteosarcoma cells in vitro

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Osteosarcoma (OS) is a rare primary bone cancer arising among the adolescence and it is worsen upon metastasis. The important biomarkers that are usually involved in the invasion and metastasis of cancer cells are the cell adhesion molecules (CAMs). Therefore, this study aims to elucidate the expression profile of the adhesion molecules involved in metastatic OS cells isolated from OS patients. Normal human osteoblast (NHOst), osteosarcoma cell line (MG-63), non-metastatic and metastatic primary cell culture are used in this study. Morphological observation was carried out using hematoxylin and eosin (H&E) staining. The expression of ICAM-3 and VCAM-1 biomarkers was assessed by imunostaining (IHC) and western blot (WB) techniques. The results of H&E staining showed that all four types of cultured cells possess distinct morphological differences. Further analysis through immunostaining and western blotting showed the expression of VCAM-1 is higher compared to ICAM-3. The IHC results showed that the expression and intensity of VCAM-1 is slightly higher compared to ICAM-3 in all cancer cell line. Comparing the normal NHOst to all cancer cell lines, both the VCAM-1 and ICAM-3 biomarkers are significantly under expressed. In conclusion, we have showed that the expression of the two biomarkers is different although both proteins are involved in OS disease. Moreover, we managed to demonstrate the different metastatic characteristic of OS primary culture in comparison to normal cell lines. These findings are important for future exploration targeting these biomarkers and their characteristics as therapeutic mediators to treat OS.

Keywords: cell adhesion molecules, osteosarcoma, protein expression.

TUE-163
Molecular genetic analysis of the *Drosophila* Mig-10/RIAM/Lamellipodin (MRL) adapter protein in experimental metastasis

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The invasion of cancer cells into surrounding tissues plays a causal role in tumour progression and is the initial step in tumour metastasis, which clinically is the most important process in the progression of cancer. For invasion to occur, cells must detach from the epithelium, acquire and regulate both their motile properties and affinity for other cell types as they migrate to a new location. Several lines of evidence indicate that the Mig-10/RIAM/Lamellipodin (MRL) family of adapter proteins transduce signals derived from growth factor receptors, via interactions with Ras GTPases and/or phospholipids, resulting in changes in the actin cytoskeleton, increased lamellipodia protrusion, cell

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motility and altered cell adhesion properties. *Drosophila* encodes only one MRL protein, encoded by *pico*, which we previously showed to have a role in the regulation of actin dynamics. Although aberrant recruitment of the actin cytoskeleton is implicated in the motility and dissemination of cancer cells, the contribution of specific actin regulators to cancer metastasis is not fully understood. Here we report that overexpression of Pico can promote invasion of Ras^{V12}-induced tumours in a *Drosophila* model of cancer metastasis, characterised by JNK-dependent elevation of MMP1 expression leading to basement membrane degradation at sites of cell invasion. Notably, the ability to promote invasion is shared with a subset of actin regulators, including Enabled, Scar and Chicadee/Profilin, which physically interact with Pico, and Mal/SRF, a downstream transcription factor that promotes actin reorganisation. We will also discuss data obtained using a panel of site-directed mutants in Pico designed to abolish binding to specific MRL-associated proteins, and a fluorescent SRF-dependent reporter gene, which provides a readout the activation state of transcriptional signalling. Importantly, our findings are consistent with the known roles of MRL proteins in promoting lamellipodia-like structures and in intracellular signalling, and point to the potential involvement of MRL proteins in tumour cell invasion and metastasis.

Keywords: None.

TUE-164

Molecular tools for ecological risk assessment of pharmaceutical drugs released in hospital waste water and surface water

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Most pharmaceutical compounds are excreted partially transformed or even unchanged *via* urine and faeces of patients under medical treatment. As hospital effluents reach the municipal sewer network generally without preliminary treatment, they can reach environment and affect animals, plants, but also human health *via* drinking water. Although the concentrations are low (ng/L to µg/L), no data exist concerning their ecotoxicological impact. In this study biomarkers of early effect were performed on hepatic cells (HepG2) and on mammary cells (MCF7): cell viability using cell proliferative assay and genotoxicity (DNA breaks and DNA adduct) using comet assay and DNA P^{32} post-labelling method, respectively. These data were compared with two standardized bioassays: algaltoxkit (*Solenastrum capricornutum)* and microtox® (*Vibrio fischeri*). Cells were exposed to increasing amount of individual drug or in mixture during 24, 48 or 72 h. The time-exposures of bacteria and algae were ranged between 5-30 min and 72 h, respectively. Finally, the same tests were used to check the potential toxicity of Hospital waste water. Samples from a Hospital located in Girona (Spain) were collected during 3 consecutive months. Water samples from the urban WWTP that receives Hospital wastewater were also collected (inflow and outflow). Depending of the relative proportion of the drugs/mixtures applied on cells or aquatic organisms, either antagonistic or additive effects were observed. A non-monotonic dose- response (hormesis) on cell viability was observed when HepG2 were exposed to TAM alone or in presence of CIP. CP induced antagonistic effects when it was added to mixtures. The same was observed with microtox® when the bacteria were exposed to the mixtures. Exposure of cells to individual drugs does not induce any DNA breaks, whereas when cells were exposed to drugs in mixture a dose dependant increase of DNA breaks was observed even with the lowest doses. TAM induces a dose related increase of DNA adduct formation in hepatic cells whereas a non-monotone response was observed in mammary cells. CIP and CP increased DNA adduct formation and modify also the pattern of DNA adduct.

Keywords: Biomarker, DNA binding, ecotoxicity.

TUE-165

mRNA binding proteins regulate BRCA1 mRNA translation in CML cells

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The BCR-ABL1 oncoprotein plays a major role in the development and progression of chronic myeloid leukaemia (CML). To study the role of BCR-ABL1, we employed mouse progenitor cell line expressing high level of BCR-ABL1 corresponding to drug-resistant cells from blast crisis of the disease. We previously demonstrated that CML progression correlates with increased aneuploidy resulting from affected mitosis. This was caused by BRCA1 downregulation leading to decreased expression of protein members of spindle assemble checkpoint [1]. BRCA1 tumor suppressor regulates crucial cellular processes involved in DNA damage repair and cell cycle control. In this study we investigated the mechanisms responsible for BCR-ABL1-mediated BRCA1 downregulation. Herein we found that downregulation of BRCA1 protein is associated with enhanced half-life and increased levels of BRCA1 mRNA in a BCR-ABL1 transformed cell line and in CML primary cells from patients. Lowered luciferase synthesis level under BRCA1 3'UTR control indicated that BRCA1 mRNA translation is affected in BCR-ABL1 expressing cells. Silencing and overexpression studies suggested that mRNA binding proteins regulates BRCA1 mRNA translation and stability. Studies based on site directed mutagenesis revealed that phosphorylation pattern plays a key role in determining of HuR effect on BRCA1 mRNA translation in BCR-ABL1 expressing cells. Currently we use BRCA1 Stellaris RNA FISH probes and SmartFare RNA probes to visualize BRCA1 mRNA together with mRNA binding proteins. Altogether, we showed a novel mechanism affecting BRCA1-dependent signaling in CML, in which BCR-ABL1 expressing cells modulate translation of BRCA1 mRNA, leading to protein downregulation. This may ultimately contribute to genomic instability and provide justification for targeting PARP1 and/or RAD52 to induce synthetic lethality in CML.

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TUE-166
Multitargeted tyrosine kinase inhibitors in regulation of growth and survival of acute myeloid leukemia cells

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In spite of continuous progress in therapy of acute leukemia, treatment failures still occur frequently. Mutations affecting genes for tyrosine kinases and their signaling pathways result in abnormal proliferation and lead to acute myeloid leukemia (AML). The application of preparations curbing the impact of this disorder might contribute to further improvement of its curability.

The aim of this work was to determine the influence of selected inhibitors of c-KIT receptor tyrosine kinase on growth and survival of AML. We compared the antitumor activities of the multitargeted tyrosine kinase inhibitors (imatinib, nilotinib, midostaurin and dasatinib) to determine which inhibitor causes the strongest cytostatic and cytotoxic effect on AML cells.

The human AML cell lines HL-60, GDM-1, Kasumi-1 and MV-4-11 were cultured in RPMI 1640 containing 10% or 20% fetal bovine serum. Cell proliferation was determined by hemocytometer counts and MTS assay. The investigated tyrosine kinase inhibitors were also examined for their cytotoxic potential and ability to induce tumor cell apoptosis or necrosis. The percentage of apoptotic cells was determined by differential staining with Hoechst No. 33258 and propidium iodide (PI) and FACS analysis using Annexin V/PI staining.

The exposure of acute myeloid leukemia cells to investigated inhibitors at the concentration ≥ 0.01 μM resulted in dose-dependent suppression of proliferation compared to the control. Imatinib, nilotinib, midostaurin and dasatinib completely inhibited growth of AML cell lines. Both differential staining and FACS analysis showed independently that investigated inhibitors induce apoptosis of AML cells. The percentage of apoptotic cells was increased in a dose-dependent manner by treatment with inhibitors.

Midostaurin and dasatinib are more potent inhibitors of cellular proliferation than imatinib and nilotinib. Moreover, they demonstrate stronger proapoptotic effects. Tyrosine kinase inhibitors such as imatinib, nilotinib, midostaurin and dasatinib represent a promising class of therapeutic agents for the treatment of AML.

Keywords: acute myeloid leukemia, receptor c-KIT, tyrosine kinase inhibitors (TKIs).

TUE-167
Muscarinic acetylcholine receptors mediate rps6 phosphorylation in SNU-407 colon cancer cells via two distinct pathways

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Muscarinic acetylcholine receptors (mAChRs) are involved in the regulation of diverse cellular functions, including cell growth and proliferation. In this study we examined the signaling pathways by which mAChRs activate ribosomal protein S6 kinase 1 (S6K1), a key regulator of cell growth and proliferation, in SNU-407 colon cancer cells. Treatment of the cells with carbachol stimulated phosphorylation of T389 of p70 S6K1 in a time-dependent manner. The PI3K inhibitor LY294002 or the mTOR inhibitor rapamycin almost completely blocked carbachol-stimulated S6K1 activation, indicating that mAChRs mediate S6K1 activation through the PI3K/AKT/mTOR pathway. We observed, however, that carbachol-stimulated phosphorylation of ribosomal protein S6 (rpS6), a downstream target of S6K1, was only partially inhibited by LY294002 or rapamycin. When the cells were treated with the MEK inhibitor U0126, carbachol-stimulated rpS6 phosphorylation was abolished in the presence of either of these inhibitors. These results suggest that the ERK pathway as well as the PI3K/AKT/mTOR/S6K1 pathway is responsible for mAChR-mediated rpS6 phosphorylation in SNU-407 colon cancer cells.

Keywords: Muscarinic acetylcholine receptor, Ribosomal protein S6 phosphorylation, SNU-407 colon cancer cell.

TUE-169
New anticancer compounds from sea cucumbers: molecular mechanisms of action

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Background: Cytotoxic activity of sea cucumber glycosides against different types of cells and cell lines including human tumor cell lines has been studied for many years. However, the molecular mechanism(s) of antitumor action of triterpene glycosides on the cancer cells remains unclear. Present work reports a continuation of investigations of monosulfated triterpene glycoside, cucumarioside A_2-2, isolated from the Far-Eastern sea cucumber *Cucumaria japonica*. It describes a study of glycoside anticancer activity *in vivo* and glycoside interaction with mouse Ehrlich carcinoma cells *in vitro*.

Methods: The cytotoxicity of cucumarioside A_2-2 and its effect on apoptosis, cell cycle, DNA biosynthesis, p53 activity, multidrug resistance (MDR), and glycoside antitumor action against Ehrlich carcinoma was studied.

Results: Cucumarioside A_2-2 influences tumor cell viability at micromolar concentrations. The EC_{50} for glycoside estimated by non specific esterase assay and MTT assay was 2.1 and 2.7 mM, respectively. Cucumarioside A_2-2 at sub-cytotoxic range of concentrations exhibits a cytostatic effect by blocking cell proliferation and DNA biosynthesis in the S phase. It may induce apoptosis in tumor cells through caspase-dependent way bypassing activation of p53-dependent segment. It was found that cucumarioside A_2-2 blocks the activity of membrane transport P-glycoprotein in the mouse Ehrlich ascites carcinoma cells. It increases the upload and intracellular concentration of cytostatic doxorubicin, and prevents in this way an efflux of acticancer drug from the cancer cells.

Conclusion: The anticancer and pro-apoptotic properties of cucumarioside A_2-2 may be due to direct interaction of the glycoside with tumor cells. The *in vivo* anticancer effect of cucumarioside A_2-2 may be associated with the ability of the drug to arrest the cell cycle in the S phase and induce programmed tumor cell death. Since the interaction with cancer cells results in decrease of MDR, this glycoside may be considered as potential inhibitor of multidrug resistance of tumor cells and can be used in combined anticancer therapy.

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Keywords: antitumor activity, apoptosis, cell cycle.

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TUE-170

New benzo[b]furane and dicarboximide derivatives with anticancer activity - studies on the mechanism of action in human cells

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There is a permanent need for new anticancer drugs with improved pharmacological profiles. Following that demand, Screening Laboratory at Department of Bioorganic Chemistry (CMMS PAS, Lodz) tested a set of 80 benzo[b]furanes or dicarboximides developed in Department of Medical Chemistry of Warsaw Medical University. Among them, several entities exhibited remarkable cytotoxic properties [1,2,3]. Interestingly, some compounds displayed selective toxicity towards human leukemia cells (K562, HL-60, MOLT-4) and were non-toxic to adherent cancer cells (HeLa, CFPAC) neither to normal endothelial cells (HUVEC). We have shown that these compounds induce the programmed cell death (apoptosis) in leukemia cells, which is the desired mechanism of action for anticancer drugs. To give further explanation of the mechanism of cytotoxicity of the novel compounds, the profiling of gene expression (TaqMan Human Apoptosis Array; Applied Biosystems) was performed in leukemia cells treated with the active compounds. The studies showed significantly increased expression (2-fold or more, $p < 0.05$) of several genes involved in regulation of apoptosis. The ability of these compounds to interact with DNA was explored by circular dichroism spectroscopy and by a plasmid DNA cleavage with restrictases. The selected compounds inducing apoptosis of leukemia cells, possibly by intercalating to DNA, can be considered promising leads for further development of anticancer drugs.

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Keywords: cytotoxicity, apoptosis, leukemia.

TUE-171

New role for p130Cas as a regulator on the cellular responsiveness to TGF-β-induced growth inhibition in cancer cells

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Transforming growth factor-beta (TGF-β) suppresses the initiation of tumors by causing the cell cycle arrest and the loss of the anti-proliferative function of TGF-β is a hallmark of many cancers. Here we report that p130Cas plays a role in determining the cellular responsiveness to TGF-β-induced growth inhibition in cancer cells. Analysis of the tyrosine phosphorylation levels of p130Cas revealed higher levels of phosphorylation in cancer cell lines (MCF7 and A375) than in corresponding normal cell lines (MCF10A and MEL-STV). Contrast to normal cells, the cancer cells showed the resistance to TGF-β-induced not only Smad3 phosphorylation and p21 expression but also growth inhibition. However, silencing p130Cas was sufficient to restore Smad3 phosphorylation and p21 expression as well as the susceptibility to TGF-β-induced growth inhibition. Interestingly, overexpression of p130Cas accelerated TGF-β-induced epithelial-mesenchymal transition. In sum, our results suggest that elevated expression or/and phosphorylation of p130Cas contributes to the resistance to TGF-β-induced growth inhibition and thus to the initiation and progression of human cancers that harbor an active integrin signal.

Keywords: Cancer, p130Cas, TGF-beta.

TUE-172

NF-kB and p53-dependent signalling pathways are involved in cellular response to ionizing radiation

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Signalling pathways that depend on p53 or NF-kB transcription factors are essential components of cellular response to stress. Both proteins, which participate in regulation of the expression of numerous genes involved in cell cycle arrest, DNA repair, apoptosis, immune response and inflammation, can be activated by the same stimuli and the final cellular outcome is determined by the crosstalk between them. Here we aimed to characterize the effect of ionizing radiation (IR) on regulation of expression of genes controlled by these transcription factors.

U2-OS human osteosarcoma cell line was used as an experimental model. The clonogenic assay was performed to characterize its radiosensitivity, and then two doses: 4 Gy and 10 Gy (LD_{50} and LD_{100}, respectively) were used for further analyses. Activation of the NF-kB and p53 pathways was monitored by Western-blotting. Expression of selected target genes dependent on NF-kB and/or p53 was assessed by qRT-PCR at different time points after irradiation (from 30 minutes to 24 hours). Moreover, analysis of RelA (NF-kB subunit) and p53 binding to promoter regions of selected genes was performed using ChIP-qPCR.

Exposure of the U2-OS cells to IR resulted in strong activation of both NF-kB and p53 pathways, which was observed as IkBa phosphorylation (Ser32) and subsequent NF-kB nuclear translocation, and by p53 accumulation (irradiation with 10 Gy resulted in faster and stronger activation of NF-kB). As a consequence, both transcription factors were recruited to promoter regions of analyzed target genes (e.g. *IL8*, *CDKN1A*). In general, IR increased expression of NF-kB-dependent genes: exposure to 10 Gy resulted in increased activation of genes involved in negative feedback loop (e.g. *TNFAIP3*). In contrast, p53-dependent genes (e.g. *HDM2*) were activated after exposure to 4 Gy and repressed after exposure to 10 Gy.

We concluded that both p53 and NF-kB-dependent pathways were activated in cells exposed to IR, yet specific mechanism of the response was affected by dose of radiation (and putatively the level of radiation induced DNA damage).

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Keywords: Cancer signaling, Signaling pathway, transcription factors.

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TUE-173
Notum inhibits TRAIL-induced cytotoxicity

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Our immune system provides a key barrier against tumour formation, for example releasing anti-tumour substances in the extracellular environment. TRAIL is a powerful factor produced by the immune cells, which selectively kills tumour cells but not normal cells.

On the other hand tumours can release molecules contributing to cancer development and progression. Notum is a secreted phospholipase with target selectivity towards specific GPI-anchored proteins such as Glypicans. It has been found to be over-expressed in tumours with constitutively active Wnt signalling but rarely in normal adult tissues.

Our results show that Notum protects cancer cells from TRAIL mediated anti-tumour activity. Here we present data on the mechanism of action of Notum on TRAIL and on the molecular players involved. These findings could contribute to explain why some tumours are resistant to TRAIL and suggest the possibility that inhibiting Notum could potentiate TRAIL based anti-tumour therapies.

Keywords: Glypicans, Notum (Notum Pectinacetylesterase Homolog - Drosophila), TRAIL (TNF-related apoptosis-inducing ligand).

TUE-174
Novel evidence that extracellular nucleotides direct Rhabdomyosarcoma metastasis and A1R identified as a novel receptor for extracellular AMP

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Purinergic nucleotides including Adenosine triphosphate (ATP) play a pivotal role in intracellular energy transfer. Evidence accumulates that they can be also released form the cells in particular if cells are damaged and cell membranes are leaky. For example both necrotic and apoptotic cells release ATP and other nucleotides that constitute 'danger signals' belonging to the family of alarmines. This happens in particular at sites of mechanical stimulation, hypoxia, tissue injury or inflammation. Extracellular nucleotides (ATP, UTP, ADP) activate specific purinergic (P2) receptors, and adenosine interacts with its own receptors called P1 receptors (A1, A2A, A2B, A3). Interestingly, the receptor for AMP is still unidentified.

Rhabdomyosarcoma (RMS) is the most common sarcoma in children. RMS accounts for approximately half of all pediatric soft tissue sarcomas and 15% of all pediatric solid tumors. Tumor microenvironment has a key role in supporting tumor growth and affects host-tumor interaction. Recent data show that solid tumors exhibit a gradient of adenosine concentration from the centre to the periphery, that is higher than the surrounding healthy tissue. In addition, many tumors over-express enzymes involved in the catabolism of extracellular nucleotides and in the generation of adenosine.

The aim of this study was to investigate the effect of purinergic nucleotides and their receptors on migration, invasion and proliferation of RMS cells. We employed in our studies several alveolar and embryonal RMS cell lines and first evaluated them for expression of membrane receptors for extracellular nucleotides. Next we performed several functional studies on cells exposed to ATP, ADP, AMP such as i) chemotactic responsiveness to purine gradient, ii) effect of purines on adhesion, iii) phosphorylation of AKT and MAPKp42/44 kinases and iii) effect of purines on cell proliferation. In some experiments we employed receptor inhibitors and applied shRNA strategy to assess involvement of A1 receptor.

We report for a first time that the purine nucleotides: ATP, ADP, AMP and adenosine, which are released during physiological stress from damaged cells, affect the migration of rhabdomyosarcoma cells. Rhabdomyosarcoma cells are characterized by high expression of purinergic receptors P2Y and adenosine receptors A1 and A2A. While ADP and ATP act through P2Y1 receptor adenosine interacts with A2A receptor. We also report for a first time employing selective inhibitors and shRNA strategy that AMP signals through A1 receptor.

Keywords: cancer, metastasis, rhabdomyosarcoma, purinergic signaling.

TUE-175
Novel mTOR isoform and tumorigenesis

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The mTOR plays a striking role in signal transduction and regulates cell growth, metabolism, proliferation and survival in response to nutrients and growth factors. Deregulation of mTOR signaling pathway has been associated with numerous pathologies, including many human cancers, Alzheimer′s disease, obesity and type II diabetes.

There is only one gene encoding mTOR. Therefore the diversity of the mTOR-mediated signaling is conferred by two functionally and structurally distinct multiprotein complexes with the well known mTORalpha in their core. Previously in our Laboratory for a fist time mTOR splicing isoform (mTORbeta) was identified. This isoform differs from well known mTORalpha leaking HEAT and partially FAT domains but carrying intact Kinase domain. It has been shown that mTORbeta is an active protein kinase that mediates downstream signaling through complexing with Raptor and Rictor proteins and, significantly, is a potential oncogene.

Recently, we have identified a novel mTOR isoform – mTORdelta. Importantly, this isoform has another principle of isoform formation then mTORbeta (unpublished data). Deletion in kinase domain resulted in frame shift within 3' - region and finally translation of unique protein sequence which is absent in mTORalpha. So, current research proposal is focused on clarifying the involvement of mTORdelta in tumorigenesis. Characterization of oncogenic and signaling activities of mTORdelta and its expression in cancer will offer new perspective target for cancer therapy includes rapamycin insensitive tumor types.

Early using RT-PCR of various cell lines we have identified a novel splicing isoform mTORdelta. The aim of this study was to characterize the functional role of mTORdelta in tumorogenesis. So, initially, we have generated polyclonal antibody against mTORdelta and specific antibodies against Raptor and Rictor which are applicable in various immunoassays. Then we have shown by Western-blot analysis that mTORdelta expressed in different cell lines *in vivo*. Next, HEK293 stable cell line overexpressing mTORdelta has been generated. To examine the oncogenic potential of mTORdelta the various assays have been done: MTT assay to analyze the cellular activity and proliferation; Soft Agar Assay to measure cell anchorage-independent proliferation potential; Scratch Migration Assay to study the migration capacity of cells. In result, HEK293 stable cell line overexpressing mTORdelta demonstrates a low proliferative potential and not considerable migration capacity in compare to control HEK293 cell.

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In contrast to mTORbeta isoform, mTORdelta can inhibit cell proliferation. To elucidate the mechanism by which cell proliferation may be inhibited by mTORdelta now we are exploring its effect on the cell cycle progression by flow cytometric analysis.

Keywords: cell signalling, mTOR, splicing isoform.

TUE-177
Novel selective inhibitor against CDK9, a new target in antitumor therapy

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Background: Cyclin-dependent kinase 9 (CDK9) was discovered in the early 1990s as a member of cdc2-like serine/threonine kinase family. In the past two decades CDK9 activation/malfunction was observed in proliferative disorders, infections, cardiological diseases and – in combination with other kinases – in inflammation. Thus CDK9 is now an attractive target for kinase inhibitor drug research in various indications including antitumor therapy.
Objective: The aim of our study was the identification and the biological evaluation of new, potent small-molecule CDK9 inhibitors as anticancer agents.
Results: In primary screen compounds from Vichem's NCL, (library of kinase inhibitors) were screened for 18 cell lines with *CellTiter-Glo* Luminescent Cell Viability Assay. The ratio of survived cells and the untreated samples (positive control) was examined. We further investigated the effective compounds which showed more than 75% inhibition in 10 μM and determined their IC_{50} values for NSCLC cell lines. Compounds which had IC_{50} value lower than 2 μM were chosen for additional experiments. Using the DiscoverX panel of KINOMEscan Technology Platform the potential target of the hit compounds was identified. We developed IMAP FP technology based in-vitro assays for screening the analogues of the hit compound on CDK2, CDK4 and CDK9 kinases. We screened these compounds at 10 μM concentration on three kinases then we determined IC_{50} values of the most effective ones. In this way the selectivity of the hit compounds were revealed also. Clonogenic assay was performed to elucidate the colony forming ability and to describe the proliferative potential of drug treated cancer cells. The apoptotic effect, inhibition of migration of tumor cells, solubility and permeability of hit compounds were also determined.
Conclusions: Promising lead molecules have been identified and characterized in order to achieve newly synthesized, selective CDK9 inhibitors.

Authors are grateful to National Innovation Office (NIH, Hungary) for financial support, grant numbers: NKFP_07_A2-NANODRUG and TECH_08_A2-STEMKILL.
Keywords: antitumor therapy, Cyclin-dependent kinase 9, non-small cell lung cancer.

TUE-178
Nuclear localization of formyl peptide receptor 2 in human lung cancer cells

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GPCRs are the largest group of membrane receptors in eukaryotes. They exhibit seven transmembrane-spanning domains, three extracellular and three intracellular domains. Several agonists may interact with GPCRs triggering signalling cascades via G-protein-associated activation. Conventional models of GPCRs signalling describe receptor activation at plasma membrane level leading to downstream signalling. Nevertheless, GPCRs may localize to and signal at the nuclear membrane.

Formyl-peptide receptors 1, 2 and 3 (FPR1, FPR2, FPR3) belong to the GPCR super-family and are coupled to pertussis toxin (PTX)-sensitive Gi proteins. WKYMVm, a modified peptide isolated by screening synthetic peptide libraries, binds FPR2 with high efficiency. FPR2 activation results in different intracellular responses in a ligand-specific fashion[1]. These include the transactivation of many Tyrosine Kinase Receptors, such as EGFR[2], c-Met[3] and VEGFR.

We identified by analysis *in silico* of FPR2 sequence a nuclear localization signal (NLS) 227-KIHKK-231 in the third intracellular loop. Western blot analysis performed on purified nuclear proteins and immunofluorescence assays confirmed the nuclear localization of FPR2. We used a receptor binding assay to investigate specific binding of WKYMVm on purified nuclei of CaLu-6 cells. The experiments performed by using I^{125}-WKYMVm revealed a binding that was specifically displaced by increasing concentration of unlabeled ligand. The saturability of the nuclear membrane binding sites for WKYMVm was tested by adding increasing concentration of I^{125}-WKYMVm and receptor binding analysis predicted a Kd = 245pM. Moreover, exposure of purified CaLu-6 nuclei to WKYMVm for different times induced the rapid phosphorylation of Myc and c-Jun, which was prevented by WRWWWW, a selective antagonist of the receptor, supporting that FPR2 is functionally expressed onto CaLu-6 nuclei. We also performed site directed mutagenesis experiments on putative NLS to identify amino acid residues involved in the nuclear localization of FPR2. The consensus sequence 227-KIHKK-231 was mutated in 227-KIHAK-231 (FPR2mut3) and 227-KIAAA-231 (FPR2mutbis). These were transfected in HEK293T cells (FPR2 null) and both western blotting experiments and immunofluorescence assays showed that FPR2mutbis did not localize onto nuclear membrane of HEK293T transfected cells, suggesting that the sequence 229-HKK-231 plays a key role in FPR2 nuclear localization.

These data support new concepts on function and regulation of FPR2 which could have an impact on development of new therapy.

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Keywords
cellular signalling, GPCR, nuclear localization.

TUE-179
OncoFinder, a unique method enabling qualitative and quantitative analysis of intracellular signaling pathway network activation using transcriptomic data

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Analysis of the complete transcriptomes is complicated by the problems linked with difficulties in understanding of the overall physiological (functional) features basing on the observed large-scale gene expression profiles. We propose a new biomathemati-

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cal method, OncoFinder, which for the first time enables performing both quantitative and qualitative analysis of the intracellular signaling pathway activation (SPA). This method is universal and may be used for the analysis of any physiological, stress, malignancy and other user-defined conditions at the molecular level. In contrast to other techniques, OncoFinder utilizes an algorithm that distinguishes the activator/repressor role of every gene product in each pathway. This unique feature makes it possible to quantitatively characterize activation of each signaling pathway in a given biosample. We show that the relative importance of each gene product in a pathway can be assessed using kinetic models for "low-level" protein interactions. For the first time, OncoFinder technique showed a strong potential to neutralize differences between the experimental data obtained using different transcriptomic methods like NGS and microarray hybridization, using most of the commercially available platforms. This approach also allowed us to characterize new SPA signatures as the better markers of cancer progression compared to the individual gene products. OncoFinder also makes it possible to correlate SPA with the success of anticancer therapy of the individual patients. To facilitate using OncoFinder platform by the biomedical society, we created a software package available freely for the academic research community. Some aspects of the enclosing technology were published by us recently: (Buzdin et al., http://journal.frontiersin.org/Journal/10.3389/fgene.2014.00055/abstract); (Zhavoronkov et al., http://journal.frontiersin.org/Journal/10.3389/fgene.2014.00049/abstract); Suntsova et al. PNAS 2013; Spirin et al. Leukemia 2014 (In press). The additional information about the software is available upon the request at: http://www.insilicomedicine.com/ and http://pathwaypharmaceuticals.com/. The authors enthusiastically support building international scientific collaborations and partnerships

Keywords: drug discovery, intracellular interactome, signaling pathway network profiling by screening transcriptomes, proteomes and epigenomes.

TUE-180
OTX2 controls the proliferation of cerebellar granule cell precursors

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Transit amplification of granule cell precursors (GCPs) is one of the most important processes of cerebellar development. In a short lapse of time, each precursor will divide symmetrically, in average, eight times, making it prone to mutations which may result in medulloblastoma (MB) formation, the most common pediatric brain tumor. Otx2, a homeodomain transcription factor, is expressed in GCPs throughout their maturation with a posterior-high to anterior-low gradient. Otx2 function in cerebellum development and MB formation is still poorly understood. Otx2 overexpression is observed in 75% of MB. Ectopic expression of Otx2 in medulloblastomas cell lines increases their tumorogenicity, while Otx2 inhibition in xenograft tumors extends animal survival. Moreover, in vitro studies indicate that Otx2 may directly activate cell cycle genes and inhibit differentiation in MB cells. Together, these data suggest that this factor may act as an oncogene in MB formation by driving uncontrolled GCPs proliferation. In this study, we investigated the role of Otx2 in cerebellum development and GCPs proliferation using a conditional Otx2 KO mouse model. This system allows tamoxifen-inducible Otx2 ablation at the time of cerebellum formation (between E16.5 and P21), without affecting early development, where Otx2 plays essential functions. We triggered Otx2 ablation at postnatal day 1 (P1) and analyzed the consequences at P3 and P5. In parallel, we performed a transcriptomic analysis of Otx2 KO cerebellum to identify Otx2 targets in this organ. We show that Otx2 invalidation consistently results in a reduction of the posterior cerebellum size and in a decrease in the number of proliferating GCP. We are now assessing the function of Otx2 in a murine model of MB. Understanding the role and identifying the targets of Otx2 in normal and tumoral cerebellar GCPs will allow to develop new therapies to fight against MB.

Keywords: Cerebellum, Granule cell precursors, Otx2.

TUE-181
Oxidative stress induces S-glutathionylation of STAT3 and impairs its phosphorylation: in vivo and in vitro study

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STAT3 is a transcription factor constitutively activated in highly malignant solid and hematological tumor. Activated STAT3 may participate in oncogenesis by stimulating cell proliferation and inducing resistance to apoptosis, as well as promoting tumour angiogenesis, invasion, and migration. The tight association of STAT3 activation with transformation and tumor progression makes STAT3 a potential therapeutic target. Recently, we identify naturally occurring sesquiterpene lactones as potent inhibitors of both IL-6-inducible and constitutive STAT3 activation in different cells lines. These terpenes induce the drop in intracellular GSH concentration. Furthermore, the glutathione ethylene ester, the cell permeable GSH form, prevents their inhibitory action on STAT3 phosphorylation. The disturbance in the intracellular redox state induces S-glutathionylation of STAT3. These findings suggest that these compounds is able to induce redox-dependent post-translational modification of cysteine of STAT3 protein in order to regulate its function.

Many reports support the idea that STAT3 S-glutathionylation might represent the key event in redox STAT3 modulation. However, a number of questions still remain unanswered with regard to the molecular aspects of STAT3 S-glutathionylation, and it is unclear whether there is more than one cysteine residues active for glutathionylation.

In order to clarify S-glutathionylation of STAT3 we perform an *in vitro* study.

We demonstrated that the combined treatment with diamide, a thiols specific oxidant, and GSH induces S-glutathionylation of STAT3 in the recombinant purified form. This effect was completely reversed by treatment with dithiothreitol, indicating that S-glutathionylation of STAT3 was related to formation of protein-mixed disulphides.

Since STAT3 was glutathionylated in the presence of oxidants, we examined whether this modification might affect STAT3 phosphorylation using *in vitro* kinase assay. As expected, S-glutathionylation significantly decreased the level of STAT3 tyrosine phosphorylation and the addition of a reducing agent before JAK2 incubation almost completely rescued STAT3 phosphorylation level. The addition of the bulky negatively charged GSH moiety impairs JAK2-mediated STAT3 phosphorylation very likely interfering with tyrosine accessibility thus affecting protein structure and function. This suggests the possible cross-talk between phosphorylation and glutathionylation and points out that STAT3 is susceptible to redox regulation. The data presented herein confirm the occurrence of a redox dependent regulation of STAT3 but more research is needed to identify the

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cysteine residues mainly involved in S-glutathionylation of STAT3.

Keywords: Oxidative stress, S-glutathionylation, STAT3 signaling.

TUE-182
p53-specific E3 ubiquitin ligase MDM2 interacts with lysine methyltransferase Set7/9

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TA53 protein is one of the most important tumour suppressors in mammals, also dubbed as 'guardian of the genome'. Acting as a transcription factor, p53 controls proliferation, metabolism, aging and apoptosis. The specificity of p53 response is controlled by a variety of post-translational modifications (PTMs). As a result, differentially modified p53 can selectively interact with various partner proteins at the same time. For example, dynamic lysine methylation of the C-terminal regulatory region in p53 by a number of lysine methyltransferases (KMTs) is one of the critical PTM that confer the specificity to the p53 activity.

One of these p53-modifying KMTs is Set7/9 (SETD7, Set9). This KMT, by methylating p53, on K372 enhances its subsequent acetylation, which, in turn, contributes to its stabilisation and activation. The main negative regulator of p53 is an E3 ubiquitin ligase MDM2, which blunts the p53-specific response by several means. Besides ubiquitinylation of p53, Mdm2 also interacts with various proteins that influence p53 activity. Given that Set7/9 increases p53 acetylation and Mdm2 decreases it, it is plausible that Set7/9 competes with Mdm2 for binding to p53.

In the present work we demonstrated a physical interaction between MDM2 and Set7/9. By using a panel of Set7/9 and MDM2 deletion mutants we have characterized this interaction in details using several approaches. At first, we expressed the recombinant GST-MDM2 and GST-Set7/9 deletion variants in bacteria and incubated the purified proteins with nuclear extract obtained from U2OS (human osteosarcoma) and H1299 (human lung carcinoma) cell lines. The interaction of MDM2 and Set7/9 was visualized by western blotting using anti-MDM2 and anti-Set7/9 antibodies. To validate these finding *in cellulo* we performed co-immunoprecipitations of co-transfected Flag-MDM2 and Flag-Set7/9 variants from U2OS and H1299 cells. Collectively, we demonstrated a novel interaction between MDM2 and Set7/9 and identified the protein regions responsible for this interaction. The role of this molecular interaction is discussed.

This study was supported by grants from the Goverment of Russian Federation no. 11.G34.31.0069, RFBR #14-04-32242 mol_a and RFBR #13-04-01024 A and from MCB Program at Russian Academy of Sciences.
Keywords: methylation, p53 tumor suppressor.

TUE-183
PAMAM coated magnetic nanoparticles as CpG-ODN carriers for activation of toll-like receptor 9

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Gene therapy is a powerful technique in the treatment of many life threatening diseases such as cancer. The major challenge in this therapy is to seek a safe and efficient delivery carrier for various gene materials. Carriers are designed to protect these molecules from degradation, improve their stability and facilitate the delivery of them to the site of action.

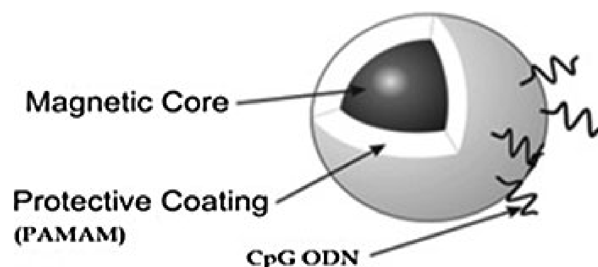

Fig. 1. Pathogen mimicking metal oxide nanoparticle.

CpG-oligodeoxynucleotide, the synthetic 24mer single stranded agonist of TLR9, containing unmethylated cytosine–phosphate–guanosine motifs (CpG-ODN) possess potential antitumor activity. This research aims to develop an appropriate carrier, with the ability of interacting with and delivering unmethylated CpG-ODNs into cells to activate toll-like receptor 9 (TLR9), which can be a very important process for therapy of cancers.

In our study, we utilized different generations of a three-layer magnetic nanoparticle composed of a Fe_3O_4 magnetic core, an aminosilane (APTS) interlayer and a cationic poly (amidoamine) (PAMAM) dendrimer to enhance the delivery of CpG-ODN molecules (Figure 1). The characterization of synthesized nanoparticles was performed by X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR), Transmission electron microscopy (TEM), and ZETA potential analyses.

Magnetic nanoparticles at different generations were loaded with CpG-ODN. Loading of CpG-ODN onto PAMAM coated magnetic nanoparticle was followed by agarose gel electrophoresis. The characterization of CpG-ODN-loaded nanoparticles was carried out by Nanodrop spectrophotometer, X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR), and ZETA potential analysis. Cellular internalization of bare nanoparticles and CpG-ODN-loaded nanoparticles were studied using different breast cancer cell lines, and cytotoxicity was determined by XTT analysis.

Results showed that the CpG-ODN-nanoparticles composites were successfully synthesized, can enter into tumor cells and inhibited cell growth in dose-and time-dependent means. In addition, more positive functional groups at the surface will make higher generations (G7, G6, and G5) of PAMAM coated nanoparticles a more suitable delivery system for CpG-ODN.

Taken together, our findings indicate that the developed system can be used for high transfection efficiency and effective targeting of TLR-9 in breast cancer cell lines, conferring a simple and noninvasive approach for cancer immunotherapy.

Keywords: Cancer immunotherapy, CpG-ODN, TLR-9.

TUE-184
Peculiarities of mTOR kinase subcellular localization in the human epithelial cells

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The mammalian target of rapamycin (mTOR) is a highly conserved serine/threonine kinase. It is one of the central links of several signaling networks, which provide signal transduction from hormones, growth factors and nutrients into the cell. mTOR is a part of two distinct complexes mTORC1 and mTORC2, which participate in the regulation of translation initi-

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ation, autophagy, ribosome biogenesis, transcription, and cell survival. The specific realization of these functions is provided by separation of mTOR substrates to the different cellular compartments. Deregulation of mTOR signaling was detected in several human diseases, including different types of cancer, type II diabetes and neurodegenerative disorders. Nowadays much attention is paid to the studying of mTOR subcellular localization. It will help to understand functioning of signaling networks and its possible therapeutic correction.

Different human epithelial cell lines, such as MCF-7, MCF-10A, HeLa, HepG2, A549, and also histological sections of the normal and malignant human breast tissues were used in the research. Colocalization of mTOR and different types of the cytosceletal proteins was studied by double immunofluorescent analysis and then visualized with confocal or fluorescent microscopy. To verify interconnection of mTOR kinase and cytokeratins we performed co-immunoprecipitation and PLA.

Application of anti-mTOR N-terminal antibodies detected fibrils-like structure in MCF-7 cells. So, possible colocalization of mTOR and different cytosceletal proteins was tested. We didn't reveal obvious relation between the location of mTOR kinase and β-tubulin. The convincing colocalization of mTOR and β-actin also was not detected. However, there was a strong colocalization of mTOR and cytokeratins. Application of anti-pan-cytokeratin antibodies revealed such colocalization in a set of the human cell lines and epithelial cells of the human breast tissue samples. Also the use of a series of alternative fixation and permeabilization regimens did not alter the link between mTOR kinase and keratins. Then we compared reaction of the anti-N-terminal mTOR antibodies, generated in our laboratory, with commercially available ones. Under identical conditions of the experiment all tested antibodies recognized mTOR kinase at the fibrils of the intermediate filaments. Co-immunoprecipitation along showed that anti-mTOR antibodies precipitated keratins from lysates of MCF-7 cells. Obtained data were confirmed by PLA.

So, it was shown cytoplasmic localization of mTOR that is in accordance to results of other authors. But for the first time we discovered the colocalization of mTOR kinase and cytokeratins and revealed that it is a general phenomenon in human epithelial cells.

Keywords: Cytokeratins, Human epithelial cells, mTOR.

TUE-185
Phosphatidylcholin/sphingomyelin metabolizm crosstalk inside the nucleus chromatin after the cisplatin in vivo action

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It is known that the phospholipids represent a "minor component" of chromatin. It has been highlighted that these lipids are metabolized directly inside the nucleus thanks to the presence of the enzymes related to their metabolism such us neutral sphingomyelinase, sphingomyelin-synthase, reverse sphingomyelin-synthase and phosphatidylcholine–specific phospholipase C.

The present research was aimed to investigate the crosstalk of the lipid metabolisms in chromatin isolated from rat liver and thymus in vivo under the antitumour platinum drug cisplatin action. The possible mechanism of the crosstalk was discussed considering the recent literature in the field.

The in vivo 24-hour effect of cisplatin on rat liver and thymus cells nuclear phospholipids was investigated. Rat liver nuclear phospholipids were fractionated by microTLC technique. The quantitative estimation of fractionated phospholipids was carried out by computer program FUGIFILM Science Lab. 2001 Image Gauge V 4.0, which was destined for densitometry.

The small decrease of phosphatidylcholine quantity (14%) and the significant diminution of sphingomyelin (44%) in liver chromatin preparations as well as the reverse changes of these phospholipids in thymus chromatin preparations (decrease of phosphatidylcholine content over 40% and sphingomyelin quantity near 14%) was established.

The results confirm the probable influence of cisplatin on activity of sphingomyelinase and sphingomyelin-synthase and show that in chromatin exists a phosphatidylcholine/sphingomyelin metabolism crosstalk which regulates the intranuclear ceramide/diacylglycerol pool. The results also indicate that cisplatin may affect on phosphatidylcholine/ sphingomyelin crosstalk mechanism which exists in nuclei.

Keywords: chromatin, cisplatin, phospholipids.

TUE-186
Phosphorylation of CacyBP/SIP by casein kinase II – regulation by calcium binding protein S100A6

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CacyBP/SIP (Calcyclin binding protein/Siah-1 interacting protein) was originally purified from Ehrlich ascities tumor cells as an S100A6 ligand (Filipek & Wojda 1996; Filipek & Kuznicki 1998). Up to now, many papers have been published regarding tissue and cell distribution and binding partners of the CacyBP/SIP protein. Among these partners are members of calcium binding proteins from the S100 family, proteins involved in ubiquitination such as Siah-1 and Skp1, cytoskeletal proteins such as tubulin, actin, tropomyosin, and ERK1/2 kinase (Schneider & Filipek, 2011). Interestingly, the interaction of CacyBP/SIP with ERK1/2 kinase, due to phosphatase activity of CacyBP/SIP, results in dephosphorylation of ERK1/2 and has an influence on phosphorylation of the Elk-1 transcription factor. There is no data up to now as to the physiological role of the interaction between CacyBP/SIP and calcium binding protein, S100A6. Since the theoretical analysis showed that CacyBP/SIP might be phosphorylated by casein kinase II (CKII) on threonine 184, which is located close to the S100A6 binding site, in this work we examined whether CacyBP/SIP is phosphorylated by CKII and whether S100A6 has an effect on CacyBP/SIP phosphorylation/activity, and in consequence on cell growth and proliferation. CKII plays a central role in the control of a variety of pathways engaged in cell proliferation, transformation, apoptosis and senescence. Interestingly, in highly proliferating cells/tumors, an up-regulation of CacyBP/SIP and S100A6 is observed. Thus, phosphorylation of CacyBP/SIP by CKII and its regulation by S100A6 might be considered as one of the factors involved in cell proliferation/tumorigenesis. This work was supported by grant rom the National Science Center (NZ1/00595) and by statutory funds from the Nencki Institute of Experimental Biology.
Keywords: CacyBP/SIP, phosphorylation, S100A6.

TUE-187
PML involvement in breast cancer cell proliferation and fate

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Breast cancer is the most frequent cancer worldwide for females. Currently, the cancer stem cell (CSC) hypothesis suggests that

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many cancers, including breast cancer, are sustained by a subpopulation of cells that share common properties with embryonic and adult stem cells. CSCs are responsible for the tumor's self-renewal, heterogeneity and resistance to therapy. Because of their unique features CSCs are promising targets for novel anticancer therapeutic approaches. Recent studies showed that promyelocytic leukemia (PML) protein regulates the function of hematopoietic stem cells (HSCs) and leukemia initiating cells (LICs)[1]. Apart from the hematopoietic system, PML has been found to regulate the stem cells of the nervous system and the mammary gland[2]. PML is the core component of subnuclear structures, called PML-nuclear bodies, which are involved in various cellular functions including proliferation, apoptosis, senescence, transcription and self-renewal. PML is a well-established tumor suppressor gene and its expression is lost in primary breast cancer tissue samples[3]. However, it was recently found that PML is overexpressed in a subset of breast cancers[4]. In an effort to understand the molecular mechanisms underlying the involvement of PML in breast CSCs regulation we studied the effect of PML overexpression in breast cancer cell lines. Using two different experimental conditions, monolayer and mammosphere culture (CSCs enrichment), we compared the gene expression profile of breast cancer cells overexpressing PML. Primary data show that PML overexpression represses proliferation of breast cancer cells cultured in both conditions. Differentially expressed genes involved in proliferation, apoptosis, DNA repair and differentiation are analyzed by transcriptome profiling.

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Keywords: PML, breast cancer.

TUE-188
Potential side effects of resveratrol and baicalein in prostate cancer at physiological levels: a whole-transcriptome study using LNCaP cell line

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Introduction: Most flavonoids are known for their antioxidant qualities. These compounds act as agonists or antagonists of ligand-dependent transcription factor called aryl-hydrocarbon receptor (AHR). Besides direct transcriptional regulation, antioxidants can interacts with estrogen receptor (ER) and alters the expression of ER responsive genes. The mechanism of this is yet not well understood, whereas it was reported to be dose-dependent. The current study focuses on the processes affected by flavonoids at physiological levels that can lead to male reproductive system dysfunction. In the study, ER and AHR positive human prostate cancer cell line LNCaP was used as a model to investigate global gene expression in male reproductive cells, exposed to two natural AHR ligands, baicalein and resveratrol, in presence and absence of oestradiol.

The Study: RNA was extracted from cells, treated with oestradiol, baicalein, resveratrol at physiological concentrations at several time points, following cDNA synthesis. Chromatin was immunoprecipitated using ERα antibodies and HTP sequencing was applied for all samples. Analysis of the sequencing data revealed that the expression of thousands of genes was significantly regulated in LNCaP cells after treatment with baicalein and resveratrol. Among the genes with the highest expression rate change after resveratrol and baicalein treatments were such genes as *CCND2, CDH11, COL1A2, DLC1, EPHA4*. In addition, baicalein showed remarkable influence on ER ability to bind DNA and the expression of ER regulated genes. Interesting gene expression patterns ($p < 0.05$) were observed for the genes associated with carcinogenesis, endocrine disruption and spermatogenesis. RNA-seq and ChIP-seq results refer to the direct action of baicalein and resveratrol on ER signaling, without the need for AHR activation and suggest a set of ER-responsive genes directly regulated by baicalein and resveratrol.

Conclusions: A set of unique genes regulated by baicalein and resveratrol demonstrate the specific action of flavonoids at physiological concentrations in male reproductive tissues, whereas the ER-responsive genes, directly regulated by baicalein reveal their potential role in endocrine disruption, male infertility and prostate cancer. The results of whole transcriptome study suggests both positive and potential side effects of studied flavonoids. *In vivo* studies are currently being performed to analyse the results of the current study.

Keywords: baicalein, LNCaP, resveratrol.

TUE-190
Prediction of Bevacizumab drug efficacy on the treatment of colon cancer using bioinformatic algorithm OncoFinder

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One of the major problems of modern oncology deals with the efficiency of choosing of anticancer therapy that would be successful for treatment of an individual patient. A recently developed bioinformatic platform termed OncoFinder makes it possible to create a model of individual target drug efficacy to the patient basing on the high-throughput gene expression data. Microarray or Next Generation Sequencing – based gene expression profiles of tumor samples are investigated against datasets obtained for the normal tissue samples isolated from healthy donors. OncoFinder algorithm assesses the degree of pathological changes in the pro- and anti-mitotic signaling pathways and the ability of target anticancer drugs to compensate these changes. Bevacizumab is a target drug that belongs to the family of "mabs", i.e. therapeutic monoclonal antibodies. Bevacizumab binds to and inhibits the biological activity of human*vascular endothelial growth factor* (VEGF) protein, which is involved in many processes accompanying cancer development, including vascularization. Bevacizumab is routinely used for treatment of colorectal, kidney, non-small cell lung cancer, and certain brain tumors.We investigated the utility of OncoFinder algorithm in predicting the clinical efficiency of Bevacizumab for treatment of the patients with colon cancer. The gene expression profiles of the cancer tissue specimens taken from 26 patients that were either good or poor responders to the Bevacizumab treatment were processed with the OncoFinder platform and compared to the normal tissues. We show here that the OncoFinder-generated measures of potential drug utility termed "Drug Scores" differed greatly among the

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responder- and non-responder groups ($p < 0.003$). These results evidence that the OncoFinder platform if highly effective for prediction of the efficiency of colon cancer treatment with Bevacizumab.

Keywords: None.

TUE-191

Preferential binding of mutant p53 proteins to supercoiled DNA containing structural motifs in vitro and in cells

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It was observed that the role of wild type p53 protein (wtp53) as a tumor suppressor depends on sequence specific interactions with promoters of target genes. This type of specific binding to DNA is performed by central DNA-binding domain (DBD) of p53 protein. Hot spot mutant p53 proteins (mutp53) are not able to bind to DNA sequence specifically because of mutations in their DBD domains. However, the binding of mutp53 proteins to DNA is assumed to be involved in mutp53-mediated repression or activation of several mutp53 target genes. The study of sequence specific binding of p53 protein revealed an importance of DNA topology in selective binding to responsive sequences. Structure specific binding of p53 protein is executed mainly by C-terminal DNA-binding domain (CTDBD), which is active also in mutp53 proteins.

In our work we investigated the importance of DNA topology on mutp53-DNA recognition *in vitro* and in cells by study of the interactions of seven hot spot mutp53 proteins with topologically different DNA substrates (supercoiled, linear and relaxed) containing and/or lacking mutp53 binding sites (mutp53BS). We used in this study a variety of techniques based on electrophoresis and immunoprecipitation. Our results show that all seven hot spot mutp53 proteins (R175H, G245S, R248W, R249S, R273C, R273H and R282W) retained the ability of wtp53 protein to preferentially bind supercoiled DNA, while linear or relaxed circular DNA was a poor substrate. The preference of mutp53 proteins for circular DNA at native negative superhelix density (supercoil-selective binding) was further supported by competition experiments with linear DNA or relaxed DNA *in vitro* and *ex vivo*. Similar results were obtained with DNA containing specific mutp53 binding site from promoter of *MSP/MST1* gene. Monoclonal antibodies specific for C-terminal or N-terminal epitopes of p53 protein were used for confirmation of important role of CTDBD domain in recognition of DNA topology. The preferential binding of mutp53 protein to supercoiled mutp53BS was explored also in cells by using chromatin immunoprecipitation. Moreover, an influence of DNA topology in p53 regulation of *BAX* and *MSP/MST1* promoters was detected by the luciferase reporter assay *in vivo*.

This contribution brings new information on mutp53 binding to topologically constrained DNA substrates and their biological consequences. Our work could be helpful for wider understanding of mutp53 protein functions in carcinogenesis.

Keywords: DNA topology, mutant p53 protein, protein – DNA interactions.

TUE-192

Preparation of fluorescent IgG monoclonal antibody conjugated PHB coated magnetic nonoparticles for imaging and cancer therapy

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Conjugation of monoclonal antibodies to magnetic nanoparticles is an effective method for cancer diagnosis and treatment. In this study the fluorescent IgG monoclonal antibody was conjugated to Polyhydroxybutyrate coated magnetic nanoparticles (PHB-MNPs). The conjugation process was carried out with the surface activation method of EDC (ethyl (dimethylaminopropyl) carbodiimide) and NHS (N-Hydroxysuccinimide). Fluorescent IgG monoclonal antibody conjugated PHB-MNPs were formed and incubated with MCF-7 cells prior to imaging. After washing the cells with PBS, they were observed by confocal laser scanning microscope. As a results, this method may be a useful method for detection of tumor cells, especially by MRI techniques.

Keywords: Fluorescent IgG monoclonal antibody, PHB, magnetic nanoparticle, cancer, imaging.

TUE-193

Prognostic relevance of cholinergic signalling changes in head and neck squamous cell carcinomas

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The traditional view of acetylcholine (ACh) acting solely as neurotransmitter has changed based on the findings demonstrating that non-neuronal cholinergic system is vital for various types of cells such as epithelial, endothelial and immune cells. Changes in the expression of ACh-linked genes were causally related with cell proliferation. So, excess of ACh is expected to exacerbate cholinergic inputs and by this means accelerate tumour growth. This research was addressed to explore a possible link of cholinergic signalling changes with head and neck cancer. For this, paired pieces of head and neck squamous cell carcinoma (HNSCC) and adjacent non-cancerous tissue (ANCT) were compared for their mRNA levels for ACh-related proteins and ACh-hydrolyzing activity. The measurement in ANCT of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities (5.416 ± 0.501 mU/mg protein and 6.442 ± 0.595 mU/mg protein) demonstrated that upper respiratory tract is capable of controlling the availability of ACh. In HNSCC, AChE and BChE activities dropped to 3.584 ± 0.633 mU/mg ($p = 0.002$) and 4.002 ± 0.418 mU/mg ($p = 0.001$). Moreover, tumours with AChE activity above 1.801 mU/mg (50th percentile) and BChE activity below 3.061 mU/mg were associated with increased patient overall survival. ANCT and HNSCC differed in mRNA levels for AChE-T and AChE-H, $\alpha7$ and $\alpha5$ of nAChR, and M3 and M2 mAChR. Our results suggest that the low AChE activity in HNSCC was associated with poor overall survival. So, the ChE activity level of head and neck carcinoma can be used as a reliable prognostic marker.

Keywords: cancer, cholinergic signaling, head and neck carcinoma.

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TUE-194
Prognostic value of caspase activity in breast cancer tumor progression

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Caspases are the main components of apoptosis (programmed cell death) and are generally divided into regulatory caspases 8, 9, and effector caspases, such as caspase 3, 6, and 7. The universal mechanism of activation of caspases involves their aggregation and self-cleavage that ultimately results in cell death. It is generally accepted that apoptosis avoidance is one of the strategies of successful tumor progression. Accordingly, lower activity of caspases inhibits apoptotic processes and is usually associated with predisposal to several tumors including breast cancer. The aim of this study was to evaluate association of protease activity of the regulatory and effector caspases with breast cancer and to further investigate its possible correlation between grade of an oncopathology and caspase activity. We used breast cancer tissues derived from patients with various grades of breast cancer progression ranging from the first to the third grade. There were total 43 samples of breast carcinoma diagnosed in patients at Oncology Clinic of Republic of Karelia (Russia) between 2011 and 2013, and 30 normal breast tissue controls. Caspases -3, -6, -8, -9 activity assessment in a tumor tissue and peripheral blood lymphocytes was carried out at the different stage of breast carcinogenesis for determination of caspases activity. Caspase activity was measured using fluorogenic substrate while cellular apoptosis was assessed by means of cytofluorometric assay. For tissue caspases, there was statistically significant correlation between downregulation of caspases activity and degree of tumor progression. This difference held true for caspases 3, 6, 8, and 9. In contrast, we saw no decrease in activity for caspases 6 and 9 from peripheral lymphocytes. Furthermore, caspases 3 and 8 showed increase in activity in peripheral lymphocytes. Specifically, there was statistically significant difference between stages 1-3 of tumor progression for caspases 3 and 8. Overall, these data show that the activity of caspases in tissue can be used as prognostic parameter for breast cancer tumors.

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Keywords: breast cancer, caspases, prognostic marker.

TUE-195
Protease Nexin-1 enhances migration and invasion of C6 glioma cells through up-regulation of urokinase plasminogen activator and matrix metalloproteinase-9/2

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Protease Nexin-1 (PN-1) or Serpine2 is a physiological regulator of extracellular proteases as thrombin and urokinase (uPA) in the brain. Recently, several experimental reports indicated that PN-1 is also implicated in some human cancers and further identifed as a substrate for matrix metalloproteinase (MMP)-9, a key enzyme in tumor invasiveness. In our study, we investigated the role of PN-1 in migration and invasive potential of glioma cells, using the rat C6 glioma cell line as stable clones transfected with pAVU+27 vector espressing PN-1 short-hairpin RNA. We find that PN-1 knockdown enhanced *in vitro* migration and invasiveness of C6 cells which also showed a strong gelatinolytic activity by *in situ* zymography. PN-1 silencing did not alter as prothrombin whereas increased uPA, MMP-9 and MMP-2 expression levels and gelatinolytic activity in conditioned medium from stable C6 cells. Selective inhibitors for MMP-9 (Inhibitor I) or MMP-2 (Inhibitor III) abolished the migration and invasive ability of PN-1 silenced cells in migration and matrigel invasion assays. Moreover, exogenous recombinant PN-1 added to the culture medium of silenced cells suppressed MMP-9 and MMP-2 gelatinolytic activity thus validating the specificity of PN-1 silencing strategy. Phosphorylation levels of extracellular signal-related kinases (Erk1/2 and p38 MAPK) involved in MMP-9 and MMP-2 signaling were increased in PN-1 silenced cells. Our findings suggest that PN-1 affects glioma cell migration and invasiveness through regulation of uPA and MMP-9/2 protein levels which contribute to the degradation of extracellular matrix during tumor invasion.

Keywords: Cell invasion, Matrix Metalloproteinases, Protease Nexin-1 (Serpine2).

TUE-198
Proteome and metabolome analysis of ovarian cancer ascites as cell-cell communication medium

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Ovarian cancer ascites is a native medium for cancer cells that allows investigation of the secretome of cancer cells in their natural environment. On the one hand, this medium is of interest as a promising source of potential biomarkers, on the other hand, as a medium for intercellular communication. Studies of the ascites with the use of omics technologies can help to understand the peculiarities of cancer cell activity in the organism and elaborate new therapeutic approaches. The aim of this study was to elucidate specific features of malignant ascites proteome and metabolome. Comparison of malignant ascites with those of cirrhosis allowed the revealing of the components specific for malignant ascites and omitting those that belong to systemic response to the ascites formation.

A novel combined approach to protein fractionation prior to mass spectrometry analysis allowed the identification of 1632 and 1139 proteins in ovarian cancer and cirrhosis ascites, respectively, 663 proteins were specific for malignant ascites. Importantly, this value was several times higher than the earlier published data. Interesting results were obtained by the functional analysis of proteomic data. We demonstrated that the major differences between cirrhosis and malignant ascites were observed for the cluster of spliceosomal proteins as well as large number of RNA-binding proteins was found in the malignant ascites.

In our GC/MS-based metabolomic analysis of the ovarian cancer and cirrhosis ascites, 129 compounds were found, and 89 of these compounds were identified as known metabolites. Forty-one compounds were considered to be statistically different

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according to the nonparametric Wilcoxon–Mann–Whitney test ($p < 0.05$) with Benjamini and Hochberg adjustment for p-values. Over one third of these compounds were identified only in the malignant ascites. The major significant differences between the malignant and control ascites were observed for fatty acids and their derivatives, which are known to be among the key components of intercellular signaling.

In summary this study extended our knowledge of the protein and metabolomic composition of the ovarian cancer ascites and revealed its specific features which were associated with the function of the ascitic fluid as a medium of interaction between the malignant cells and their environment.

Keywords: Ovarian cancer ascites, Proteomics, Metabolomics.

TUE-199
Proteomic and protein glycosylation changes associated with TGFBR2 deficiency in MSI colorectal cancer

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Members of the transforming growth factor (TGF)-signaling pathway are common targets for mutation in colon cancers. Deregulation of this pathway appears to play an important role in colon carcinogenesis by affecting TGF-mediated growth inhibition, apoptosis, and differentiation as well as other TGF-regulated processes. In particular, inactivating mutations of the TGF-ß-receptor type II (TGFBR2) occur in more than 90% of microsatellite unstable (MSI) colon cancers. In order to systematically analyze TGFBR2-deficiency-associated changes of the biochemical phenotype of MSI colon carcinoma cells we used the TGFBR2-deficient MSI colon carcinoma cell line HCT116 as a model system. Stable clones conferring doxycycline (dox)-inducible expression of a single copy wild type *TGFBR2* transgene were generated by recombinase-mediated cassette exchange (RCME). By applying a click-chemistry approach with azido-derivatized amino-acids or monosaccharides we specifically labeled newly synthesized proteins or post-translational glycan modifications after dox-induction as well as in uninduced cells and clicked a biotin residue to the metabolically labeled proteins or oligosaccharide chains. Finally we extracted the labeled proteins by streptavidine-coupled magnetic beads. Identification of the bound proteins was achieved by nano-HPLC-coupled Orbitrap mass spectrometry. A total of 76 proteins was found to be expressed in a TGFBR2-dependent manner: 40 individual proteins were only expressed in TGFBR2-deficient cells and 36 individual proteins were exclusively found in TGFBR2-proficient cells. For 19 proteins altered sialylation and for 21 proteins altered fucosylation was found.

Altogether this study, based on a combination of three technologies, i.e. RCME, click-chemistry and mass spectrometry, provides a versatile platform to analyze proteomic as well as posttranslational modifications caused by MSI-relevant target genes like TGFBR2. On the one hand this approach can help to systematically puzzle out the consequences of tumor-specific mutations in a major signalling pathway as exemplified by the TFGBR2 tumor suppressor, on the other hand this approach facilitates the identification of tumor markers that could be used for diagnostic and therapeutic applications.

Keywords: colon cancer, microsatellite instability, TGF-beta signalling.

TUE-201
Quantitative expression analysis of the apoptotic gene BCL2L12 in breast cancer: association with clinical and molecular prognostic parameters

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Introduction: Apoptosis is a highly orchestrated, genetically regulated form of cell death, the impairment of which is crucial in breast cancer (BC) development and progression. *BCL2L12*, a member of the *BCL2* family of apoptosis-related genes, has been studied in various malignancies, revealing its potential role as a tumor biomarker. It has been recently found that *BCL2L12* is subjected to alternative splicing, resulting in the generation of 13 alternatively spliced variants. The aim of this study was the quantification of *BCL2L12* splice variants 1 and 2 (v.1 and v.2) expression at the mRNA level and the assessment of their biomarker potential in BC.

Methods: Total RNA was extracted from 40 pairs of BC and normal tissues. Thereafter, RNA was reverse transcribed into first-strand cDNA, which in turn was used as template in a SYBR Green based Real-Time PCR assay. Relative quantification analysis was conducted using the comparative C_T (2^{-ddC_T}) method, and the associations of *BCL2L12* variants expression with various clinopathological parameters, were evaluated by statistical analysis.

Results: *BCL2L12* v.1 mRNA levels were found to be significantly ($p = 0.003$) higher in malignant compared to their matched non-cancerous breast tissues. Moreover, *BCL2L12* v.1 demonstrated increased expression in premenopausal women ($p = 0.026$) as well as in those with early TNM stage tumors ($p = 0.039$). Interestingly, significant *BCL2L12* v.1 upregulation ($p = 0.044$) was observed in triple negative BC. Regarding *BCL2L12* v.2, a negative correlation with patients' age was found (($r_s = -0.376$; $p = 0.017$), whereas increased *BCL2L12* v.2 expression levels were associated with advanced tumor grade ($p = 0.022$) and ER-negativity ($p = 0.01$).

Conclusion: Our preliminary results indicate a possible involvement of *BCL2L12* v.1 and v.2 in BC progression and suggest their potential as biomarker in this malignancy.

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Keywords: Apoptosis, Breast Cancer.

TUE-203
Redox role of STAT3 in cellular survival during oxidative stress

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Signal Transducer and Activator of Transcription 3 (STAT3) is a transcription factor that is essential for embryogenesis and is involved in the development and maintenance of several tissues,

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including the heart. In the heart, STAT3 is vital for ischaemic preconditioning, an adaptive process in response to transient hypoxia that prevents cardiomyocyte apoptosis, a contributory factor in the progression of ischaemic heart disease.

Recently, a small pool of STAT3 was found to reside in the mitochondria and thought to modulate mitochondrial output by way of the electron transport chain (ETC). Deletion of STAT3 from the heart causes a reduction in the activity of complexes I and II of the ETC. In cells derived from mouse embryos we observed modest decreases in complex I and II stimulated respiration as well as complex I activity upon STAT3 deletion. The mitochondria are responsible for the majority of ROS generated in most cells, which is caused by electron leakage from primarily complexes I and III of the ETC. The mitochondria therefore contribute largely to the intracellular redox potential (IRP).

In the stressed heart, decreased oxygen tensions alter the IRP and can cause a concomitant increase in the production of reactive oxygen species (ROS). We have shown that STAT3 is directly sensitive to ROS. Oxidation of conserved cysteines by peroxide decreased STAT3 binding to consensus serum-inducible elements (SIE) in vitro and in vivo. A version of STAT3 that cannot be oxidized has been created (RI-STAT3) as have MEFs expressing RI-STAT3 and wild type STAT3 (WT STAT3) on a STAT3 null background. MEFs expressing RI-STAT3 have a reduced growth rate and appear not to sense oxidative stress as readily as wild-type STAT3 (WT-STAT3).

Our aim is to understand the role of STAT3 in ischaemic preconditioning. We seek to fathom if oxidation of conserved cysteines within STAT3 alter STAT3 mediated gene expression and indeed if the later is coupled to STAT3's ability to maintain mitochondrial integrity and ATP production in the face of oxidative stress.

Keywords: cell survival, Oxidative stress, STAT3.

TUE-204
Redox-mediated P-gp and MRP1 transport activity in human CD19+ lymphocytes

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In the last decade, there are a lot of experimental data about close relationship between tumor progression and cellular redox state. It is known that the alteration of the redox balance plays a key role in cell response to the antitumor agents action and is an important regulator of the expression of protein-transporters associated with multidrug resistance (MDR). However, the question about the influence of redox disbalance on the P-gp and MRP1 functional activity in various human subsets of immunocompetent cells still hasn't been studied in detail. So, necessity for such investigations is important for explaining a possible failure of the conventional chemotherapy and modifying its approaches taking into account the nature of the MDR transporters functioning under redox state changing and for development an adequate treatment strategy under B- or T-cell leukemia.

In current work the influence of the substrates of membrane transport proteins P-gp and MRP1 are using under the treatment of B-chronic lymphocytic leukemia (analogs fludarabine and cladribine, doxorubicin and vincristine) on the reactive oxygen species (ROS) accumulation in the donor's total lymphocyte population, $CD19^+$ (B-cells) and $CD19^-$ (T- and NK-cells) subpopulations is investigated, as well as the P-gp and MRP1 transport activity in these cells is estimated. So these enable to describe P-gp and MRP1 functioning under the redox-balance changing that is mediated by the anticancer drugs metabolism.

It is shown that P-gp transport activity is reduced in donor's B-lymphocytes as compared with the T-and NK-cells, moreover redox balance is shifted toward oxidants in this conditions. In intact donor's $CD19^+$ and $CD19^-$ lymphocytes the P-gp functionality depends on the redox balance. ROS level alteration relative to the physiological range (under the drugs influence) simultaneously determines the P-gp transport function in donors B-lymphocytes.

In turn, MRP1 functional activity in donor's $CD19^+$ and $CD19^-$ lymphocytes is almost equally as after drugs action as in intact cells. The accumulation of ROS lead to the activation, but ROS level decrease – to the inhibition of the MRP1 transport activity in the $CD19^+$ and $CD19^-$ intact cells. Shift the redox balance (relative to physiological range) towards oxidants results in decrease MRP1 transport activity in both subpopulations and towards antioxidants – increase only in B-lymphocytes.

Thus, the membrane transporters P-gp and MRP1 are involved in maintaining the physiological redox state in donor's intact lymphocytes. But there is a change in the functioning of these proteins in the $CD19^+$ cells under overrunning of this state.

Keywords: MRP1, P-gp, ROS.

TUE-205
Resveratrol enhances oxidative stress-induced apoptosis in mouse neuroblastoma Neuro 2a cells

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Resveratrol, (3,4′,5-trihydroxy-trans-stilbene) is a natural polyphenolic compound, produced naturally by some plants in response to several harmful factors such as attack by pathogens, UV radiation or increased oxidative stress. It is considered to be one of the strongest exogenous antioxidant with numerous properties. Many extensive investigations proved that this extraordinary polyphenol might slow progression or even prevent chronic diseases – cardiovascular, ischemic, neurodegenerative, diabetes. As the latest data show it also might improve chemotherapy.

The aim of our research was to evaluate the effects of resveratrol on nitric oxide- and hydrogen peroxide-induced oxidative stress in neuroblastoma cells (Neuro-2a). Resveratrol was added to the cells culture medium 3 and 12 hours before H_2O_2 or NOC-18 treatment. After 24 hours of incubation of Neuro-2a cells alone with H_2O_2 or NOC-18 respectively we observed reduced cell viability in dose-dependent manner. Concentration of the oxidants which caused 50% reduction in cell viability (IC_{50} value) was 0.037 mM and 0.27 mM for H_2O_2 and NOC-18, respectively. Resveratrol combined with oxidizing factors reduces cell viability substantially. Our data indicate that resveratrol in combination with H_2O_2 and NO increases the percent of apoptotic cells. Resveratrol more effective intensifies apoptosis in Neuro-2a cells in combination with nitric oxide-releasing compound than with hydrogen peroxide. We proved that resveratrol increases apoptosis via changes in mitochondrial membrane potential. Results demonstrate that impact of resveratrol on neuroblastoma cells depends both on concentration and time of incubation.

In conclusion, resveratrol in oxidative stress conditions, similar as are in tumor environment, intensifies apoptosis in Neuro-2a. This natural polyphenol might be applied in chemotherapy but still more evidences and further investigations of resveratrol action and its molecular targets are needed to use is as a drug.

Keywords: neuroblastoma, oxidative stress, resveratrol.

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TUE-206

RGD-modified streptavidin selectively binds to and inhibits growth of cancer cells

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Nowadays the important role of cell adhesion molecules in invasion of metastatic tumor in surrounding tissues as well as in the growth of tumor blood vessels is well known. In particular the increased invasiveness and metastasizing that is typical for vertically growing melanoma is known to be mediated by specific integrins. So, integrin αvβ3 which is overexpressed in melanoma cells with high metastatic potential as well as in endothelial cells of tumor blood vessels and exhibits low expression level in normal melanocytes used to be an attractive target for melanoma diagnostics and therapy. Integrin αvβ3 is known to recognize the RGD peptide sequence which has been found in a wide variety of its natural ligands.

A number of expression vectors analogous to constructs described in [1, 2] has been constructed. These vectors provide the production of the streptavidin fused with RGD-bearing peptides (SR10, SR13, SR15) in *E.coli.* These proteins were demonstrated to bind selectively to murine (B16F10) and human (MeWo) melanoma cells as well as to endothelial cells (HUVEC).

In order to estimate kinetics of binding of each protein with receptors on the MeWo and HUVEC cell surface we've analyzed fluorescence intensity of cell population after incubation with FITC-labeled proteins SR10, SR13, SR15. We noted a characteristic feature – sharp decline in fluorescence intensity after 45 minutes of incubation. Probably this effect is due to the internalization of integrin receptors bound with SR10, SR13 or SR15. Internalization was confirmed by confocal fluorescent imaging of fixed MeWo and HUVEC cells. Luminous spots were detected on the membrane and in the compartment which is typical for endosome location. Moreover, it was noted that increase in incubation time leads to increase in fluorescence intensity both on the cell surface and in cytoplasm. Internalization observed might be caused by activation of the integrin receptors after binding with ligand. It's known that activated receptors undergo endocytosis with the following recycling [3].

An influence of different concentrations of SR proteins on cell viability was estimated by XTT-test. It was demonstrated that incubation of HUVEC in the presence of 1.5 μM SR15 leads to two-fold decrease in cell proliferation rate. The maximum decrease in MeWo proliferation rate (up to 18%) was achieved by exposition of cells to SR13 in 0.075 μM concentration.

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Keywords: integrins, melanoma, RGD.

TUE-207

RHOC-gtpase promotes colon cancer cell invasion in matrix metalloproteinase dependent manner

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Introduction: RhoC GTPase is a member of the Ras superfamily of small GTPases which are involved in a wide range of cellular processes including polarization, migration, adhesion, vesicular trafficking, transcriptional regulation and, cell cycle. There are numerous studies which show that RhoC was overexpressed in many tumor cells especially during epithelial mesenchymal transition as well as in tumor invasion and metastasis. However the exact mechanism has not yet been identified how RhoC promotes invasion and metastasis. Matrix metalloproteinases (MMPs) are secreted and membrane bound endopeptidases which enhance the invasive and migratory potency of cells by removing extracellular barriers and releasing pro-metastatic molecules. In addition, MMPs are inhibited by endogenous tissue inhibitors of metalloproteinases (TIMPs) which are the major regulators of these proteases.

Purpose: The aim of this study is to investigate the effects of RhoC on invasive capacity of human primary (HCT-116) and metastatic (SW620) colon cancer cells via MMP expression and secretion levels.

Methods: RhoC gene was silenced by siRNA transfection in HCT116 and SW620 colon cancer cells. The depletion of RhoC mRNA and protein expression levels was confirmed with Real Time PCR and Western blot techniques, respectively. After siRNA mediated RhoC silencing; i) MMP-2, MMP-7, MMP-9, MMP-14, TIMP-1, TIMP-2 mRNA expression levels were detected with Real Time PCR ii) MMP-2, MMP-7, MMP-9 and TIMP-2 protein expression levels were investigated with Western Blot. iii) The activity levels of secreted MMP-2 and MMP-9 were determined with Gelatin Zymography. iv) The invasive capacity of HCT-116 and SW620 colon cancer cells was assessed by cell invasion assay kit.

Results: The knockdown of RhoC by siRNA strongly reduced MMP-7 protein expression levels in HCT-116 and also suppressed the secretion of proMMP-2 and proMMP-9 levels in both HCT116 and SW620 cells. Furthermore, the inactivation of RhoC resulted in upregulation of TIMP-1 mRNA expression levels in SW620 cells. Consistent with these results, cell invasion also decreased in both RhoC downregulated HCT-116 and SW620 cells.

Discussion: We predict that RhoC promotes invasion by both activating MMP-2, -7, and -9 and decreasing TIMP-1 levels in colon cancer cells. Therefore development of the new therapies that block RhoC activity may be effective to prevent the invasion of colon cancer cells.

Keywords: Colon Cancer Cells, Matrix Metalloproteinases, RhoC.

TUE-208

RNase A caused rearrangement of intracellular pathways in tumor cells providing restoration of normal cell proliferation and differentiation

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Recently, we have shown that the pancreatic RNase A is capable of inhibiting tumor and metastasis growth and one of the mechanisms is associated with the change of miRNA profiles in the

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blood and tumor cells (Mironova et al, 2013). Here, in order to find intracellular targets of RNase A, we performed an analysis of whole transcriptome of Lewis lung carcinoma tissue after treatment of tumor-bearing mice with RNase A by high-throughput sequencing using SOLiD 5.5 platform.

Analysis of sequencing data revealed that inhibition of tumor and metastasis growth by RNase A is accompanied by up-regulation of 320 genes and down-regulation of 645 genes in tumor cells. Top 20 of the mostly down-regulated genes includes snoRNA class (both C/D and H/ACA boxes) elevated in tumor cells; genes encoding proteins with cell-growth promoting and transforming activity (*PARK7*), genes encoding proteins functioned as negatively regulators of MAP kinase superfamily and tumor suppressor (*DUSP6*), which is associated with cellular proliferation and differentiation, anti-apoptotic genes (*LCN2*). Among top 20 of the mostly up-regulated genes are negative regulators (*FAM89B*) of TGF-beta signaling known to exert metastasis-promoting activity associated with epithelial-to-mesenchymal transition, modulation of cancer microenvironment and extracellular matrix components, inflammation and immune suppression at the later stage of tumor progression. Also we observed the increase in expression of genes encoding phosphatidylserine receptors (*JMJD6*) involved in phagocytosis of apoptotic cells, p53-inducible proteins (*STEAP3*), regulators of p53/TP53 stability (*USP10*).

Obtained data give the evidence that RNase A caused in tumor cells rearrangement of intracellular pathways associated with malignant transformation and tumor escape from immunological surveillance towards normalization.

Keywords: Cancer signaling, non-coding RNA, sequencing.

TUE-209
ROCK specific inhibitor fasudil suppresses head and neck squamous carcinoma growth by stimulating gene expression and protein secretion of the chemokine CXCL14/BRAK

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Ras-homologous small GTPase (RhoA) and Rho-associated coiled-coil-containing protein kinase (ROCK) are key regulators of the endocytic and exocytic trafficking of molecules in cells. Activation of this pathway promotes tumor invasion and metastasis. Fasudil is a specific inhibitor of ROCK, which has been approved for the treatment of cerebral vasospasm. We previously reported that fasudil has anti-tumor activity by stimulating Chemokine CXCL14/BRAK(BRAK) secretion in fibrosarcoma cells. But effect of Head and Neck Squamous Carcinoma cells (HNSCC) are not clear. We investigated the effects of Fasudil on BRAK secretion and gene expression in HNSCC cells.

We examined the effect of Fasudil on tumor growth. HSC-3 cells were inoculated subcutaneously into both sides of the dorsolateral regions of female mice (5 weeks old). These mice were daily-administered Fasudil, i.p. (50 mg/kg/day). Fasudil suppressed the growth of HSC-3 in vivo (n = 6). Next, we examined the effects of fasudil on the secretion of BRAK protein by using ELISA in HSC-3 cells. The secretion of BRAK protein was significantly increased by treatment with fasudil in HSC-3 cells. In order to determine the effects of Fasudil on the gene expression of BRAK by using qPCR in HSC-3 cells. The gene expression of BRAK was significantly increased by treatment with Fasudil (25 μM) in HSC-3 cells.

These results suggest that Fasudil inhibits tumor growth by stimulating secretion of BRAK protein and gene expression of BRAK in the HNSCC cells. These results suggest that HNSCC therapy using fasudil may have clinical efficacy.

Keywords: Cancer related proteins, Cancer signaling, CXCL14/BRAK.

TUE-210
Role of C3G in tumorigenesis: cross-talk with p38alfa MAPK pathway

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C3G is a guanine nucleotide exchange factor (GEF) for Rap1 and R-Ras, but it also plays functions independent of its GEF activity. It is essential for embryonic development due to its role in integrin-mediated adhesion and migration. The function of C3G in human cancer is controversial. It suppresses malignant transformation induced by several oncogenes in NIH3T3 cells, but it can also mediate it, as it occurs in papillary thyroid carcinoma. In addition, C3G inhibits migration of highly invasive breast carcinoma cells.

p38 MAPKs are activated by several stimuli leading to the regulation of different cellular functions, including migration and invasion. p38a MAPK is the most abundant isoform and essential for embryonic development. It can act as a tumour suppressor, but it can also promote migration, invasion and survival of tumour cells in some cases.

We have identified a functional interaction between C3G and p38a MAPK in the regulation of cell death and adhesion in mouse embryonic fibroblasts (MEFs) and in chronic myeloid leukemia cells. In these systems, C3G acts through the inhibition of p38a MAPK in order to either activate or inhibit apoptosis, depending on the stimulus. Thus, we have also evaluated by *in vitro* assays whether this C3G/p38a cascade regulates migration and invasion of MEFs and HCT116 colon carcinoma cells. We found that C3G knock-down promotes migration and invasion through the enhancement of p38a activation via a Rap-1 independent pathway. MMPs might be important mediators. These results indicate that C3G is an inhibitor of migration and invasion, while p38a will mediate these processes. So, C3G would impair the development of metastasis acting as a tumour suppressor. However, we have also found that C3G promotes tumour growth of HCT116 cells, both *in vitro* and *in vivo,* regardless of the presence of p38a. Moreover, in this model, p38a also favours tumour growth. Thus, C3G or p38a Knock-down, as well as p38a chemical inhibition, highly decrease anchorage independent growth and tumour growth in xenograft assays. We are currently evaluating the potential mediators of C3G and p38a actions and the mechanisms involved.

Keywords: C3G, CANCER, MAPK.

TUE-211
Role of GS28 on sodium nitroprusside-induced cell death in human cervical carcinoma cells

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We observed the decreased expression of GS28 (golgi SNAP receptor complex 1) in ischemic hippocampus of rat brain. GS28 is involved in ER-golgi transport of proteins synthesized in ER, but almost unknown in another role. In this study, we examined the role of GS28 and its molecular mechanisms on sodium nitro-

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prusside (SNP)-induced cell death in human cervical carcinoma cells (Hela). GS28 siRNA-transfected (K/D) cells showed significant inhibition of cytotoxicity in the cells treated with SNP, compared with that of control cells. Pretreatment of GS28 K/D cells with p38 and JNK MAPK inhibitors reduced the inhibition of cytotoxicity in SNP–treated cells. And phosphorylation of p38 and JNK MAPKs decreased in control cells treated with SNP, but didn't decrease in that of GS28 K/D cells in immunoblot analysis. Pretreatment with ROS scavenger or iron chelator inhibited the cytotoxicity both of the control and GS28 K/D cells treated with SNP. Pretreatment of the GS28 K/D cells with pancaspase inhibitor had no effect on cytotoxicity both of the control and GS28 K/D cells treated with SNP, but autophagy inhibitors (bafilomycin and concanamycin A) reduced the cytoxicity of control cells. Taken together, GS28 has an inductive role on SNP-induced cell death via modulation of p38 and JNK activity in human cervical carcinoma cells.

Keywords: gs28, mapk, nitroprusside.

TUE-212

Role of p53 homologues p63 and p73 in CXCR5 gene regulation in human breast carcinoma cells under genotoxic stress

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Tumor cells often demonstrate overexpression of various chemokine receptors which is associated with their increased survival and migration potential. Recently, CXCR5-CXCL13 co-expression was identified as a negative prognostic factor in breast cancer patients with lymph node metastases. We set out to investigate CXCR5 attenuation as a possible therapeutic strategy for advanced metastatic breast cancer. We have previously shown that p53 could repress CXCR5 expression, CXCR5 promoter activity and cell migration in a breast cancer cell line. In the current study we examined the possible role of two other p53 family members, p63 and p73, in this process. p53 expression in MCF-7 breast cancer cells was suppressed by lentiviral transduction of an shRNA vector. CXCR5, p53, p63 and p73 expression was measured by QRT-PCR and by Western blotting. In order to activate p53-dependent protective and apoptotic mechanisms, we used DNA-damage agent methyl methanesulfonate (MMS).

P53 suppression in MCF-7 cells led to elevated expression of both p53 homologues, especially the p73. MMS exposure for 24 hours caused dose-dependent decrease in CXCR5 expression despite the low level of wild-type p53, indicating alternative activation of p53-dependent pathways, probably through p63 or p73. Our results led us to suggestion that genotoxic stress in p53-deficient cells causes reactivation of p53 pathway through p63 and p73 systems. To understand individual roles of p63 and p73 in CXCR5 regulation, we are generating derivatives of MCF-7 cells with different combinations of p53, p63 and p73 inactivation using the CRISPR-Cas9 system. For enhanced genome editing specificity and for minimized off-target activity, the single nicking D10A Cas9 mutant is being employed.

Keywords: Chemokine receptors, Expression regulation, Tumor supressors.

TUE-214

Smad proteins differentially regulate the activity of murine protein serine-threonine kinase 38 (MPK38)

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We have demonstrated previously that murine protein serine-threonine kinase 38 (MPK38/MELK), a member of AMP-activated protein kinase (AMPK) family, stimulates TGF-beta signaling in a kinase-dependent manner via Smad(s) phosphorylation. Based on this finding, we speculated that MPK38 activity is subjected to Smad regulation in which Smad proteins positively or negatively regulate the activity of MPK38. In this study, we demonstrate that Smad2/3/4 have a positive role, whereas Smad7 has a negative role, in the regulation of MPK38 activity and function. Wild-type Smad2/3/4 markedly increased the stability of MPK38 compared to control expressing empty vector, whereas Smad7 decreased the MPK38 stability. However, MPK38-mediated phosphorylation-defective Smad mutants (Ser^{245} of Smad2, Ser^{204} of Smad3, Ser^{343} of Smad4, and Thr^{96} of Smad7) had no such effect. We further analyzed the effects of Smad proteins on the complex formation between MPK38 and Trx, a destabilizer of MPK38, using RNA interference. Results showed that Smad2/3/4 decreased the complex formation, whereas the complex formation was increased in the presence of Smad7, indicating a differential role of Smad proteins for regulating the stability of MPK38. Consistently, ectopic expression of wild-type Smads (Smad, 2, 3, 4, and7), but not the MPK38-mediated phosphorylation-defective Smad mutants, differentially regulate MPK38-dependent ASK1, TGF-beta, and p53 signaling in a dose-dependent fashion by stabilizing or destabilizing the MPK38 protein, suggesting Smad proteins as potential regulators of MPK38 in cells.

Keywords: Smad proteins, ASK1, TGF-beta, and p53 signaling, MPK38/MELK.

TUE-215

Sox9 is required for basal cell carcinoma tumorigenesis

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The epidermis comprises a variety of stem/progenitor cell populations within different epidermal compartments, which in the last years have been intensively studied for their potential in initiating basal cell carcinoma (BCC). The key molecular event driving the formation of BCCs is constitutive Hedgehog signaling. Independent of the histologic subtype, all BCCs express the transcription factor Sox9, which is thought to be a direct downstream target of Hedgehog signaling. Sox9 is required not only for the formation of the multipotent epidermal stem cell population, but also for maintaining the stem cell pool for each recurring adult hair follicle cycle. Here we addressed the hypothesis that Sox9 is the key Hedgehog target required for BCC initiation and/or maintenance.

Loss of Ptch1 leads to upregulation of Hedgehog signaling and formation of BCCs. Here, through the combination of a Ptch1 conditional allele ablated under the control of Keratin5 cre followed by grafting to immunodeficient mice, we verified that BCCs result.

Preliminary evidence indicates that concomitant ablation of both Ptch1 and Sox9 results in hair follicle-deficiency as previously reported in epidermal Sox9 knockout studies but more importantly, prevents development of BCC. These data reveal

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that Sox9 is a critical player in BCC pathogenesis. However, we cannot exclude the possibility that the blockage of BCC tumorigenesis might be the effect of loss of the cellular origin as Sox9 ablation also results in the loss of the multipotent stem cell population.

This study significantly contributes to the understanding of molecular cues underlying BCC carcinogenesis and has revealed a potential new therapeutic target.

Keywords: basal cell carcinoma, Sox9, transcription factor.

TUE-216
Standardization of collagen zymography method for determining matrix metalloproteinases -1, -8 and -13 activities

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Introduction: Matrix metalloproteinases (MMPs) are a large family of Zn^{+} and Ca^{2+}-dependent endopeptidases which are subdivided into collagenases, gelatinases, stromelysins, matrilysins and membrane-type according to their substrate specificity, primary structure and cellular location. MMPs are responsible for degradation of several extracellular matrix (ECM) components and cleavage of non-ECM molecules such as growth factors, integrins, and receptors. Therefore they involved in both physiological and pathological processes. MMP-1, -8 and-13, known as collagenases, are capable of degrading collagen type I, II, III, VII, VIII, X, XI, and processing some cytokines. Collagen zymography is an electrophoretic technique based on SDS-PAGE principle. It provides the detection of pro and active forms of collagenases in the same gel.

Objective: The goal of this study was to standardize collagen zymography method and determine the sensitivity of collagen zymography for each collagenases.

Method: Commercially obtained type I and type II collagen were used as substrate for comparing which substrate is the most sensitive to detect collagenases. Also, type I collagen was purified from rat tail tendons. The SDS-PAGE gels containing different substrate concentrations (0.15, 0.3 and 0.6 mg/ml) were prepared and recombinant human collagenases (rhMMP-1, -8 and -13) were loaded different concentrations (1-100 ng), and finally electrophoresed with molecular weight marker to identify band sizes for pro and active forms of each MMPs. Following electrophoresis and renaturation steps, the gels were incubated in developing buffer for 24 h and 48 h. Each experiment was repeated six times. Densitometric analysis of the lytic bands were quantified and standard curves were plotted as activity unit versus amount of rhMMP-1, -8 and -13. After standardization of the method, human thyroid cancer (8505C) and normal thyroid (Nthy-ori 3-1) cell lines were examined by using collagen zymography method.

Results: The purified type I collagen was determined as the best suitable substrate than commercial type I and type II collagen. The best clear lytic bands were obtained with 0.3 mg/ml type I collagen substrate concentration after 48 h incubation. We found that the lowest collagenase activity can be detected in these conditions was 1 ng. The quantifications of rhMMP-1, -8 and -13 bands showed linear standard curves ranged from 1 to 10 ng concentrations. According to collagen zymography results, proMMP-1 was found in thyroid cancer cell line.

Conclusion: In this study, we improved collagen zymography method by using type I collagen that purified from rat tail tendons. We also generated the standard graphs of each collagenases to quantify the activities of collagenases in unknown samples.

Keywords: Collagen Zymography, Collagenases, Matrix Metalloproteinases.

TUE-217
Sterically stabilized cationic liposomes for efficient glycotargeted gene delivery to hepatocytes

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This study focused on the assembly, characterization, and *in vitro* evaluation of lectinized gene delivery lipoplexes for improved transfection efficiency via asialoglycoprotein receptor (ASGP-R) mediated endocytosis, with the design capacity for hepatocytic corrective gene therapy. Cationic liposomes based on two cholesteryl cytofectins, 3β-[*N*-(*N'*, *N'*-dimethylaminopropane)-carbamoyl] cholesterol (Chol-T) and *N*, *N*-dimethylaminopropyl-aminylsuccinylcholesterylformylhydrazide (MS09), and the neutral lipid dioleoylphosphatidylethanolamine (DOPE), were prepared. Stealth liposomes were prepared by steric stabilization using 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] ($DSPE\text{-}PEG_{2000}$) grafting while hepatocyte ASGP-R targeting was achieved using the cholesteryl-β- -galactopyranoside (Chol-β-Gal) functionality.

Cryo-TEM and DLS studies revealed that PEGylation generated smaller and denser aggregated lipoplexes than their non-PEGylated counterparts. All formulations were in the range 138 – 250 nm with narrow particle size distributions (polydispersity indices were <0.4). An increase in PEGylation was accompanied by decreased particle size and ζ-potential ($P < 0.001$). Liposomes bound and efficiently compacted pCMV-*luc* DNA as evidenced in band shift and ethidium bromide intercalation assays respectively, while nuclease digestion assays demonstrated that the degradative effect of serum on lipoplex-associated DNA was minimal. MTT [3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide] and AlamarBlue (AB) reduction studies, showed that the lipoplexes elicited a dose-dependent cytotoxic effect in both HEK293 and HepG2, with cell viability remaining above 70% (MTT) and 50% (AB). The *Ricinus communis* (RCA_{120}) agglutination test demonstrated that the galactosyl residues on the targeted lipoplexes were well exposed and accessible as shown by the selective and time-dependent agglutination and inhibition by the competitive binder, -galactose. Targeted lipoplexes were able to deliver pCMV-*luc* DNA up to 300% more effectively ($P < 0.001$) compared to their untargeted equivalents. An increase in PEGylation of the Chol-β-Gal liposomes was accompanied by an increase in luciferase activity. MS09 Chol-β-Gal 2% PEG and MS09 Chol-β-Gal 5% PEG complexes were able to achieve on average, 9% (N/P 2.3) and 20% (N/P 3.5) greater transfection levels in HepG2 cells than the commercial transfection reagent TurboFect (TF), respectively. Competitive inhibition studies produced a decline ($P < 0.001$) in luciferase activity of >83%, confirming targeting specificity and ligand-directed ASGP-R-mediated uptake of the targeted lipoplexes in HepG2 cells.

In vitro studies strongly suggest that PEGylated ASGP-R directed lipoplexes should be developed further for application *in vivo*.

Keywords: Cationic Liposome, Glycotargeting, PEGylation.

TUE-218
Strict targeting of receptors by strereospecific monoclonal antibodies

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Until now, various kinds of monoclonal antibodies (mAbs) directed against target antigens have been established and widely utilized for many purposes. Lately, mAbs have also attracted

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attention as potent therapeutic medicines because of their strict specificity for particular antigens. The global market for therapeutic mAbs is expanding and is likely to be worth more than US $70 000 million in 2016. However, there is one big problem. Most established mAbs recognize the primary structures of antigens. If membrane receptors are the target antigens, stereospecificity of mAbs is of critical importance. However, practical protocols for generating stereospecific monoclonal antibodies (ssmAbs) have yet to be established. To address this question, we developed a new hybridoma technology termed the "stereospecific targeting (SST)" technique, which features two pivotal steps. One is employment of DNA immunization. The target antigens must be expressed in the mouse body retaining their original conformational structures and then be recognized by the immune system to sensitize B lymphocytes so that they mature to generate conformation-specific antibodies. Another point is the usage of antigen-expressing myeloma cells. If DNA-immunized B lymphocytes are selected by myeloma cells based on B cell receptors (BCRs), the selected B lymphocytes could produce stereospecific antibodies, since antigens expressed on the surface of myeloma cells keep their structures intact. Finally, myeloma cell-B cell complexes are selectively fused by electrical pulses to generate hybridoma cells secreting ssmAbs. The advantage using electric fusion is that only attached cells are preferentially fused. As a test, we selected EphA2 as an antigen, which is overexpressed by multiple types of epithelial tumors. As a result, the formation of myeloma cell-B cell complexes could be identified based on immunofluorescence analysis after targeting sensitized B lymphocytes with intact antigens expressed on myeloma cells Moreover, the desired mAb was successfully obtained, reacting with EphA2-expressing MDA-MB-231 cancer cells, but lacking cross-reactivity with recombinant EphA2. There is one more important point for this new technology. The SST technique may also be applicable for selective production of "human" ssmAbs by using transgenic mice producing human antibodies. Since the SST technique is very simple, ready generation of human ssmAbs is very feasible.

Keywords: monoclonal antibodies, receptors, stereospecificity.

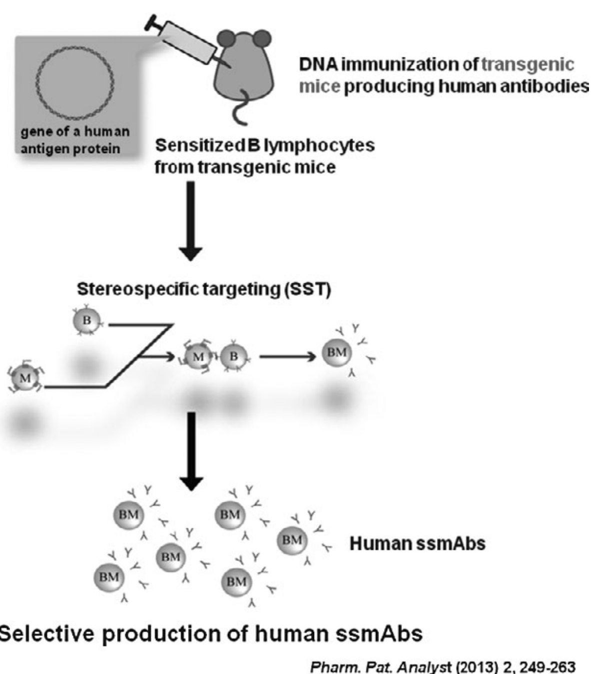

Fig. 1.

TUE-220
Synergistic and antagonistic effect of heat shock-induced HSF1 on expression of NF-κB-dependent genes

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As the result of stress conditions, different signal transduction pathways are activated in cell that determine the mechanisms responsible for adaptation and survival. Among them are two major pathways, regulated and executed by NFκB and HSF1 transcription factors, which are critical for growth, development and response to the treatment of cancer and other human diseases. Both signaling pathways can interfere with each other, but mechanism of this regulation is not clear yet. Here we aimed to identify NFκB-regulated genes, which expression is affected by HSF1, in positive or negative way.

Expression of NFκB-dependent genes was analyzed in U2OS human osteosarcoma. These cells, either control and preconditioned with hyperthermia (HS) to activate endogenous HSF1, were stimulated with TNFα cytokine and the expression of TNFα-induced genes was analyzed by the expression microarrays. Actual sites of HSF1 binding to chromatin were detected in cells subjected to HS by chromatin immunoprecipitation assay coupled with DNA sequencing (ChIP-Seq approach). Bioinformatics analysis was also made for prediction of hypothetical κB and HSE motifs in promoter regions of analyzed genes.

We observed, that 324 genes changed their expression upon stimulation with TNFα; 191 genes were up-regulated while 133 genes were down-regulated compared to untreated control. Hypothetical κB motifs were found in proximal promoters of 114 of these genes (this group putatively represents set of genes regulated by NFκB). We found, that expression of 187 of TNFα-modulated genes was affected by the hyperthermia pre-treatment. Two modes of co-effects were observed: synergistic/additive (strengthen of TNFα effect) and antagonistic (reduction of TNFα effect). In general, among genes co-affected by TNFα and HS, the κB motif was present in proximal promoters of 74 genes, while the HSE motif and/or actual HSF1 binding was observed in regulatory regions of 52 genes. We identified 27 co-affected genes with binding sites (either hypothetical or actual) for both transcription factors. Among them there were 10 genes, for which observed effect of HS on their TNFα-induced expression was synergistic/additive (eg *EGR1, FOSB, RRAD*) and 17 genes with antagonistic effect (eg *CCL2, CCL20, CD83, IL8, TNF*).

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Keywords: HSF1, Hyperthermia, NFκB.

TUE-221
System Architecture of membrane-anchored Ras - big insight on the nanoscale

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The small GTPase Ras is highly mutated in human cancers and thus a major driver of cell transformation and tumorigenesis. In

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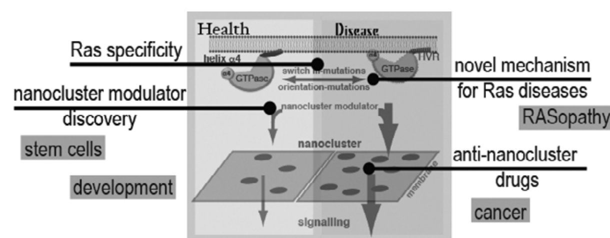

Fig. 1.

the plasma membrane approximately 40% of Ras proteins are organized into nanoscopic signaling platforms, called nanocluster. Ras nanocluster are dynamic proteo-lipid complexes that contain 6-8 Ras proteins and they are essential for Ras signaling. Lateral segregation of Ras paralogs to distinct nanocluster furthermore contributes to their specific activity. However, little is known about the composition, functioning and physiological relevance of Ras nanocluster.

Using biochemical, molecular cell biological, fluorescence superresolution microscopic and computational methods, we investigate how paralog-specific Ras nanoclusters are regulated. In addition, we exploit chemical screening to generate new tools to study Ras nanocluster and explore the potential to generate new lead compounds against Ras in cancer.

Our latest results revealed an unprecedented mechanism of Ras over-activation in cancer. Certain tumor associated mutations increase Ras activity by uniquely augmenting its nanoclustering, as not other biochemical activities are significantly affected. These findings validate Ras nanocluster as drug-targets. We therefore use customized nanoclustering-FRET biosensors to identify compounds that specifically affect the membrane organization of Ras and thus its signaling. This recently lead us to the discovery of cancer stem cell active drugs, which specifically affect K-ras signaling.

Our work aims at the complete description of the Ras signalling architecture on the membrane. We expect that improved understanding of Ras nanoclustering has special potential to yield innovative cancer-drug targets, –biomarkers and -drugs. Based on our evidence for nanoclustering of other classes of membrane anchored signalling proteins, we propose a broad implication of this mechanism in biology.

Keywords: Cancer signaling, microscopy, Ras.

TUE-222
Systematic characterization of drug-induced adaptive responses in melanoma

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Activation of BRAF via a V600E mutation is the most prevalent genetic change in human melanoma, found in at least 50% of tumors. The $BRAF^{V600E}$ oncoprotein induces constitutive activation of pro-mitogenic RAF/MEK/ERK signaling. Therapy with RAF inhibitors such as vemurafenib leads to rapid tumor regression in most patients, but the duration of responses is highly variable with frequent relapse to lethal drug-resistant disease. It is increasingly clear that innate resistance of melanoma cells to RAF inhibitors involves adaptive responses that reactivate ERK or induce pro-growth pathways such as the PI3K/AKT cascade. Thus, understanding and ultimately preventing adaptive responses is a key to durable therapy. Systematic data comparing $BRAF^{V600E}$ tumor cells is generally lacking and is not known whether adaptation to different MEK and RAF inhibitors is fundamentally similar. It is also unclear whether the key difference between sensitive and resistant cells involves drug potency (difference in IC_{50}) or the fraction of cells that are responsive (E_{max}).

We develop a systematic approach to profiling drug adaptation that combines high density time- and dose-dependent multiplex biochemical measurement with single cell assays and statistical modeling, with the overall goal of (i) characterizing variability in adaptation with time, dose and genotype, (ii) discovering new or poorly characterized adaptive mechanisms, and (iii) demonstrating the effectiveness of a high-throughput approach involving multiplex measurement and computational modeling. Our analysis indicates that responses to RAF inhibitors are remarkably diverse and involve multiple cell signaling kinases that can be up or down-regulated over time, often in different directions in different cell lines, and extend well beyond the RAS/MEK/ERK and PI3K/AKT known to influence responses to RAF inhibitors in melanoma cells. We characterize the role of JNK/c-Jun in adaptation and show that RAF and JNK inhibitors induce synergistic cell killing. Single-cell studies show that JNK inhibition prevents a subset of vemurafenib–treated cells from becoming quiescent, thereby promoting apoptosis and increasing E_{max}. These findings have potential therapeutic significance and reveal the value of studying drug adaptation using systematic biochemical, single-cell and modeling methods.

Keywords: Adaptive response in melanoma, BRAF inhibitor therapy, Data-driven modeling.

TUE-223
Tamoxifen mediates premature senescence in human breast cancer and colon cancer cells through CK2 inhibition

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Cellular senescence is an important tumor suppression process in vivo. Tamoxifen is a well-known anti-breast cancer drug; however, its molecular function is poorly understood. Here, we examined whether tamoxifen promotes senescence in breast cancer and colon cancer cells for the first time. Human breast cancer MCF-7, T47D, and MDA-MB-435 and colorectal cancer HCT116 cells were treated with tamoxifen. Senescence was measured by SA-β-gal staining and based on the protein expression of p53 and $p21^{Cip1/WAF1}$. The production of reactive oxygen species (ROS) was determined by staining with CM-H_2DCFDA and dihydroethidium (DHE). As results, tamoxifen promoted senescence phenotype and ROS generation in MCF-7 and HCT116 cells. The ROS scavenger, N-acetyl-L-cysteine (NAC), and the NADPH oxidase inhibitor, apocynin, almost completely abolished this event. Tamoxifen inhibited the catalytic activity of CK2. Overexpression of CK2α antagonized senescence mediated by tamoxifen, indicating that tamoxifen induced senescence via a CK2-dependent pathway. A well-known CK2 inhibitor, DRB, also stimulated ROS production and senescence in MCF-7 cells. Finally, experiments using T47D (wild-type p53) and MDA-MB-435 (mutant p53) cell lines suggested that tamoxifen induces p53-independent ROS production as well as p53-dependent senescence in breast cancer cells. Taken together, these results demonstrate that tamoxifen promotes senescence through a ROS-p53-$p21^{Cip1/WAF1}$ dependent pathway by inhibiting CK2 activity in breast cancer and colon cancer cells.

Keywords: protein kinase CK2, senescence, tamoxifen.

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TUE-225
The antiproliferative effect of nucleus-directed maspin on breast cancer cells

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Background: Mammary Serine Protease Inhibitor (Maspin) belongs to the serpin family and is classified as a class II tumor suppressor protein. Maspin demonstrates antiapoptotic, antimetastatic and antiangiogenic properties. However, maspin expression is downregulated in malignant breast cancer and in most breast cancer cell lines. Many reports suggest that the nuclear fraction of maspin is responsible for anticancer effect rather than cytoplasmic maspin. It may be connected with influence of nuclear maspin on transcription factors activity or on nuclear enzymes, for example histone deacetylase (HDAC).

Observations: The aim of our research was to study an effect of maspin localization and level of expression on breast cancer cells. Maspin localization and Ki-67 protein expression was evaluated in samples from patients with breast cancer. Moreover, three breast cancer cell lines – MCF-7, MDA-MB-231, SKBR-3, and normal epithelial cell line from breast - MCF10A were transfected with plasmid encoding maspin-EGFP (fusion protein locates in cytoplasm) and maspin-NLS-EGFP (fusion protein with nuclear localization signal locates in nucleus) and Ki-67 protein expression was evaluated. We observed the inhibitory effect on cell proliferation of maspin located in nucleus both in tissue samples from patients with breast cancer and in breast cancer cell lines. A statistically significant negative correlation was observed between nuclear maspin and Ki-67 expression in patients with breast cancer. Furthermore, a correlation was found between nuclear maspin and loss of Ki-67 protein in breast cancer cell lines. In normal epithelial cells from breast, there was no effect on cell proliferation. The anti-proliferative effect of nuclear maspin on breast cancer cells was statistically significant in comparison to cytoplasmic maspin. Because of low transfection efficiency and lethal effect of high level of nuclear-localized maspin, we decided to use lentiviral system, which ensures higher efficiency of gene delivery and allows to manipulate the level of protein. This approach will provide homogeneous material for further analysis and will allow to investigate differences in cell cycle and in both enzymes and signaling pathways activity depending on maspin localization.

Conclusions: Understanding the molecular mechanisms of maspin activity depending on its subcellular localization may broaden our knowledge about functions of this protein and its role in cancer progression. Moreover, evaluation of the maspin localization may be use as a prognostic factor in breast cancer.

Keywords: breast cancer, maspin.

TUE-226
The *C. elegans* EGF homolog LIN-3 acts as guidance cue for anchor cell invasion

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During development, the epidermal growth factor (EGF) acts in multiple tissues as stimulator of cell proliferation, growth, differentiation and survival. Cancer cells take advantage of EGF signalling by expressing increased levels of EGF receptors (Mendelsohn and Baselga, 2000). Furthermore, EGF receptor signalling is implicated in controlling the invasiveness of metastatic cancer cells (Stock et al.; Yang et al.). The development of the C. elegans vulva is an excellent in vivo model to study cell invasion as a normal developmental process. During the third larval stage, a specialized uterine cell, the anchor cell (AC), invades through two basal laminae into the vulval tissue in order to connect the uterus with the developing vulva. AC invasion is a robust process, which is secured by a number of redundant signaling events that have not all been elucidated yet. Using tissue specific RNAi experiments and mutant analysis, we show that the EGF homolog LIN-3 produced by the vulval cells guides the AC during its invasion into the vulval epithelium LIN-3 EGF acts in parallel with a ventral Netrin signal to polarize the AC towards the ventral midline. Thus, the combined action of multiple guidance signals enable the AC to breach the basal laminae and invade the vulval cells at a precise location.

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Keywords: None.

TUE-227
The coordinated action of BIK, p53 and ROS in DNA damage-induced cell death

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Cancer continues to be a leading cause of death along with cardiovascular diseases as a major health problem for the affected individuals and a socioeconomic burden for the society. Despite increased success with the help of fast sequencing of tumor genomes, smart targeted-therapies and increased screening and prevention efforts, de novo or acquired resistance to therapy remains to be a major factor negatively affecting prognosis and survival in cancer patients. In addition to surgery, conventional chemotherapy and radiotherapy constitutes the two main axes of cancer therapy strategies. These therapies trigger the elimination of cancer cells by inducing mitochondrial programmed cell death pathway. Mitochondrial cell death is regulated by BCL-2 protein family members and the release of cytochrome *c* into cytosol is the point of no return for commitment to cell death. Many chemotherapeutics and radiotherapy target DNA and cells respond to DNA damage with cell cycle arrest, senescence, DNA repair or cell death. DNA damage can also activate oncogenes and induce tumorigenesis. As a proapoptotic BH3-only protein, BIK binds to antiapoptotic BCL-2 proteins and neutralizes them to promote cell death. The expression of BIK can be regulated via p53-dependent or p53-independent mechanisms. In addition, reactive oxygen species (ROS) dictates cell death or survival in response to DNA damage, either by damaging cellular structures or by regulating gene expression. Our studies delineated how BIK is regulated in different p53 backgrounds in colon cancer cells and how this phenomenon is regulated by ROS formation in response to DNA damage.

Keywords: Cell death, DNA damage, ROS.

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TUE-228

The cytotoxic analysis of free doxorubicin and doxorubicin loaded PHB-MNPs on sensitive and doxorubicin resistant MCF-7 cell lines

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Doxorubicin is an antracycline antibiotic. It is a commonly used anticancer agent in many cancer types and its most serious side effect is damaging the heart cells. Therefore targeting of this chemotherapeutic agent with a suitable nano carrier mediated drug delivery system is important. Aim of this study, the cytotoxic effect of free Doxorubicin and Doxorubicin coated MNPs were compared on sensitive and Doxorubicin resistant breast cancer MCF-7 cells. In this study, Polyhydroxybutyrate (PHB) coated magnetic nanoparticles (PHB-MNPs) were synthesized by in situ coating method. Doxorubicin was loaded onto the PHB-MNPs. Cytotoxicity analysis of Doxorubicin loaded PHB-MNPs were performed on Doxorubicin resistant MCF-7 breast cancer cells. The cytotoxicity levels of free Doxorubicin and Doxorubicin loaded MNPs on both of the cell lines were tested by XTT cell proliferation assay. Doxorubicin loaded PHB-MNPs were about 2.5 fold more cytotoxic as compared to free drug on resistant MCF-7 cell line (1 μM Doxorubicin) *in vitro*. Therefore Doxorubicin loaded PHB-MNPs lead to overcome the drug resistance.

Keywords: Doxorubicin, drug resistance, breast cancer, cytotoxicity.

TUE-229

The effect of a phospholipase C gamma inhibitor on the proliferation and phenotype of Du145 prostate cancer cells

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Introduction: Prostate cancer remains the second most common cause of cancer related death among men, highlighting the need for new therapies. Many cancer cellular functions have been discovered to be regulated by phospholipase C (PLC) gamma activation, suggesting that it represents an important therapeutic target for development of anticancer drugs. Here, we investigate the influence of a newly developed, small molecule PLC gamma inhibitor, with or without taxane therapy, on the growth and survival of sub-populations of a prostate cancer cell line.

Materials and Methods: Cells were incubated 48 h with Paclitaxel (5 nM) and PLC gamma inhibitor (1 microM) alone or in their combination.The viable cells were determined by the MTT assay. Flow cytometric analysis of cells positive to anti-CD44, anti-CD54, and propidium iodide staining was performed to characterise apoptotic Du145 sub-populations 48 h after inhibitor treatment.

Results: Treatment of the DU145 prostate cancer cell line with the PLC gamma inhibitor resulted in cell cycle arrest with minimal increase in apoptosis. Sub-populations of prostate cancer cell lines have unique phenotypes (with CD44 + cells being more proliferative and CD54 + cells serving as better CD8 + T cell targets). We examined the effects of the PLC gamma inhibitor on these subpopulations and found that exposure decreased the percentage of both CD44+ (p = 0.00007) and CD54+ (p = 0.009) sub-populations. In contrast, treatment with Paclitaxel only effected CD44+ cells (p = 0.0002). Combination treatment of the PLC gamma inhibitor and Paclitaxel however had synergistic effects on both CD44+ and CD54+ DU145 cells (p = 0.005 and p = 0.0002, respectively).

Conclusions: These results suggest that a combination of PLC gamma inhibitor and Paclitaxel could be a novel strategy for the treatment of prostate cancer.

Keywords: prostate cancer, phospholipase C inhibitor.

TUE-230

The effects of calcitriol on N-MYC downstream regulated gene-2 and sodium-iodide symporter gene expressions in undifferantiated human anaplastic thyroid cancer cell line

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Introduction: Anaplastic thyroid cancer is one of the most aggressive human cancers. The loss of expressions in sodium-iodide symporter (NIS) and thyroid-stimulating hormone receptor causes to the failure of conventional therapies. Thus, it is important to develop differentiation-inducing agents which can be used together with low cytotoxicity chemotherapeutics. Calcitriol (1,25-dihydroxyvitamin D_3) is the most active form of vitamin D and affects both directly and indirectly some gene and protein expressions. It has significant roles in apoptosis, angiogenesis and inflammation. N-Myc Downstream Regulated Gene-2 (NDRG2) is a differentiation marker which has tumor suppressor functions such as reduction of tumor growth, invasion and metastasis as well as induction of differentiation. Sodium-iodide symporter (NIS) is a membrane bound glycoprotein which is known to be a hallmark of differentiation of thyroid cancer. Thus, the loss of differentiation is correlated with decrease in NIS expression and function.

Objective: The aim of this study is to compare differences of the basal gene expression levels of NDRG2 and NIS in anaplastic thyroid cancer (8505C) and normal thyroid cancer (Nthy-ori-3-1) cell lines and to investigate whether calcitriol has effects on NDRG2 and NIS gene expression levels in 8505C cells.

Material and Methods: The basal gene expression levels of NDRG2 and NIS were detected with Real Time PCR in both cell lines. The IC 50 value of calcitriol was evaluated with WST-1 test. Then, 8505C cells were treated with calcitriol for 24, 48 and 72 hours and the differences in NDRG2 and NIS gene expressions were analyzed with Real-Time PCR. All experiments were performed in triplicate.

Results: Basal NDRG2 and NIS gene expressions were found significantly increased in Nthy-ori-3-1 cells compared with 8505C cells (p = 0.002 and p = 0.008, respectively). According to WST-1 test results, the IC 50 value of calcitriol was 60 μM which resulted in 62%, 63% and 56% reduction in cell viability at 24, 48 and 72 hours, respectively. Therefore, 8505C cells were incubated with 60 μM calcitriol by 24, 48 and 72 hours and no significant differences were found in NDRG2 and NIS gene expressions between control and calcitriol treated groups.

Conclusion: Basal NDRG2 and NIS gene expressions were found significantly lower in 8505C cells. However, calcitriol treatment did not show any significant difference in NDRG2 and NIS gene expressions in 8505C cells. This could be partially explained

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by vitamin D receptor (VDR) polymorphism which affects negatively the translocation of VDR into the nucleus.
Keywords: Anaplastic thyroid cancer, calcitriol, N-myc Downstream Regulated Gene-2.

TUE-232
The effects of ceranib-2 on cancer and non-cancer cell ultrastructure

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Ceramide induce cell death on high levels on the contrary of sphingosine-1-phosphate high levels that lead to cell proliferation. Therefore, ceramide/sphingosine-1-phosphate ratio is critical for cell viability. Ceramidases are regulatory enzymes of this ratio. Ceranib-2, an inhibitor of human ceramidase, was used in this study to detect the effects of a ceramidase inhibitor on cell ultrastructure in cancer and non- cancer cell lines. The effects of ceranib-2 on H-Ras 5RP7 and 3T3 cells were determined using a transmission electron (TEM-FEI Tecnai Bio-TWIN) microscopic assay. 5RP7 and 3T3 cells tretaed with IC_{50} concentrations of ceranib-2 for 24 hours were fixed with 2,5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 and left in buffer overnight at +4°C. After being embedded in agar and post fixation in %2 osmium tetroxide, cells were dehydrated in graded ethanol: 70, 90, 96 and 100%. Then cells were embedded in EPON 812 epoxy and sectioned on ultramicrotome (LEICA UC6), stained with uranyl acecate and lead citrate and observed on TEM.

It has been showed cell death caused by ceranib-2 inducing apoptosis on 5RP7 cells with 3 μM and 5 μM on 3T3 cells. As apoptotic sparks were detected condensed nucleus, blebbs on cell membrabe and apoptotic bodies. Ceranib-2, may be succesful in drug desingning for cancer treatment.
Keywords: Cancer treatment, Ceramidase inhibitor, Ceranib-2.

TUE-233
The Hedgehog receptor Patched functions in multidrug transport and chemotherapy resistance

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The Hedgehog (Hh) signaling pathway controls cell differentiation and proliferation. It plays a crucial role during embryonic development, but is also involved in cancer development, progression, and metastasis. The Hh receptor Patched is an Hh target gene that is over-expressed in many aggressive cancers. We recently demonstrated that Patched is involved in the efflux of drugs such as doxorubicin, a chemotherapeutic agent used for clinical management of recurrent cancers, suggesting that Patched could contribute to chemotherapy resistance of cancer cells (Bidet et al. 2012, patent WO2012-080630). We proposed Patched as a new target for cancer treatment, and we developed a screening based on the ability of molecules 1/ to inhibit growth of yeast over-expressing human Patched in medium containing doxorubicin, 2/ to increase doxorubicin cytotoxic effect on cancer cell lines over-expressing Patched. We showed that some molecules extracted from Mediterranean sponges are able to increase doxorubicin cytotoxic effect on human metastatic melanoma cell lines by inhibiting drug efflux activity of Patched.

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Keywords: CANCER, Chemotherapy Resistance, Hedgehog signaling.

TUE-234
The highly flexible and heterogeneous nature of E1A from human Adenovirus (HAdV) characterized at atomic resolution through NMR

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The small DNA tumor viruses encode some of the most versatile hub proteins like the E1A protein from human Adenovirus (HAdV). The E1A protein is essential for productive viral infection in human cells and a vast amount of data are available on its interactions with host proteins. Up to now no high-resolution information on the full-length E1A protein is available despite its important biological role.

Here we present the NMR characterization of the entire 243 residue long 12S isoform of the E1A protein from HAdV (E1A-12S). The protein results very heterogeneous in terms of structural and dynamic properties with highly flexible modules. This study opens the way to characterize the many interactions in which this protein is involved.
Keywords: None.

TUE-235
The human cathelicidin peptide LL-37 increases intracellular calcium in cancer cells by activating the BKCa channel

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Human cathelicidin hCAP18 and its C-terminal peptide LL-37, initially characterized by its antimicrobial properties, is a multifunctional protein affecting both nonmalignant and malignant cells. Among other activities, it increases cellular mobility, involved both in wound healing and dissemination of tumor cells, by a mechanism still to be discovered.

Since calcium signaling contributes to cell migration we investigated the impact of LL-37 on the calcium concentration in the MDA-MB 435s cancer cell line. We found that LL-37 dramatically increased intracellular calcium. This may be explained by direct activation of calcium-channels. Alternatively the activation of potassium channels may induce a membrane hyperpolarisation by K + -exportation and increase the driving force for calcium entry. Patch clamp analysis demonstrated that LL-37 induced an outward current that was blocked by iberiotoxin, a specific inhibitor of the mechanosensitive BKCa potassium channel. Likewise, iberiotoxin inhibited the induction of calcium influx and cell mobility by LL-37, demonstrating the indirect mechanism by the activation of BKCa.

The all-D enantiomer of LL-37 showed identical activities as the natural L-peptide, which basically excludes an interaction with a specific protein receptor. In agreement with previous findings from others, we propose that LL-37 exerts its function

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through its association with the cellular membrane, and alteration of the membrane structure.

To identify which calcium-channels were involved, we performed a candidate approach using pharmacological inhibitors and RNA interference. Surprisingly, the ionotropic ATP-gated P2X7 receptor, previously shown as being activated by LL-37, did not contribute. Instead, LL-37 induced calcium influx using members of the TRP family and the Orai1 channel.

LL-37 is highly expressed in a variety of cancer forms. Our findings may contribute to explain how this peptide may contribute to malignancy.

Keywords: calcium channels, LL-37, malignant transformation.

TUE-236
The influence of GSTP1 gene expression level in tumor on clinical outcome in childhood neuroblastoma patients

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Neuroblastoma (NB) is the most frequent childhood malignancy characterized by high clinical heterogeneity ranging from spontaneous remission to rapid tumor progression and death. Glutathione S-transferase P1 (GSTP1) enzyme is involved in phase II metabolism of many carcinogens and anticancer agents as well as in the regulation of c-Jun NH2-terminal kinase-mediated cell signaling. In tumor cells high GSTP1 expression level could enhance detoxification of chemotherapeutic agents and associate with drug resistance, failure of cancer chemotherapy along with poor survival of patients. The aim of our study was to estimate the influence of GSTP1 gene expression level in tumor on clinical outcome in childhood NB patients.

The case group comprised 75 patients with histologically confirmed NB aged 1.5-175 months (I-II stages: 13; III-IV stages: 62; MYCN gene amplified: 26.6%). Patients were treated according to risk groups under the international standard protocols. Response to chemotherapy was assessed according to INRC. Primary tumor tissue obtained from patients at diagnosis was used for molecular-genetic analysis. The GSTP1 gene expression level was analyzed by TaqMan real time RT-PCR and normalized to the expression level of house-keeping gene GAPDH. MYCN gene amplification was detected by FISH method.

We observed that GSTP1 expression level increases proportionally to the stage of disease ($p = 0.12$) with maximum value in stage IV. Also GSTP1 expression level was higher in MYCN-amplified tumors than in those without MYCN amplification ($p = 0.09$). We did not notice an association between demographic characteristics of the NB patients (age at diagnosis and sex) and GSTP1 expression level. Primary resistant tumors had significantly higher levels of GSTP1 gene expression compared to chemotherapy sensitive tumors ($p = 0.01$), regardless MYCN status. ROC analysis revealed that GSTP1 expression level in tumor is an important marker which is associated with clinical outcome of primary childhood NB patients (Se = 75.8 %; Sp = 62.5 %; AUC = 0.71; $p = 0.002$). Tumors were categorized into two groups (high or low GSTP1 expression) based on cutoff point - optimal criterion, that was determined by ROC analysis. High GSTP1 expression was associated with reduced event-free survival (EFS) in NB patients. The 2-year EFS rate for NB patients with high GSTP1 expression was 38 % compared to 71 % for low GSTP1 expression ($p = 0.042$).

Our results suggest the possibility that GSTP1 is involved to the clinical behavior of NB, which is consistent with its known roles in drug resistance development, cell cycle and cell death regulation. Target inhibition of GSTP1 could be very promising strategy for NB treatment.

Keywords: Glutathione S-transferase P1, neuroblastoma.

TUE-237
The influence of p73 isoforms on cell viability and proliferation

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The p53 family includes three proteins: the tumour suppressor p53 and its two siblings, p73 and p63, which play an important role in carcinogenesis and also in embryogenesis. All proteins of the family are expressed as multiple isoforms due to existence of two promoters and alternative splicing of N-terminal and C-terminal region. All proteins of the family have similar domain architecture. Half of the isoforms have N-terminal transactivation domain necessary for transcriptional activation of target genes. Central DNA-binding domain is highly conservative among family members, so transcriptionally active p63 and p73 isoforms with the full-length N-terminal domain (TA-isoforms) can transcriptionally activate many p53 targets including proapoptotic and cytostatic genes and so act as tumour suppressors. The isoforms lacking full-length N-terminal domain because of transcription from an alternative promoter or alternative splicing mostly act as oncogenes because of competition with TA-isoforms for responsive elements in promoter regions of target genes and also because of similarity between their oligomerization domains that allows them to form inactive heterooligomers with TA-isoforms (dominant-negative inactivation). The balance of different isoforms can impact in tumour development. The C-terminus of the p53 family protein is responsible for protein – protein interactions. Some isoforms of p63 and p73 have also additional C-terminal domain called sterile α motif which is present in many proteins involved in embryogenesis. The length of C-terminus also affects the level of transcriptional activity of TA-isoforms, for example the activity of full-length TAp73α is much less than of shorter TAp73β and TAp73γ isoforms. Nowadays there are many works devoted to the properties and functions of particular isoforms, and there few studies comparing the impact of p73 isoforms with different C-terminal domains on cell viability and cell cycle. To find it out we stably overexpressed TA- and ΔN-p73 isoforms with different C-termini (full-length α isoform and shorter β and γ isoforms) in HCT116 human colon carcinoma cells and tested their influence on the level of apoptosis and cell cycle. TAp73 isoforms induced apoptosis at similar levels, while overexpression of ΔNp73 isoforms decreased the number of apoptotic cells compared to control. Interestingly, overexpression of all p73 isoforms including ΔN-isoforms caused the decrease of amount of cells in G1-phase and accumulation of cells in G2/M-phase. This may provide additional information on the role of p73 in cell signalling pathways.

Keywords: apoptosis, Cell cycle, p53 family.

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TUE-238
The mitochondrial protein VDAC1 and apoptosis modulators as signature biomarkers for cancer diagnosis, prognosis and treatment efficacy

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Human tumors are riddled with genomic alterations that include not only mutations that disrupt a wide spectrum of genes yet also those that affect protein expression levels. Hence, such alterations can be identified at the mRNA level but most significantly at the protein expression level. A key challenge is identifying such alterations for use as biomarkers enabling early diagnosis of cancer, as well as for forecasting cancer development, treatment efficacy and to potentially guide personalized medicine. Given that in many cancers, cells undergo re-programming of metabolism and develop survival strategies involving anti-apoptosis defense mechanisms, we analyzed the expression of metabolism- and apoptosis-related proteins in several cancers. We found that a mitochondrial key player in cell metabolism and regulation of mitochondria-mediated apoptosis, voltage-dependent ion channel isoform 1 (VDAC1). is highly expressed in different cancers, such as CLL, cervix, lung, liver, thyroid cancer, glioblastoma. The expression profiles of other proteins involved in the re-programming of cancer cell metabolism and capability to evade apoptosis were also analyzed. These included the glycolysis enzyme hexokinase, the mitochondrial anti-viral-signaling (MAVS) protein and Bcl2, proteins that are all over-expressed in both solid and non-solid tumors. Unexpectedly, cancerous cells also over-express the mitochondrial pro-apoptotic proteins SMAC/DIABLO (second mitochondria-derived activator of caspases) and AIF (apoptosis-inducing factor). Moreover, we were able to predict the probability of disease based on the expression levels of VDAC1, Bcl2, SMAC/Diablo, MAVS or a combination of AIF and HK-I, by using binary logistic regression analysis. Furthermore, based on the levels of these biomarkers, we could predicate which of the CLL patients considered in this study would need drug treatment. The expression profiles of these apoptosis modulator proteins reflect the adoption of a cancer survival strategy involving anti-apoptotic defense mechanisms and thus, low susceptibility to apoptosis-mediated cell-killing compounds. When one considers VDAC1 function in cell metabolism and apoptosis, as well as its interaction with apoptosis-modulating proteins, then its over-expression in cancers is not surprising. Hence, the expression profiles of VDAC1 and selected apoptosis modulators can serve as biomarkers to forecast cancer development, treatment efficacy and potentially enable early diagnosis.

Keywords: Biomarkers, VDAC1.

TUE-240
The role of 8p deletions in breast cancer development and progression

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In a typical cancer sample, 25% of the genome is affected by arm-level loss-of heterozygosity (LOH). A large size of the chromosomal deletions suggests a "two-hit" mechanism, when co-deletion of genes, miRNA clusters, and/or regulatory regions can synergistically promote tumor growth. In our study we focused on the short arm of chromosome 8 (8p), which is deleted in more than 50% of primary breast tumors. Although various potential tumor suppressive genes have been identified across the 8p region, none fulfils the Knudson criteria. The frequent loss of 8p could not be also explained by the presence of common fragile sites.

To assess the contribution of 8p deletion to tumorigenic transformation, I combined novel genetic engineering technologies and the experimental models of cell transformation derived from non-malignant human cells. As a first step, I optimized a semi-automatic workflow for the generation of human mammary cells with targeted chromosomal deletions. Using the TALEN approach, I succeeded to introduce deletions of the predetermined genomic DNA segments in the range of 4 Mb to 33 Mb at frequencies of 10^{-4} to 10^{-5}. I then generated a set of MCF10A cell lines harboring serial LOHs of 8p chromosome. Using a high-density SNP analysis, we also confirmed the integrity of the rest of the genome of the isolated clones. Currently, we are elucidating the contribution of 8p loss to several cancer-associated phenotypes. We anticipate that experimental models of human cell transformation that mimic cancer-associated chromosomal abnormalities will provide essential reagents for maximizing the efficiency of large-scale functional genomics efforts and accelerate the functional annotation of the human cancer genome.

Keywords: chromosomal deletions, human models of cell transformation, tumorigenesis.

TUE-242
The Role of DJ1, a PTEN inhibitor, and Sodium 4- Phenyl Butyrate in cancer cell motility and migration

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DJ-1 is a 20-KDa protein that was first identified as an oncogene product responsible for a subset of familial Parkinson's disease; hence its name PARK 7. Previous studies showed that DJ-1 negatively regulated the tumor suppressor gene PTEN expression; thus promoting the phosphorylation of the PI3K/Akt signaling pathway, and activating cell proliferation and transformation. Therefore, DJ1 can be used as an indication of cancer metastasis and can be a potential therapeutic target. However, the detailed mechanism and the role of DJ-1 in cellular motility are still not fully understood. PBA or phenylbutyrate is a low molecular weight fatty acid that was shown to increase expression of many genes including anti-apoptotic genes, and it does so by binding to the Sp1 binding sites in the genes promoter. Knowing that DJ-1 has the Sp1 binding site, studies showed that phenylbutyrate can increase DJ-1 gene expression and cause growth arrest of malignant tumors as well. However, the effect of PBA on cellular motility is not fully understood. Therefore this study showed that DJ-1 increased cellular motility and migration in lung, brain, breast, and skin cancer cells by activating the PI3K pathway. We showed that this activation leads to the phosphorylation of Akt and thus activation of RhoGTPases (RhoA, cdc42 and rac) which are known to increase cellular motility. Moreover, this study also showed that PBA increase DJ-1 gene expression and leads to the decrease in cancer cells motility and migration.

Keywords: DJ1, invasion, PBA.

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TUE-243
The role of LNX2 protein in regulation of Frizzled-7 receptor signalling

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Frizzled receptors are a group of seven-pass transmembrane receptors that play a key role in the transduction of signals from secreted ligands known as Wnts. Wnt signaling plays a central role in development, regulating proliferation, stem cell maintenance and cell fate decisions. Deregulated Wnt signaling is also a major contributing factor to epithelial carcinogenesis, including breast and colon cancer. Activation of Wnt signaling, as evidenced by nuclear localisation of β-catenin, has been repeatedly described in human breast cancer. There is increasing evidence that endocytosis of Frizzled plays a key role in regulation of Wnt signaling. Endocytosis of Frizzled seems to depend on interaction with Dishevelled (Dsh), which in turn recruits components of the endocytic machinery. Upon internalization, the Fz-Dsh complex appears to dissociate indicating that post-endocytic trafficking of Frizzled receptors (and their subsequent degradation) is dependent on other proteins.

Frizzled 7 interacts with PDZ domain-containing proteins via the C-terminal binding motif. PDZ-containing proteins are abundant in cells and have the ability to form PDZ-based multiprotein complexes at the cell membrane. They are important for regulation of receptor trafficking and signal transduction. Among these proteins is LNX2, a member of RING finger-type E3 ubiquitin ligase family. It regulates Notch signalling pathway and plays an important role in tumorigenesis. We have found that LNX2 is a novel PDZ-protein interacting with Fz7. It is involved in post-endocytic trafficking of Frizzled-7 and regulates Wnt signalling in breast cancer cells.

Keywords: breast cancer, ubiquitination, WNT signalling.

TUE-244
The role of transmembrane residues in regulation of pore dilation of the P2X7 receptor channel

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P2X receptors are membrane cation-permeable channels that open in response to the binding of extracellular adenosine 5′-triphosphate (ATP). In mammals, seven P2X subunits (P2X1-P2X7) have been identified which form functional trimeric homomers or heteromers. The P2X7 subtype is the most specific in the P2X family and widely differs from other P2X subtypes. Functionally the P2X7 receptor attracts considerable interest in relation to human health and disease mainly due to the fact that the P2X7 gene is highly polymorphic and the receptor properties are changed in cancer cells. The most striking kinetic feature of P2X7 is that during sustained agonist application the receptor does not desensitize as other P2X subtypes, but its pore dilates reaching the permeability for organic cations. Formation of a pore in the plasma membrane is associated with uncontrolled influx of Ca^{2+} which can induce cell death.

The aim of our work was to investigate the role of selected amino acids of both transmembrane domains (TM1 and TM2) in the kinetics of the P2X7 receptor. We selected amino acids that have been previously shown to significantly influence kinetics of the P2X4 receptor - the most closely related receptor to P2X7 in the P2X family. Using single-point mutagenesis the selected residues were substituted to alanine, single-point mutants expressed in human embryonic kidney cells (HEK293), and agonist-induced currents were measured by whole cell patch clamp recording technique.

We found that alanine substitution of Y40 or F43 residue abolishes the ability of the P2X7 to dilate the pore. Moreover the Y40A mutant displays desensitization profile. In TM2 domain alanine substitution of G338 significantly increased sensitivity to various agonists and strongly prolonged deactivation time. Mutants L341A and G345A retain ability to dilate, but the ability was reduced. In summary, our experiments, supplemented with structural analysis, indicate that the P2X7 dilation is critically dependent on the aromatic residues in TM1 and that the G338, L341 and G345 residues of the TM2 domain are located in the signal transduction pathway that regulate pore dilation and the associated sensitization of the P2X7R.

Keywords: ion channel, P2X7 receptor, pore dilation.

TUE-245
The Sall2 transcription factor is involved in cellular stress responses

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Cellular stress responses are an integral part of normal physiology to either ensure cell survival or to eliminate damaged or unwanted cells. Aberrant cellular stress responses are tightly linked to many common diseases including cancer. Evasion of cell death can promote tumor initiation, progression, and resistance to cytotoxic therapies. Thus, a better understanding of the underlying mechanisms of cellular stress responses will help to identify key targets to interfere with these processes. Our current work suggests that Sall2, a member of the SPAL/SALL family of transcription factors, is a stress-inducible molecule involved in the cellular response to stress. Sall2 is deregulated in various cancers suggesting a role in the disease. We previously identified Sall2 as an interacting protein of neurotrophin receptors and demonstrated it is involved in neuronal differentiation. Recently we identify Sall2 as a direct target of p53 tumor suppressor. We demonstrated that p53 directly binds to the Sall2 promoter and regulates Sall2 expression under genotoxic stress. To further investigate Sall2 role in cellular stress, we use primary MEFs from Sall2-deficient mice and exposed them to metabolic (serum or glucose deprivation) or genotoxic (chemotherapeutic agents) stress conditions. We investigated for changes on Sall2 expression levels in wild type MEFs, and compare cellular stress response between Sall2-deficient and wild type MEFs. Our data suggest that Sall2 is a stress-inducible molecule and its role in cell survival depends on the cellular context. Under genotoxic stress Sall2 acts as a pro-apoptotic factor, while under metabolic stress Sall2 acts as a pro-survival factor. Further comprehension of Sall2 regulation and its direct targets under stress conditions is essential to fully understand its role in normal and disease states.

Keywords: cell survival, cellular stress, Sall2.

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TUE-246
The scaffolder 14-3-3 sigma as a new modulator of TGF-beta signaling for growth inhibition

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Transforming growth factor-β (TGF-β) has a potent antiproliferative effect on a wide variety of cells, and insensitivity to this growth-inhibitory effect is closely associated with progression of tumors. To identify new target genes regulated by TGF-β, we performed cDNA microarray analysis using mRNA from normal Eph4 mammary epithelial cells and TGF-β-resistant Eph4-Ras cells treated with/without TGF-β and isolated 14-3-3 sigma as a new candidate gene induced by TGF-β. Our data show that TGF-β-mediated upregulation of 14-3-3 sigma is dependent on Smad3 not p53. 14-3-3 sigma functional study demonstrate that upon TGF-β stimulation, 14-3-3 sigma serves as an adaptor to bridge the E3 ligase anaphase promoting complex/ cyclosome (APC/C) to Cdc25A, which results in Cdc25A polyubiquitination and subsequent degradation. Our results uncover a previously unrecognized role for 14-3-3 sigma in the regulation of anti-proliferative TGF-β signaling and provide molecular insight into the mechanisms by which 14-3-3 sigma-APC/C target Cdc25A for degradation.

Keywords: 14-3-3 sigma, APC/C, TGF-beta.

TUE-247
The SH3 domain protein is a novel regulator of PKC/MAPK signaling in fission yeast

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Protein kinase C (PKC) is a calcium-dependent enzyme that phosphorylates serine and threonine amino acid residues on its substrate proteins, and is highly conserved in all eukaryotic organisms from yeast to man (Nishizuka *et al*, 1977). Numerous studies have clarified that PKC is involved in a wide range of physiological functions, and serves as a molecular target for anti-cancer drugs since it has been identified as the receptor for phorbol ester tumor promoters.

In our laboratory, we have been studying the signal transduction system using *Schizosaccharomyces pombe* (*S. pombe*) which is an excellent model to study the mechanisms of cellular proliferation and cancer in humans. Through molecular genetic screening using FK506, which is an immunosuppressive drug that inhibits calcineurin, we showed that Pck2, a protein kinase C homologue in *S. pombe*, acts upstream of Pmk1 MAPK (ERK homologue).

In this study, we identified that Skb5, a Src homology 3 (SH3) domain protein, as a regulator of Pck2 signaling by utilizing the cytotoxicity induced by Pck2 overproduction. Since Skb5 has been reported to bind to Mkh1, which is the MAPKKK found in Pmk1 MAPK signaling or Ptc1, which is the MAPK phosphatase for Sty1 or Pmk1, we assumed that Skb5 negatively controls the Pck2/MAPK signaling pathway by physically interacting with these proteins. However, the exact mechanisms of the physical/ functional interactions between Skb5 and these signaling molecules are poorly understood. Therefore, we attempt to uncover the physiological significance of Skb5 binding to both the MAPK activator Mkh1, and the MAPK suppressor Ptc1. In addition, Skb5 has proven to be implicated in the regulation of the stress-activated MAPK Sty1/Spc1 pathway, thus posing Skb5 as a key adaptor molecule in the PKC/MAPK signaling in yeast.

Keywords: Fission Yeast, MAPK, Protein Kinase C.

TUE-248
The TRPM4 channel regulates the Epithelial-Mesenchymal transition in prostate cancer cells

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The Transient Receptor Potential Melastatin 4 (TRPM4) is a Ca^{2+} activated and voltage-dependent monovalent cation channel, which depolarizes the plasma membrane, thereby modulating Ca^{2+} influx through Ca^{2+}-permeable pathways. TRPM4 overexpression has been described in a number of cancers, including prostate cancer and it has been suggested that TRPM4 promotes cancer progression in these cells. Nevertheless, whether TRPM4 is involved in prostate cancer progression and the underlying molecular mechanisms remain unknown. In this work we analyzed the consequences of TRPM4 knockdown in the expression of epithelial-mesenchymal transition (EMT) markers (E-Cadherin, N-Cadherin, Vimentin, β-Catenin, Fibronectin1, ZEB1, SNAIL1 and SLUG) and related biological processes such as cellular migration and invasion. TRPM4 knockdown in PC3 and LnCaP prostate cancer cells resulted in a significant change in the expression pattern of EMT marker genes. These changes are compatible with a reversal of the EMT process in these cells. Also, TRPM4 knockdown produced a diminution in cell invasion and migration in PC3 cell line. To explore the underlying mechanism, we evaluated the activity of the β-Catenin and GSK3-β signaling pathway in these cells. TRPM4 knockdown cells have decreased total β-Catenin protein levels, and an increase in S33, S37 and T41 β-catenin phosphorylation as well as an increase in S9 GSK3-β inhibitory phosphorylation, suggesting that a down-regulation of components of the canonical WNT signaling pathway could explain the relationship between TRPM4, EMT and cell invasion.

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Keywords: Epithelial-Mesenchymal Transition, Prostate Cancer cells, TRPM4 Channel.

TUE-249
The Wilms Tumor protein 1 represses the expression of its pro-apoptotic co-factor, ZNF224, in leukemic cells

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ZNF224 is a pro-apoptotic zinc-finger protein in Chronic Myelogenous Leukemia (CML), which is able to bind the Wilms Tumor protein 1, WT1, acting as a co-activator on pro-apoptotic WT1 target genes while suppressing WT1 mediated transactivation of anti-apoptotic genes.[1] Recently, we reported ZNF224 as negatively regulated by Bcr/Abl fusion protein kinase. Indeed, we demonstrated that Bcr/Abl forced expression in Acute Myelogenous Leukemia cell line KG1 (Bcr/Abl negative), lead to a decreased ZNF224 expression. Moreover, an increased ZNF224 expression was shown in Bcr/Abl positive CML cell line, K562, treated with Bcr/Abl inhibitor (Imatinib) or with PI3K inhibitor

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(Wortmanin), thus indicating ZNF224 as negatively regulated by Bcr/Abl through the downstream activated PI3K pathway. In addition, literature data reported a positive regulation by Bcr/Abl kinase and PI3K pathway on the transcription factor WT1, which results highly expressed in Bcr/Abl positive CML cell line K562.[2] This data lead us to consider WT1 as a putative transcription factor downstream Bcr/Abl and PI3K pathway, regulating ZNF224 transcription. At first, to confirm this hypothesis, we conducted bioinformatics analysis on a 1000 bp region of the ZNF224 promoter, thus reporting three putative WT1 binding sites. Then, we performed Chromatin Immunoprecipitation assays in K562 cells. The chromatin was immunoprecipitated with WT1 antibody and WT1 binding on ZNF224 promoter was confirmed. Finally, to investigate the role of WT1 on ZNF224 promoter activity, we performed a luciferase assay in K562 cells. The obtained results showed a decreased ZNF224 promoter activity when WT1 was overexpressed and an increased promoter activity in K562 cells treated with Imatinib. In addition, K562 cells treated with Imatinib and overexpressing WT1 showed no significant increase in ZNF224 promoter activity, thus corroborating WT1 as a putative transcription factor downstream Bcr/Abl and PI3K pathway, regulating ZNF224 transcription.

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Keywords: Zinc-finger protein; Imatinib; Chronic Myelogenous Leukemia.

TUE-251
Tissue mechanics modulate glioma cell aggression

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Gliomas are the most common primary intracranial tumors in adults, accounting for more than 50% of all primary adult brain cancers. The most aggressive of these tumors are glioblastoma multiforme (GBM) with mean patient survival of 14.6 months. Diffuse invasion of glioma cells into healthy brain tissue prevents complete surgical resection of the tumors and contributes to a high rate of tumor recurrence and invariable lethality. Interestingly, upon clinical presentation, primary brain tumors typically exhibit high intracranial pressure and MRI elastography and vibrometry indicate that brain tumors might be quite stiff. In this respect, many solid state tumors exhibit elevated extracellular matrix (ECM) stiffness and display increased interstitial pressure. Elevation of ECM force is a known driver of malignancy and its inhibition reduces tumor aggression in many cancer types. Yet, the significance of such altered physical force to brain pathophysiology remains unclear. Using primary human tissues and various mouse models of GBMs, we tested whether the microenvironment of GBMs is mechanically different from that of non-malignant human brain tissue and whether ECM stiffness and mechanotransduction increases with glioma grade. Furthermore, we examined whether ECM stiffness per se modulates gene and protein expression that is associated with increased cell aggression, fostered invasion, and improved survival of primary GBM cells. Our nano-mechanical mapping of transgenic and orthotopic mouse models of human GBMs showed that brain tumors are stiffer than normal brain tissue and that the ECM of orthotopic mouse models with the most aggressive human GBM cells are stiffer than those injected with the less aggressive cells. Furthermore, nano-mechanical analysis of freshly-excised patient samples (6-10 cases per subtype) of different grades (0 – 4) revealed an increase in ECM stiffness with glioma grade. Consistently, we observed an increase in mechano-signaling with grade, as indicated by marked increase in pFAK^{397}, pMLC, and YAP. Furthermore we found that increase in ECM stiffness enhanced the expression of genes and proteins that are associated with increased glioma cell aggression, such as CD44, fibronectin, hyaluronic acid, mushashi-1, and CTGF. These findings argue that GBM aggression may be an adaptive response to a high force microenvironment which favors the growth and survival of highly-aggressive cells. Our work is the first to definitively report and present the consequences of altered biophysical force in gliomas.

Keywords: glioma, mechanotransduction.

TUE-252
TP53 codon 72 and 240 polymorphism and P53 expression: an association with oral squamous cell carcinoma

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Oral squamous cell carcinoma (OSCC) is the leading cause of death in the developing countries like Pakistan. This problem aggravates because of the excessive use of available chewing products. In spite of widespread information on their use and purported legislations against their use, the Pakistani markets are classical examples of selling chewable carcinogenic mutagens. Reported studies indicated that these products are rich in reactive oxygen species (ROS) and polyphenols. TP53 gene is involved in the suppression of tumor. It has been reported that somatic mutations caused by TP53 gene are the foundation of the cancer. This study aims to find the loss of TP53 functions due to mutation/polymorphism caused by genomic alteration and interaction with tobacco and its related ingredients. Total 260 tissue and blood specimens were collected from OSCC patients and compared with age and sex matched controls. Mutations in exons 2-11 of TP53 were examined by PCR-SSCP. Samples showing mobility shift were directly sequenced. Two mutations were found in exon 4 at nucleotide position 108 and 215 and one in exon 7 at nucleotide position 719 of the coding sequence in patient's tumor samples. These result in substitution of proline with arginine at codon 72 and serine with threonine at codon 240 of p53 protein. These polymorphic changes, found in tumor samples of OSCC, could be involved in loss of heterozygocity and apoptotic activity in the binding domain of *TP53*. The interpretations could be helpful in establishing the pathways responsible for tumor formation in OSCC patients.

Keywords: Missence Mutation, OSCC, TP53 gene.

TUE-253
Transarterial chemoembolization for liver cancer patient: good or bad?

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Hepatocellular carcinoma (HCC) is one of the most common and lethal cancers worldwide. It has high mortality rate as most patients were diagnosed at an advanced stage in which only lim-

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ited choices of treatment are available. At early stages, several effective treatments include tumor resection, liver transplantation, and radiofrequency ablation. However, only 20-30% of patients are eligible for such curative treatments. Therefore, transarterial chemoembolization has become the standard non-curative treatment for HCC patients who are not suitable for receiving curative treatments.

Transarterial chemoembolization (TACE) is a surgical procedure consisting of the injection of cytotoxic drugs (Chemotherapy) and the obstruction of blood supply of the tumor via transarterial embolization (TAE). Since the blood supply for liver tumors is mainly through the hepatic arteries whereas the liver parenchyma is supplied by portal veins, the emobolization of the hepatic artery will block nutrient supply and localize the cytotoxic drug to the tumor, thereby reducing the tumor growth. Although TACE can enhance the survival rate of HCC patients, its long term effect is unsatisfactory. Therefore, we are aimed to elucidate the possible mechanisms responsible for such failure.

Growing evidence suggests that HCC is originated from cancer stem cells (CSCs) that are a subpopulation of cancer cells with stem cell properties. They can self-renew, differentiate into multiple lineages and adapt to extreme micro-environments. In addition, CSCs are resistant to chemotherapies and extreme environments, leading to tumor maintenance and recurrence. Therefore, we are sought to find out the role of CSCs in the treatment of HCC after TACE.

Among Chemotherapy, TAE and TACE, TACE is the most effective method to inhibit the tumor growth of HCC. However, none of them can completely eradicate the tumor. Among all the well-known CSCs markers, we found that $CD44^{+}Epcam^{+}$ HCC cells were enriched in mice with HCC tumors after the treatments of either chemotherapy, TAE or TACE. Highest enrichment of $CD44^{+}Epcam^{+}$ HCC cells was observed in TACE treated mice, suggesting that chemotherapy and TAE synergistically enriched such cell population. By genome-wide sequencing we identified IGFBP1 as a potential candidate responsible for the enrichment of cancer stem cells after TACE treatment. Indeed, IGFBP1 was found to be up-regulated in HCC tumors after TACE *in vitro* and *in vivo*. Ectopic expression of IGFBP1 increased the proliferation rate and the sphere forming potential of HCC cells, suggesting that deregulated expression of IGFBP1 may enhance the tumorigenicity and the stemness of CSCs in HCC tumors. This study provides mechanistic explanation for the adverse outcomes of TACE treatment and reveals possible therapeutic approaches for improving the TACE.

Keywords: cancer stem cell, HCC, TACE.

TUE-254
Transcriptional and epigenetic regulation of osteopontin (SPP1) expression in glioma cells

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Clinical and experimental studies show accumulation of microglia/macrophages in gliomas and their contribution to tumor progression. Re-programmed brain macrophages support tumor growth, invasion, induce immunosuppression and modulate response to cancer treatment. We identified osteopontin (known as secreted phosphoprotein 1 - SPP1) as one of the factors released by glioma cells which induces pro-invasive activation of microglia and creates the immunosuppressive tumor milieu. Osteopontin is overexpressed in many cancers including high grade gliomas and its expression inversely correlates with patient's survival. To investigate molecular mechanisms underlying regulation of osteopontin expression in glioma cells, we performed a computational analysis of the human *SPP1* gene promoter that revealed potential binding sites of transcription factor GLI1 (glioma-associated oncogene homolog 1), a Hedgehog signaling effector implicated in tumorigenesis. Using chromatin immunoprecipitation (ChIP) we confirmed binding of GLI1 to the *SPP1* gene promoter in U87MG, LN18 and primary glioblastoma WG4 cell, but not in nontransformed human astrocytes. Knockdown of *GLI1* expression in U87MG astrocytoma cells decreased the osteopontin expression at the mRNA and protein level. Previous studies on mouse embryos demonstrated binding of Oct4 (octamer-binding transcription factor 4), involved in maintaining stem cell pluripotency, in the first intron of *Spp1* gene. Using ChIP we showed that OCT4 binds to the first intron of the *SPP1* gene promoter in U87MG, LN18 and WG4 glioblastoma cells but not in normal human astrocytes. This finding implicates OCT4 in regulation of SPP1 expression in glioma cells. Accordingly, we found the increased level of osteopontin in populations enriched in glioma stem-like cells isolated by flow cytometry as side population or grown as cancer spheres. To investigate involvement of epigenetic mechanisms in regulation of osteopontin expression, we treated glioma cells with inhibitors of histone modifying enzymes (trichostatin A, 3-Deazaneplanocin A). Inhibition of histone deacetylases (HDACs) led to increase of the osteopontin expression in LN18 glioblastoma cells at the mRNA and protein level. Conclusions: Our results demonstrate re-establishment of the stem cell-type, transcriptional regulation of osteopontin expression in glioblastoma cells, in particular in glioma stem-like cells.

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Keywords: glioblastoma, osteopontin, transcriptional regulation.

TUE-255
Transcriptional factors p53 and p63 regulate expression of orphan nuclear receptors NR4A

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The p53 protein and its two homologues p63 and p73 are transcriptional factors regulating expression of many genes. The TP63 gene encodes two main isoforms: TAp63 and ΔNp63 which are expressed from alternative promoter. The p63 protein is known to act in the development of limb and skin both in mice and humans. In addition, p63 was shown to play role in tumorigenesis, but the relative impact of p63 in tumorigenesis remains unclear and needs further investigation. Search for new transcriptional targets of TAp63 and ΔNp63 can provide more clear understanding of the p63 role in tumorigenesis. Microarray gene expression analysis of cell lines with different status of p63 identified a number of potential target genes. One of these genes is the NR4A3 gene encoding a member of the steroid-thyroid hormone-retinoid receptor superfamily. NR4A receptors are early immediate-response genes that control a variety of cellular functions, such as inflammation, proliferation, apoptosis, cell cycle regulation, cancer genesis. The main purpose of our work was to determine the influence of p53 and p63 on transcription of NR4A. Two isoforms of p63 TAp63 and ΔNp63 were expressed in Saos2-Tet-On cell lines. Western blot analysis showed elevated levels of NR4A3 protein in ΔNp63-expressing cells but not in TAp63-expressing cells. Quantitative RT-PCR showed an accumulation of NR4A3 mRNA in ΔNp63-expressing cells suggesting that the expression of NR4A3 is specifically regulated by ΔNp63.

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Since p63 is a member of the p53 transcriptional factors family, we hypothesized that p53 also affects expression of NR4A. To test this hypothesis we measured the expression levels of NR4A1, NR4A2 and NR4A3 in four cell lines U2OSp53 + and U2OSp53KD, HCT116 p53 + /+ and HCT116 p53-/- that differ by the status of p53. Quantitative RT-PCR shows up-regulation of NR4A1, NR4A2 and NR4A3 mRNA in p53-positive cells upon treatment with doxorubicin. The level of p21 mRNA, which is a well-known p53 transcriptional target, was used as a positive control. Western Blot analysis also showed increased expression of NR4A1, NR4A2 and NR4A3 in parallel with elevation of the p53 levels. Collectively, these data demonstrate that p53 and the ΔNp63 isoform regulates expression of NR4A1, NR4A2 and NR4A3 genes.

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Keywords: NR4A, p53 family, Transcription.

TUE-256
TSC2 is a negative regulator of mTORC1 in response to amino acid starvation

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mTOR Complex 1 (mTORC1) is a potent anabolic regulator of cellular growth and metabolism. When cells have sufficient amino acids, mTORC1 is active due to its lysosomal localization mediated via the Rag GTPases. Upon amino acid removal, the Rag GTPases release mTORC1, causing it to become cytoplasmic and inactive.

We show here that upon amino acid removal, the Rag GTPases also recruit TSC2 to the lysosome, where it can act on Rheb. Only when both the Rag GTPases and Rheb are inactive is mTORC1 released from the lysosome. Upon amino acid withdrawal, cells lacking TSC2 fail to release mTORC1 from the lysosome, fail to completely inactivate mTORC1, and fail to adjust physiologically to amino acid starvation.

These data suggest that regulation of TSC2 subcellular localization may be a general mechanism to control its activity, and places TSC2 in the amino acid sensing pathway to mTORC1.

Keywords: cell growth, mTORC1, TSC2.

TUE-258
UCN-01 as a new tool to block drug export in melanoma

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Melanoma is the most aggressive form of skin cancer and it is highly resistant to all current modalities of cancer therapy, including a wide variety of citotoxic drugs like methotrexate. In the last years, it has been discovered a new melanoma mechanism of resistance to methotrexate (MTX) by which this drug is sequestered into melanosomes and exported out of melanoma cells, driving to a reduced accumulation of this drug in the cytosolic compartment which is just able to induce growth arrest but

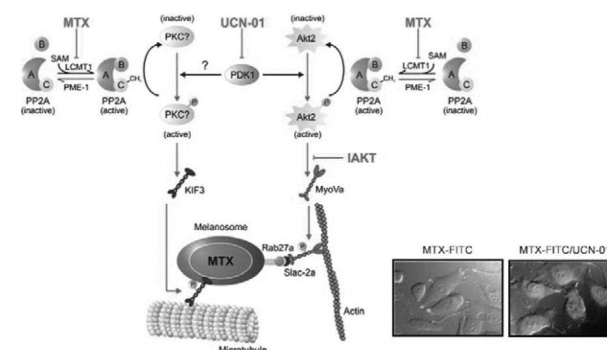

Fig. 1.

not cell death. Particularly, we found that melanoma treatment with methorexate altered melanogenesis and accelerated the exportation of melanosomas, but the cellular and molecular processes by which MTX is trapped into melanosomas and exported out of cells have not been elucidated. In this study, we identify a new methotrexate-activated molecular pathway involved in the export of melanosomas, in which the motor protein MyosinVa (MyoVa) plays a key role. The results demonstrated that melanoma treatment with MTX leads to Akt2-dependent MyoVa phosphorylation, which enhances its ability to interact with melanosomes and accelerates their exportation. Due to these findings, we designed a MTX combination therapy to increase the susceptibility of melanoma to this drug by blocking this MyoVa/Akt2 pathway. Because7-hydroxystaurosporine (UCN-01) has been shown to potently inhibit PDK1, which activates Akt by phosphorylation, we hypothesized that the inhibition of Akt2 phosphorylation by UCN-01 may result in the disruption of MTX stimulated melanosome transport. By avoiding MTX export, we observed that the E2F1 apoptotic pathway is functional in melanoma, and its induction activates p73 and Apaf1 following a p53-autonomous pro-apoptotic signalling event. *In vivo* studies in mice also indicated that low doses of UCN-01, when combined with MTX, produced a marked reduction for not only tumour growth but also melanoma metastasis. In summary, we observed that the combination of MTX and UCN-01 may represent a therapeutic option for the treatment of this evasive disease.

Acknowledgments: This work was supported by grants from Fundación Séneca, Región de Murcia (FS-RM) (15230/PI/10) and European Commission (FP7-INCO 293514). M.P.F-P has a FPU fellowship from MECD.
Keywords: Chemotherapy Resistance, Melanoma, Methotrexate.

TUE-259
Upregulation of p16 by small interfering RNA for Galpha12 inhibits cellular proliferation of HepG2 human hepatoma cells

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Heterotrimeric GTP-binding proteins (G proteins) are known as signal transducers, which convert receptor-bound signals into intracellular signals to regulate cellular responses including proliferation, differentiation and apoptosis. Galpha12 (G12), one of G protein alpha subunits, is reported to be involved in tumor cell invasion and progression. However, the precise mechanisms by which G12 regulates proliferation of cancer cells are poorly understood. Thus, we aimed to investigate the effect of G12 on

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cellular proliferation and its underlying mechanism in HepG2 human hepatoma cells. To knock down G12 expression, HepG2 cells were transfected with G12 siRNA, which showed that G12 significantly inhibited the proliferation of HepG2 cells and the anchorage-independent colony formation. Moreover, G12 siRNA restored the expression of $p16^{Ink4}$ (p16) known to be silenced in many cancer cells and arrested the cell cycle progression of HepG2 cells. In conclusion, G12 siRNA inhibits the proliferation of HepG2 cells by upregulating p16 expression, suggesting that the abnormal proliferation of HepG2 cells might be resulted from G12 signaling to suppress p16 expression of HepG2 human hepatoma cells.

Keywords: hepatocellular carcinoma, Galpha12, proliferation.

TUE-261
Visfatin exerts proliferative and anti-apoptotic effects on MCF-7 breast cancer cells

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There is accumulating evidence that visceral adiposity increases the risk of breast cancer and exacerbates its prognosis. Visfatin is a novel adipokine which is predominantly secreted from visceral fat tissue and or macrophages and plays an important role in a variety of cellular functions. Its intracellular form is the rate-limiting enzyme in NAD biosynthesis. Visfatin expression is increased in breast cancer and its high expression is associated with more malignant cancer behavior. Therefore in the present study the effects of visfatin on MCF-7 breast cancer cell proliferation and apoptosis were evaluated.

Methods: MCF-7 breast cancer cells were used as a model. Cell viability was assessed by MTT. BrdU assay was performed to measure cell proliferation after treatment with 10-200 ng/ml concentrations of visfatin. Cell proliferation was also evaluated in the presence of phosphatidylinositol 3-kinase inhibitor (LY29400), ERK1/2 inhibitor (U0126), and the inhibitor of visfatin enzymatic activity (FK866). Western blotting was performed to detect Akt and ERK1/2 phosphorylation. In order to evaluate the anti-apoptotic effect of visfatin, cells were treated with TNF-α as well as visfatin. Apoptosis was assessed by flow cytometry after double staining with Annexin V-FITC and propidium iodide. Western blotting was used to detect poly-ADP ribose polymerase (PARP) cleavage and survivin protein levels.

Results: Visfatin significantly increased cellular proliferation. This effect was inhibited by LY29400, U0126 which suggests that the effect of visfatin is mediated by ERK1/2 and Akt signaling pathway. Visfatin also significantly induced phosphorylation of ERK1/2 and Akt 10 min and 60 min after treatment, respectively, indicating a direct mechanism. Cell viability was reduced with FK866 whereas it returned back to normal by visfatin co-treatment. Visfatin treatment counteracted the apoptotic effects of TNF-α on MCF-7 cells and significantly reduced PARP cleavage. The levels of Survivin, a protein with anti-apoptotic properties, were increased by visfatin.

Conclusion: The present study shows that visfatin plays a critical role in development and progression of breast cancer by increasing proliferation of cancer cells, activation of Akt and ERK1/2, and prevention of apoptosis.

Keywords: breast cancer, visfatin.

TUE-262
WMJ-S-001, a novel aliphatic hydroxamate derivative, induces HCT116 colorectal cancer cell death through AMPK-p53-survivin signaling

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Hydroxamate derivatives have been widely studied and many lines of evidence demonstrated that they exhibit broad pharmacological activities. Recent studies reported their potential use in the treatment of cancer. However, the mechanisms by which this suppresses colorectal cancer progression remain to be elucidated. In this study, we explored the anti-tumor mechanisms of a novel aliphatic hydroxamate derivative, WMJ-S-001, in HCT116 colorectal cancer cells. WMJ-S-001 inhibited cell proliferation and induced cell apoptosis in a concentration-dependent manner. These results are associated with the modulation of $p21^{cip/Waf1}$, cyclin D1, survivin and Bax. WMJ-S-001 activated AMP-activated protein kinase (AMPK) and p38 mitogen-activated protein kinase (p38MAPK), whereas AMPK or p38MAPK signaling blockade abrogated WMJ-S-001's effects of modulating $p21^{cip/Waf1}$, cyclin D1, survivin and Bax. In addition, WMJ-S-001 time-dependently caused increases in p53 phosphorylation and acetylation. WMJ-S-001's actions on p38MAPK and p53 phosphorylation, $p21^{cip/Waf1}$, cyclin D1, survivin and Bax were reduced in cells transfected with AMPK dominant mutant (DN). Results from chromatin immunoprecipitation analysis showed that Sp1 binding to the survivin promoter region decreased while p53 binding to the promoter region increased after WMJ-S-001 exposure. Furthermore, WMJ-S-001 also suppressed tumor growth in HCT116 colorectal tumor xenograft model. In conclusion, we demonstrated in this study that WMJ-S-001 activates the AMPK-p38MAPK-p53-survivin cascade to cause HCT116 colorectal cancer cell death. The present study delineates, in part, the underlying mechanisms of WMJ-S-001 in decreasing survivin and subsequent colorectal cancer cell apoptosis.

Keywords: colorectal cancer, hydroxamate, p53.

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CSIV-02 – Cell Dynamics

TUE-264
A biomechanical feedback regulates cycles of Myosin II phosphorylation to shape tissue morphogenesis

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Myosin II motors generate forces that power cell shape changes during tissue morphogenesis. Such actomyosin networks function like a mechanical ratchet where phases of pulsed contraction associated with cell deformation alternate with phases of shape stabilization. The mechanisms for differential regulation of these networks remain unknown. Cell intercalation driven *Drosophila* ectoderm extension exemplifies the role of the dynamic regulation of Myosin II. During this process, pulsed contractions of actomyosin mesh flow towards junctions aligned in dorso-ventral axis leading to their shrinkage. A planar polarized pool of Myosin II enriched at these junctions stabilizes the deformation making the process irreversible. We investigated the role of phosphorylation of Myosin II regulatory light chain (RLC) by the Rho1-ROCK pathway. Using phospho-mimetic Myosin II RLC that bypasses upstream regulation we found that phosphorylation is necessary and is alone sufficient to stabilize Myosin II at the junctions. Spatial control over phospho-cycles through ROCK and Myosin II Phosphatase mediates planar polarized enrichment through regulation of dissociation and hence turnover of the motor. We also show that phospho-cycles generate proper pulsatility of Myosin II to drive cell deformation, whereby phosphorylation determines pulse amplitude and phosphatase mediated dephosphorylation regulates pulse frequency. Interestingly, the regulators of Myosin II themselves, namely Rho1, ROCK and Myosin II Phosphatase, incorporate in pulsatile networks suggesting that they constitute an upstream biochemical pacemaker of pulsatility. However, our experiments indicate that Myosin II pulsatility is not a simple outcome of a biochemical pacemaker but an emergent behavior of a feedback between Myosin II contractility and the regulators of its contractility.

Keywords: actomyosin contractility, cellular signalling, planar cell polarity.

TUE-265
A generic methodological framework for studying single cell motility in high-throughput time-lapse screening data

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Cell motility plays a key role in many physiological processes such as embryonic development or immune response, and is also involved in tumor invasion and metastasis.

To better understand the regulation of this biological process and detect its potential chemical disruptors, tools are needed which allow a systematic study of cell migration in a high-throughput setup.

Many assays have been specifically designed to study cell migration mechanisms. They are typically based on a method for targeting selected genes or proteins, and a measure of cellular motility. Examples include analysis of cell traces on coated layers while overexpressing genes of interest [1], wound healing assays for the investigation of gene silencing effect on cell population migratory behaviour [2], or cell tracking in low-throughput time-lapse experiments while inhibiting key enzymes [3]. However, no study evaluating single cell migration was ever realized on a large scale: published high-throughput (HT) migration screens are all based on a measure of cell motility at the population level (e.g. [2] and [4]).

Here, we propose a generic methodological framework to quantitatively study cell migration at single cell resolution in HT data. It consists of cell tracking, cell trajectory mapping to an original feature space, identification of migratory patterns using unsupervised clustering, and discriminant characterization of each experimental condition in terms of migratory behaviours (cf figure).

We apply this workflow to an existing genome-wide RNAi screen (RNA interference screen), the Mitocheck dataset. It is composed of 200 000 HeLa cell time-lapse experiments, each of them resulting from a single gene knockdown [5]. Our workflow allowed us to identify potential migration suppressors, some of which are known to be involved in cell migration.

Furthermore, we show that the application of our framework to newly generated Environmental Toxicology data allows us to study in a quantitative and fully automated way the perturbation of cell motility following TCDD (2,3,7,8-tetrachlorodibenzodioxin) exposure. This opens up the possibility to systematically screen environmental chemicals with respect to their effect on cellular migration.

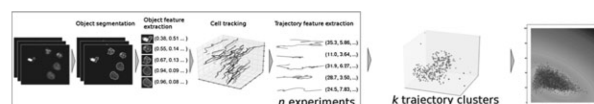

Fig. 1.

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Keywords: Bioinformatics, Cell migration, Screen.

TUE-266
A new role for CaMKIIβ in the regulation of neuronal migration

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The calcium/calmodulin-dependent protein kinases (CaMKs) represent a critical link between the external environment and cellular responses in neurons. CaMKII, one of the major CaMKs in the brain, exists as a holoenzyme composed of multiple subunits, which form a complex through their association domains. Brain CaMKII predominantly consists of the α and β isoforms, which form heteromeric or homomeric complexes. Although the functions of CaMKIIα have been extensively studied, the isoform-specific functions of CaMKIIβ remain underexplored, especially in the embryonic brain where the α isoform is not expressed.

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Here we show that CaMKIIβ is expressed from E14.5 in the cortical plate (CP) of the embryonic cerebral cortex. By using in utero electroporation, we demonstrate that CaMKIIβ knock-down accelerates the radial migration of projection neurons in the CP whereas its overexpression leads to migration defects. Interestingly, these defects are not observed when the F-actin binding domain (ΔFABD) of CaMKIIβ or its association domain necessary for the multimerization (ΔAsso) are deleted, indicating that CAMKIIβ regulates neuronal migration through its actin-bundling activity. In addition the binding of CAMIIβ to the actin cytoskeleton is regulated by Ca^{2+} fluctuations. Indeed, in cultured cells, an increase of Ca^{2+} entry induces the dissociation of CaMKIIβ from actin filaments and this is reversed when Ca^{2+} decreases. These data thus suggest that CAMKIIβ may mediate the effects of Ca^{2+} fluctuations on the saltatory movement of migrating neurons in the CP.

Altogether these data, by showing that CaMKIIβ is critical for neuronal migration during cortical development, reveal a novel function for CaMKIIβ in the mammalian brain

Keywords: Actin, CaMKIIβ, neuronal migration.

TUE-267

A theoretical study towards understanding the structural mechanism of Human Gamma-adducin and its isoforms

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Adducin is a membrane-skeletal protein which is a candidate to promote assembly of a spectrin-actin network in erythrocytes [1]. Three types of adducin proteins (alpha, beta and gamma) are encoded by three different genes, namely *ADD1*, *ADD2* and *ADD3*. All three adducin proteins contain an N-terminal protease resistant globular head domain, a neck domain and a C-terminal protease-sensitive tail domain [2]. Adducin has been suggested to be involved in kidney disease, cardiac morphogenesis and synaptic plasticity but so far no structural studies are available for these proteins. In the present work, the three-dimensional (3D) structure of gamma-adducin has been predicted by theoretical approach and its structural stability was analyzed through molecular dynamics simulation (MDS). Also, two isoforms produced by alternative splicing have also been characterized, where isoform 1 corresponds to the short protein (in which residues 576-607 are missing), and isoform 2 corresponds to the long protein (chosen as the canonical sequence). Furthermore, we also characterized the structural impact, on both adducin gamma isoforms, of a mutation found in affected individuals from a consanguineous family, presenting steroid resistant nephrotic syndrome, myocardiopathy, intellectual deficit and cataracts. Initially, the sequence of gamma-adducin was retrieved from UniProt database (ID: Q9UEY8). Due to the very low sequence similarity and low query coverage of gamma-adducin with its related structural neighbours, the homology modeling approach alone could not be used to attain the final 3D models. Hence, we used Robetta full-length protein structure server for predictions. This server parses protein chains into putative domains with the Ginzu protocol, and models those domains either by homology modeling or by ab initio modeling. Subsequently, the predicted models were subjected to MDS studies for understanding their structural stability using various parameters, such as RMSD, RMSF, etc. Four final models could be obtained for this protein which will further be compared with experimental data. This type of comparative structural analysis will be an effective tool to understand the role of new mutations found in various genetic disorders.

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Keywords: Ab initio, Adducin, MDS.

TUE-268

Accumulation of actin-binding protein zyxin in different types of adherens junctions of nontransformed and transformed epithelial cells

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The main course of our investigation is to study two distinct types of adherens junctions (AJs). Stable tangential AJs are typical of normal epithelial cells, while dynamic radial AJs are common to transformed epithelial cell and fibroblasts. Earlier we have reported of dissimilarities in regulation of tangential and radial AJ mediated by E-cadherin. Radial AJs require ROCK activity and actomyosin contractility for its formation, while tangential AJs require activity of Rac and mDia1. Our next goal was to examine distinctions in accumulation of actin-binding protein zyxin within adhesion plaques of tangential and radial AJs. To address this goal we used pairs of cell lines of same histological origin. IAR2 cells originate from rat liver explants and have normal epithelial phenotype while IAR6-1 cells derived from NDMA-treated IAR cells are moderately transformed. Along with IAR cell lines we used cells originated from human pancreatic carcinomas. Highly differentiated Capan-2 cells have virtually nontransformed morphology and form tangential AJs just as IAR2 cells do. Likewise PANC-1 cells underwent moderate degree of morphological transformation and form radial AJs similar to those of IAR6-1 cells. Cells of all four lines express E-cadherin. We examined accumulation of zyxin in mature AJs formed in cellular monolayers. It turned out that tangential AJs in IAR2 and Capan-2 cells lacked zyxin while AJs of IAR6-1 and PANC-1 accumulated it. We further tested zyxin accumulation in newly formed AJs. Specimens of narrow wound showed well-defined zyxin staining in radial AJs of IAR6-1 and PANC-1 cells whereas in case of IAR2 and Capan-2 cells there was a somewhat controversial picture of zyxin accumulation. In newly formed tangential AJs zyxin was stained either all along the contact, or in discrete patches, or in peripheral regions of contact. To clarify the way zyxin incorporates in different AJ types we examined the dynamics of its accumulation in IAR2 and IAR6-1 cells which simultaneously expressed GFP-E-cadherin and mCherry-zyxin. It became apparent that zyxin starts to accumulate in AJs along with E-cadherin in course of formation of both tangential and radial AJs. Radial AJs contained zyxin all along the period of observation while tangential AJs gradually lost it in the middle part of contact along with its elongation. Peripheral parts of tangential AJs preserved zyxin up to wound closure. On the basis of these observations we suggest that zyxin plays crucial role in linking radial AJs to dynamic actin cytoskeleton. This distinction apparently contributes to the ability of transformed epithelial cells to migrate relative to each other and normal cells.

Keywords: adherens junctions, epithelial cells, zyxin.

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TUE-269

An intercellular polyamine transfer via gap junctions in epithelial cells: implications for the control of cell proliferation and the response to stress

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Gap junctions are the only intercellular junctions in the animal kingdom that allow the direct exchange of small molecules (<1 KDa) such as ions, small metabolites and second messengers from cell to cell. Gap junctions are known to play a key role in various cellular processes like mechanotransduction, calcium signaling, and control cell proliferation. By the past, many studies have been focused on the gating of some gap channels by polyamines, especially spermine in vitro. Here we rather explore whether polyamines can be directly exchanged between adjacent cells via gap junction communications to participate in the growth control (Desforges et al, 2013). For instance, the transfer of polyamines from cell to cell can be important when quiescent epithelial cells resume cycling as a result of various stimuli including wound healing or oxidative stress. Indeed, under such conditions, quiescent cells need to increase their polyamine levels in order to resume cycling. To do so, quiescent cells can either resume polyamine synthesis or increase their active transport to resume proliferation. However, using our model, another alternative pathway can be put forward: an exchange of polyamines from cycling cells to quiescent cells through gap junctions. This hypothesis makes sense if we consider the scarce presence of free polyamines in plasma compared to the large intracellular pool. In addition, due to their small size, and to their hydrophilic nature, polyamines, especially putrescine, satisfy all the requirements to be transported from cell to cell via gap junctions. We will also discuss the potential application of the polyamine-mediated intercellular interactions during wound healing and the collective response to oxidative stress.

Reference

Desforges B, Curmi PA, Bounedjah O, Nakib S, Hamon L, De Bandt JP, Pastre D (2013) An intercellular polyamine transfer via gap junctions regulates proliferation and response to stress in epithelial cells. *Mol Biol Cell* **24:** 1529-1543

Keywords: Cancer cells invasion, gap junctions, polyamines.

TUE-270

Assessment of zinc-substituted trisulphonated phthalocyanine effect on COLO 829 cell proliferation through real-time electrical impedance-based technique

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Photodynamic therapy (PDT) is a modern approach directed to killing tumor cells mainly by apoptosis induction and reactive oxygen species production. PDT employs a photosensitiser and the use of light of an appropriate wavelength, adequate for its activation. Zinc-substituted trisulphonated phthalocyanine (ZnS3Pc) is a novel chemical compound with good photosensitiser proprieties that recommend it for use in PDT. To date, there are no studies concerning the effect of ZnS3Pc on skin fibroblast proliferation. The objective of the present study was to assess, through real-time label-free monitoring using impedance analysis, the effect of various concentrations of ZnS3Pc on the proliferation capacity of skin fibroblasts. Human skin fibroblasts from COLO 829 standardised cell line established from a patient with metastatic melanoma were seeded in two different cellular densities (5000 and 10000 cells/well) and left to adhere for 2 hours (37°C, 5% CO_2) in specially designed well plates, supplied at the bottom with sensor electrodes able of read the fluctuations in electrical impedance. ZnS3Pc was added to the wells after 4 hours in different concentrations (0.1, 2 and 4 microM). We have monitored cell proliferation using xCELLigence platform, a simple, yet powerful method that provides results in real-time, standing as a significant advantage over other traditional methods that rely on end-point analysis. The data was acquired over a 72 hours interval as a function of changes in impedance on the surface of electrodes at the bottom of the wells. Experiments were performed in triplicate. In the experimental model of fibroblasts seeded at 5000 cells/well, we have registered an activation of the cellular proliferation almost immediately after ZnS3Pc was added; this activation was best observed after 48 h of cultivation. The activation potential had a clear inverse dose-effect pattern, as the lowest concentration used (0.1 microM) activated cell proliferation more intense that the higher ones (2 and 4 microM, respectively). As for the other cellular density used (10000 cells/well) the case was opposite, namely the degree in which ZnS3Pc inhibited cell proliferation paralleled the compound concentration. Through this method we have shown a clear dependency between cell versus compound concentration in the system, thus the future medical applications of the photosensitizers must be tailored to the actual number of cell-targets. To the best of our knowledge, this is the first study that investigates the cytotoxicity of ZnS3Pc on COLO 829 cells via real-time monitoring.

Keywords: cell proliferation, Photodynamic Therapy, Phthalocyanines.

TUE-271

Bazooka (Par-3) and atypical protein kinase C regulate dynamic actomyosin networks during *Drosophila* amnioserosa apical constriction

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Cell shape changes drive tissue remodeling. Polarized activity of actin and myosin drive apical constriction, as seen for example in *Drosophila* embryonic amnioserosa cells during dorsal closure (DC). First, we characterized interactions between apical actomyosin networks and the cell polarity regulators Bazooka (Baz, the *Drosophila* Par-3), Par-6, and atypical Protein Kinase C (aPKC) (the PAR complex) during *Drosophila* DC. We found that both actomyosin networks and the PAR complex are enriched at the apical surfaces of amnioserosa cells and that actomyosin contractility is driven by cyclical assembly and disassembly of actomyosin networks. The pulsatile actomyosin networks translocate across persistent apical surface PAR complex puncta. To assess whether the PAR complex interacts with actomyosin, we characterized myosin dynamics with PAR loss- and gain-of-function perturbations. Baz enhances, whereas Par6/aPKC inhibits actomyosin. Our studies suggest that PAR proteins regulate pulsatile apical actomyosin networks mediating constriction of amnioserosa cells (David *et al*., 2010). Next, we characterized actomyosin networks during a shift in their dynamics. Amnioserosa constriction transitions from pulsatile to persistent from early to late DC. Since oscillatory networks result from delayed negative feedback, we examined whether such regulation exists in these actomyosin

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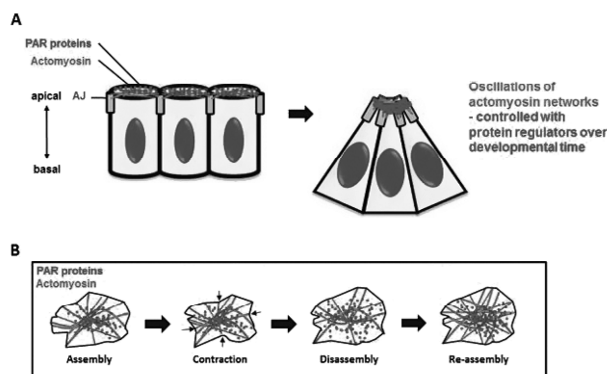

Fig. 1. (A) Apical constriction driven by apical actomyosin networks. (B) Oscillatory apical actomyosin and apical PAR surface puncta.

networks. The actomyosin inhibitor aPKC is recruited to the apical surface by actomyosin and, in turn, aPKC recruits Baz. To examine the significance of Baz – aPKC dynamic interactions, we ectopically stabilized their interactions. This inhibited the antagonism of actomyosin networks by aPKC, suggesting that Baz can act as a competitive inhibitor of aPKC. We found that interactions between Par-6 and Baz increase during DC, suggesting an increase in aPKC inhibition during DC progression. To examine whether decreased inhibition can tune oscillatory actomyosin networks, we collaborated with Qiming Wang and Dr. James Feng (University of British Columbia) to test this oscillation *in silico*. Computer modeling suggests that decreasing delayed negative feedback can transition actomyosin oscillations towards stabilized constriction. Together, these results demonstrate the requirement of Baz – aPKC interactions for their localization and for dynamic regulation of aPKC by a competitive inhibitor (David *et al.,* 2013). Finally, our research reveals a regulatory circuit to tune actomyosin behaviour during development.

Keywords: Actin and myosin, Apical constriction, Cell polarity.

TUE-272
Biophysical analysis of cellular targets for designing novel antitumour metallopharmaceuticals

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Metals are essential evolutionarily selected by nature cellular components designed to function in several indispensable molecular processes for living organisms. Metals are engaged in unique characteristics that include reaction kinetic activity, variable coordination modes, and reactivity towards a wide variety of substrates. Despite their well-established roles in numerous cellular events, such as regulation of cell macromolecular assemblies of membranes, proteins and nucleic acid topologies, in gene transcription and expression, in translation and intracellular trafficking, as well as in macromolecular crowding, their further cellular functions still remain to be elucidated. Due to their reactivity, metals are tightly regulated under normal conditions and non-physiological metal ion concentrations are associated with various pathological disorders, including cancer, autoimmune and neurodegenerative diseases. The work presented herein is devoted to characterization of selected metals that have gained considerable interest in both the development and the treatment of cancer. The characterization of yet unclear cellular targets of Boron, Gallium, Germanium, Rhodium and Gold, is emphasized as the major goal of this preliminary work. Such a characterization of major cellular macromolecular targets would pave the way of designing novel inorganic therapeutic strategies to target molecular pathways as anticancer agents. A novel hypothesis concerning the possible mechanisms of action of these inorganic drugs at the molecular level is proposed. This hypothesis is based on our newly developed theoretical model considering cell membrane phospholipids, serum and intracellular proteins and cellular nucleic acids as targets of these metal ions. Thus, the hypothesis addresses two major questions with basic cellular and therapeutic emphasis, respectively. Elucidating the molecular mechanisms of metal ion-cell biomolecule interactions would give important clues for molecular mechanisms of functioning of cellular machinery in important events such as cellular differentiation, regulation, apoptosis and evolution, besides deciphering their roles in regulation of gene expression and genome function. On the other hand, characterizing such molecular recognitions between inorganic ions and cellular biomolecules would serve as a motivation for starting research on designing novel therapeutic strategies towards biomacromolecule directed drug design with further therapeutic implications in cellular oncology research.

Acknowledgements: All the colleagues from The Central Laboratory of Middle East Technical University and Bilkent University-UNAM (Ankara) who had somehow contributed to this work are greatly acknowledged.

Keywords: bioinorganic therapeutics, biophysical analysis, cellular oncotargets.

TUE-273
Bubbly vertex dynamics: a dynamical and geometrical model for epithelial tissues with curved cell shapes

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In order to describe two-dimensionally packed cells in epithelial tissues both mathematically and physically, there have been developed several sorts of geometrical models, such as the vertex model, the finite element model, the cell-centered model, the cellular Potts model. So far, in any case, pressures have not neatly been dealt with and the curvatures of the cell boundaries have been even omitted through their approximations. We focus on these quantities and formulate them in the vertex model. Thus, a model with the curvatures is constructed, and its algorithm for simulation is provided. The possible extensions and applications of this model will also be discussed.

Keywords: computer simulation, epithelial cells, Tissue mechanics.

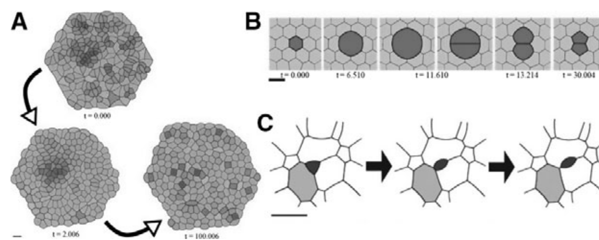

Fig. 1. (A) A typical time course of the model. (B) An example of the mitotic cell rounding. (C) A creation of a two-vertex cell.

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TUE-274
CaMKII/Androgen receptor signaling pathway stimulates MEF2 transcription factor to induce cardiomyocyte hypertrophy by testosterone

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Both Ca^{2+}/Calmodulin-dependent protein kinase (CaMKII) and androgen receptor have been involved in cardiac hypertrophy by using different action mechanisms. CaMKII regulates myocyte-enhancer factor 2 (MEF2) transcription factor, which is a key regulator of cardiac hypertrophy. However whether CaMKII/MEF2C signaling is involved on testosterone-induced cardiomyocyte hypertrophy is unknown. The aim of this study was to investigate whether testosterone activates CaMKII-MEF2C during hypertrophic process. The results show that in primary cultures of cardiomyocytes testosterone (100 nM) increased the phosphorylation of both CaMKII (Thr286) and its substrate phospholamban (Thr17). The hormone induced MEF2C nuclear retention and increase MEF2-lux activity. These effects were prevented by the use of siRNA-CaMKIId, a peptide inhibitor of CaMKII (AIP) and bicalutamide (an androgen receptor inhibitor). Cardiomyocytes transfected with a constitutively active form of CaMKII (CaMKII-T286D) increased *per se* MEF2-lux activity and posterior testosterone stimulation enhanced even more the effect of CaMKII-T286D on MEF2, this last increase was prevented by androgen receptor inhibition. These results suggest that the increase in MEF2 activity induced by testosterone in cardiomyocytes is under the control of multiple pathways such as mechanisms dependent of androgen receptor as well as calcium dependent as CaMKII activation. Cardiomyocyte hypertrophy was assessed by increases in β-myosin heavy chain and skeletal a-actin proteins, aminoacid incorporation and cell size. All these effects were increased by testosterone, but were prevented by AIP, siRNA-CaMKIId and by siRNA-MEF2C. Collectively, these are the first evidences suggesting that testosterone activate CaMKII/MEF2C to induce cardiomyocyte hypertrophy.

Keywords: CaMKII, Cardiomyocyte hypertrophy, MEF2.

TUE-275
Cell motility and molecular analysis of fibroblast spheroid

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Background: Fibroblasts exist in the connective tissue and are generally separated by collagen bundles. Some of functions in fibroblast are synthesis, secretion, and degradation of extracellular matrix (ECM) components such as collagens or proteoglycans. The ECM proteins have a great influence on not only mechanical strength but also proliferation and differentiation. As the results, the connective tissue is kept with normal, healthy conditions. In addition, fibroblasts play a critical role in wound healing. In particular, myofibroblasts interact with ECM proteins to contract the wound. Therefore, the function of fibroblasts cultured on the ECM proteins has energetically been investigated. However, the molecular mechanism of wound repair still remains to be clarified. We found that fibroblast cultured on enzyme-treated collagen spontaneously form an aggregated sphere body (spheroid). In this study, we report the cell motility and the protein expression patterns in fibroblast spheroid.

Methods: Pig type I collagen was treated with an enzyme X, and we obtained collagen preparation (N-Col) (Patent pending). Culture dish (ϕ35 mm) was coated with N-Col solution. Subsequently, mouse NIH/3T3 cells, mouse primary embryonic fibroblasts (MEF), or normal human dermal fibroblasts (NHDF) were cultured on N-Col coated dish. After 24 hrs, we observed cell morphology by using a phase-contrast microscope. To investigate the cell dynamics, we stained filamentous actin (F-actin) with DyLight 555-labeled phalloidin. To further demonstrate the relationship between spheroid and epithelial mesenchymal transition (EMT), we observed EMT protein markers stained by DyLight 555-labeled polyclonal antibodies. Finally, cell motility was monitored for a period of 24 hrs and analyzed by a time-lapse observation.

Results and Conclusion: Each of NIH/3T3, MEF and NHDF cells formed the spheroid morphology by culturing on the coated dish with N-Col. F-actin of each cell in spheroid was highly expressed as compared with that in a single cell. Besides, we confirmed that there are no apoptosis cells in spheroid. Interestingly, cell motility drastically changed by formation of spheroid. In conclusion, we demonstrated that N-Col has ability to grow sphere body of fibroblasts.

Funding: This work was supported by the Adaptable and Seamless Technology Transfer Program through target-driven R&D, Japan Science and Technology Agency [AS2414037P to K.M.].
Keywords: Collagen scaffolds, Fibroblasts, spheroid.

TUE-276
Cellular dynamic study of silica nanoparticles in living cells

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Nanotechnology is one of the most rapidly evolving fields in biology and medicine. Especially, Nanoparticles (NPs) have been widely developed and investigated for biomedical applications such as bio-imaging, diagnostics, therapeutics, and bio-analytical methods. Recently, silica nanoparticles(SNP) have been most commonly used materials due to easy surface modification and effective uptake into eukaryotic cells without any significant tox-

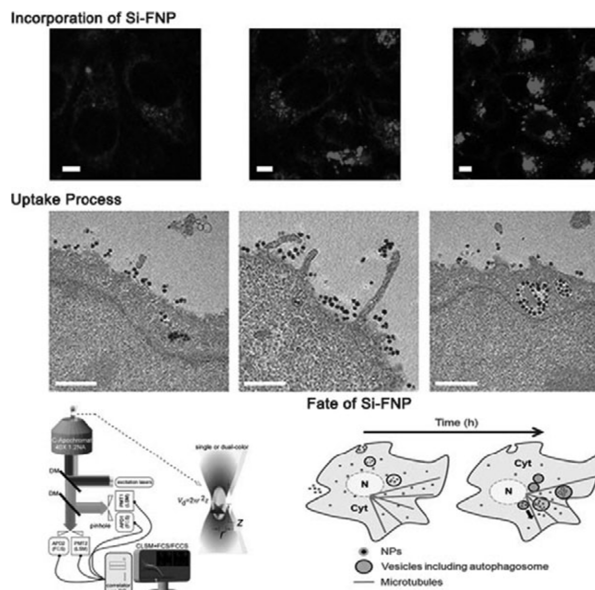

Fig. 1.

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icity. Additionally, silica-based fluorescent nanoparticles (Si-FNPs) are used as imaging and analytical standard probes to study the molecular dynamics and the property of cellular micro-environment because of high solubility, brightness and photostability. Many previous reports are only focused on understanding how cells uptake NPs, trafficking process and potential toxicity of NPs. NPs uptake into the cell by endocytosis and arrived at cytoplasm and then it is met with a variety of molecular environment like as proteins, protein complexes, vesicles, and organelles. Previous reports have showed that various NPs, including SNPs, quantum dots, and gold nanoparticles, could be uptaken and localized into the cells by endocytosis process or by artificial methods such as electroporation and microinjection. However, the intracellular dynamics and interaction with the cellular constituents were not fully understood.

In this study, complementary methodologies of fluorescence correlation spectroscopy (FCS), confocal microscopy (CLSM), and electron microscopy (TEM) was used, to describe the detailed time course of uptake process, diffusion dynamics, and localization of SNP and Si-FNPs with diameter of 50 nm. Moreover, difference of the cellular process between endocytosis and electroporation methods in the living cells was quantitatively compared.

Keywords: Silica-based fluorescent nanoparticles (Si-FNPs), Fluorescence correlation spectroscopy (FCS), Nanoparticle delivery.

TUE-277

Changes in the activity of antioxidant barrier after treatment of K562 cell line with doxorubicin - transferrin conjugate

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Doxorubicin (DOX), one of the oldest member of the anthracycline antibiotics, has been administered for over 50 years to patients with leukemias and solid tumors. However, the high unspecified DOX toxicity, related with reactive oxygen species (ROS), affects its limitation in clinical application. Therefore we proposed the usage of human transferrin as a doxorubicin carrier in order to improve the quality of doxorubicin application in conventional chemotherapy. In this study we are concentrating on the influence of doxorubicin – transferrin (DOX-TRF) conjugate on the antioxidative system in chronic erythromyeloblastoid leukemia cell line (K562). We carried out neutral red cytotoxicity assay, reduced glutathione (GSH) content and alterations in the activities of catalase and enzymes responsible for maintaining glutathione in reduced form. Exposure of leukemia cells to the investigated anticancer agents caused a time-dependent depletion of intracellular GSH, accompanied by an increase of catalase activity. Moreover, analysis of GSH-related enzymes showed a significant increase in the activities of thioredoxin reductase and glutathione peroxidase after DOX-TRF application. In contrast, glutathione reductase activity was reduced by conjugate treatment to 50%. Significant differences between the pro-oxidative actions of the investigated anticancer compounds were observed in RT-PCR experiments, which confirmed that changes in the activity of catalase and GSH-related enzymes are strictly correlated with their gene transcription changes.

Keywords: anticancer drug conjugates, reactive oxygen species, transferrin.

TUE-278

Citrullination of collagen II decreases integrin-mediated cell adhesion by receptor specific manner

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Citrullination is a posttranslational modification of arginine residues catalyzed by enzymes peptidyl arginine deiminases (PAD). Some extracellular matrix (ECM) proteins, including collagen II and fibronectin, are citrullinated in human rheumatoid arthritis *in vivo*. Interestingly, some of the anticitrullinated protein autoantibodies, a well-studied rheumatoid arthritis markers, recognize citrullinated CII and can mediate arthritis in mouse. In this study, we are interested the functional effects of citrullination on ECM proteins. Furthermore, we are searching for new *in vivo* targets of PADs by the mass spectrometric analysis of synovial fluids collected from arthritis patients.

The presence of citrullinated of collagen II in arthritis joint *in vivo* was previously reported. We demonstrate by mass spectrometric and chemical analysis that PAD2 and PAD4, rheumatoid arhtritis relevant PAD isoforms, can citrullinate GFOGER, the critical binding motif of integrins, in collagen II. Citrullination of collagen decreased the adhesion of several cell types including primary synovial fibroblasts. Interestingly, the effect of citrullination varied notably between different cell types. For further investigation, we studied how the citrullination of GFOGER affects the ligand recognition of collagen receptor integrins. The data show that citrullination of GFOGER decreases significantly the alpha10beta1 and alpha11beta1 integrin mediated cell adhesion but has only a minor effect or no effect at all in the case of integrin alpha1beta1 and alpha2beta1. The receptor specific effect of citrullination can be explained by molecular modeling based on the crystal structure of integrin alpha I domain - GFOGER complex.

Given the fact that integrins regulate numerous cellular functions like apoptosis, cell survival, and proliferation, our study suggests that the citrullination of collagen II may have a role in the pathological destruction of joints by disturbing integrin-mediated cell-matrix interactions.

Keywords: citrullination, integrin, rheumatoid arthritis.

TUE-279

Cooperation between the actin nucleators mDia1 and Arp2/3 complex controls membrane ruffling

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Polymerization of branched actin filaments mediates the formation of lamellipodia and ruffles, protrusions of the plasma membrane involved in cell migration, phagocytosis and macropinocytosis. Biochemical evidence indicates that actin-related protein 2 and 3 (Arp2/3) complex nucleates branched actin filaments upon activation by Wiskott-Aldrich syndrome protein (WASP)-family verprolin-homologous protein (WAVE)

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complex. Genetic studies demonstrate that both complexes are required for the formation of lamellipodia and ruffles. However, whether and how other actin nucleators participate in this process is still unknown. Here we show that the actin nucleator mDia1 acts in concert with the Arp2/3 complex to promote the efficient formation of ruffles. Knockdown and rescue experiments revealed that EGF-induced membrane ruffling requires mDia1 and its actin-nucleation abilities. Although activated full-length mDia1 and Arp2/3 complex were poor actin nucleators, their combination triggered explosive polymerization of branched actin arrays *in vitro*. These results suggested that mDia1 polymerizes linear actin filaments igniting autocatalytic Arp2/3-complex-dependent nucleation of branched actin filaments and ruffling. Consistently, super-resolution microscopy localized WAVE and mDia1 within EGF-induced ruffles. Hyperactivation of the Arp2/3 complex in mDia1 knockdown cells and overexpression of mDia1 in WAVE-complex knockdown cells restored EGF-induced ruffling, thus showing that mDia1 and the Arp2/3 complex cooperate in the making of ruffles. Given that the expression levels and the activity of mDia1 and the Arp2/3 complex dictate the contribution of either nucleator to membrane ruffling, it appears that cell-intrinsic and cell-extrinsic factors sculpt the lamellipodium/ruffle-making machinery.

Keywords: actin reorganisation, Arp2/3, mDia1.

TUE-280

Curcumin and neurite outgrowth: investigations for spinal muscular atrophy

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Spinal Muscular Atrophy (SMA) is a neurodegenerative disease, which is caused by deletions or mutations of the Survival of Motor Neuron 1 (*SMN1*) gene. SMN is ubiquitously expressed protein that plays a role in actin dynamics, axonal transport, and neurite outgrowth in neurons. Low levels of SMN protein leads to neurite outgrowth defects in neuronal cells and rescue of the defect is thought to be a reasonable therapeutic strategy for SMA. Neuroprotective compounds, like polyphenols and histone deacetylase (HDAC) inhibitors, may be good candidates for restoring of the defect. In the present study, it was investigated whether neurite outgrowth defects could be rescued by curcumin, which is SMN-inducing polyphenol, having HDAC inhibition activity. Curcumin was applied to wild type and SMN knockdown PC12 cell lines and neurite lengths were measured. According to our results, curcumin significantly increased the neurite lengths of the wild type but not SMN knockdown cells at 3 days of differentiation. To investigate whether neurite promotion in wild type cells was caused by increasing the SMN protein, total and nuclear SMN protein levels were analyzed in response to curcumin using Western blot and immunofluorescence staining of nuclear bodies, respectively. It was found that both total SMN level and nuclear body numbers remained unchanged. According to our results, even though curcumin failed to rescue the outgrowth defect, it lost its neurite promoting ability when the SMN level was decreased. Taken together, our results indicated that the neurite promoting effect of curcumin dependent on the SMN protein. Further studies using primary neurons and induced pluripotent stem cell-derived patient motor neurons will be valuable to understand the function of SMN protein at outgrowth mechanism.

Keywords: Curcumin, Neurite outgrowth, Spinal muscular atrophy.

TUE-281

Cx43 mutant expressing fibroblasts from oculodentodigital dysplasia patients exhibit diverse properties that predict variability in wound healing

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Connexins (Cx) are a large gene family with Cx43 being the most commonly found connexin in human tissues. Cx43 oligomerizes into hemichannels that traffic to the cell surface and form intercellular gap junction channels with neighbouring cells. To date, over 70 *Cx43* (*GJA1*) gene mutations have been discovered that cause the developmental disorder known as oculodentodigital dysplasia (ODDD). In the present study, we characterized three dermal fibroblast cell lines obtained from ODDD patients with autosomal dominant Cx43 missense mutations (L7V, G138R, G143S), and further described properties of two others (D3N and V216L). Immunofluorescent labelling revealed that the L7V, G138R and G143S Cx43 mutants trafficked to the cell surface and formed gap junction-like plaques. Dual whole cell patch clamp recordings revealed functional gap junctional conductance between cells expressing both the G138R and G143S mutations. However, the L7V mutant had no detectable conductance suggesting it was a potent dominant-negative to co-expressed wild-type Cx43. Since Cx43 is important in wound healing, we further found that mutant expressing fibroblasts had significantly reduced proliferative and migratory properties as well as an inability to correctly polarize the Golgi apparatus in an *in vitro* scratch wounding assay. All cells treated with the wound healing cytokines PDGF and TGF-β initiated a robust increase in Cx43 expression, but mutant-expressing cells failed to increase the P2 phosphorylation state, which is indicative of enhanced gap junction assembly. In response to TGF-β all fibroblasts elicited an increase in α-smooth muscle actin (α-SMA) expression suggesting that the cells retained variable ability to differentiate into myofibroblasts. However, when seeded in 3D collagen gels, the D3N and V216L expressing fibroblasts were less efficient at contracting the gels after TGF-β treatment. In parallel, a dermal punch biopsy wound healing assay was performed on a $Cx43^{I130T/+}$ mouse model of ODDD. As predicted from the collagen contraction studies with the G138R and G143S mutant expressing cells (same domain as the I130T mutant), no significant difference in wound healing was observed. In summary, in dermal fibroblasts, ODDD-linked Cx43 mutants may exert little effect on co-expressed Cx43 or be potent dominant-negatives. Thus, some ODDD patients are predicted to have delayed wound healing, an outcome that is dependent on the specific mutation they harbor.

Supported by the Canadian Institutes of Health Research to DWL.

Keywords: gap junctions, oculodentodigital dysplasia, wound healing.

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TUE-282
Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri

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Inhibition of light emitted by a bioluminiscent bacterium, *Vibrio fischeri,* is the basis for several toxicity bioassays. This study is focused on *Vibrio fischeri* bioluminiscence inhibition assay, which is often chosen as the first test in a test battery based on speed and cost considerations. Water samples from Secu and Văliug dam reservoirs, Caras-Severin county (Romania) were analysed in order to determine the toxicity level. These reservoirs represents the main source of tap water for Resita city. Standard 5, 15 minutes test were compared with a 30 minutes assay using the same organism and the same test parameter. For that purpose concentration-response relationships were determined for a set of selected substances, reflecting different modes and mechanisms of action. The values of the effective concentrations which reduce the intensity of light emission by 20% (EC_{20}) and 50% (EC_{50}) were calculated. For comparison with our samples, $ZnSO_4$ x $7H_2O$ and $K_2Cr_2O_7$ were used as reference. Based on EC_{20} and EC_{50} values, we concluded that water from Secu dam reservoir is more toxic for *Vibrio fischeri.* Our results were confirmed by reported enzymological and bacteriological results.

Keywords: bioluminescence, toxicity, Vibrio fischeri.

TUE-283
Development of II-D cell blot method

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The development of new methods that enable high-throughput analysis of protein functions comprehensively is of utmost importance. Hayman *et al.* have developed the SDS-PAGE cell blot method for the analysis of cell-adhesive proteins [1]. In their study, the proteins separated by SDS-PAGE were transferred to a membrane, where cells were then cultured. Unknown cell binding proteins were then found by observing the spots where cells were attached. However, this method is not prevalent because SDS is known as a detergent which denatures many kinds of proteins. Moreover, this method requires two blotting sheets, one for protein staining and one for cell staining. By comparing these two membranes, the protein hotspots in which cell morphology has changed are determined. When trying this method two-dimensionally, merging these two membranes and defining the hotspots could pose difficulties. In a previous paper, Manabe and Jin reported a non-denaturing II-DE method that enables the separation of proteins which preserve biological structures and functions [2].

In this study, a new method that enables comprehensive analysis of proteins functions has been developed by combining the SDS-PAGE cell blot and the non-denaturing II-DE method, and a protein that induces apoptosis in *Pieris rapae* was analyzed using the Two-Dimensional Cell Blot method. The proteins in body fluid of *Pieris rapae* were separated using non-denaturing II-DE method and were transferred to a PVDF membrane. HeLa cells were plated over the protein-transferred membrane evenly, and both the proteins and the cells stained by CBB were then observed on the membrane. From these experiments, nuclear condensation, membrane bleb and cytosol overflow were found in the cells on the hotspot. These phenomena show that the protein on the hotspot had apoptosis activity. The isoelectric point of this protein was six and was almost equal to that of "Pierisin" known as the apoptosis protein in body fluids of *Pieris rapae*. The hot spot was consistent with the area where "Pierisin" protein was expected to exist. This method using II-D Cell Blot could be useful for the comprehensive analysis of proteins that induce cell morphological changes by combining with MALDI-TOF-MS. This method is also advantageous in that it requires only a small amount of proteins, about 20 μg.

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Keywords: II-D Cell Blot Method, non-denaturing II-DE method.

TUE-284
Differential effect of Antioxidants in Cell Death-Related Oxidative Stress in wild-type MEFs

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Reactive oxygen species (ROS) that can cause cellular stress are generated in the mitochondria. Physiologically ROS function as cellular signals in different molecular pathways. However, higher concentrations of ROS are toxic to cells and can trigger apoptotic or necrotic cell death. Superoxide dismutase (SOD), glutathione (GHS) and other vitamins, such as tocopherols, are considered the primary cellular defence against oxidants. In daily life, vegetables and fruits represent the main sources of antioxidants in the humans' diet. Generally, the intake of antioxidants is believed to decelerate cell aging and loss. However, recent studies report conflicting results.

The aim of our study was to evaluate the effect of different antioxidants, including N-acetyl cysteine (NAC) that replenish intracellular GHS, the lipophilic vitamin α-tocoferol, and two SOD mimetics, MnTBAP and tempol, in scavenging ROS in MEFs in three different models of cell death, including MD-induced necrosis and STS and TNFα-induced apoptosis. Apart from MnTBAP, all other antioxidants are used as drugs or dietary supplements. The antioxidant's efficiency was measured as the capacity to decrease intracellular ROS production and to prevent cell death. The antioxidants were the most efficient in scavenging MD-induced ROS, followed by STS-induced ROS, whereas they were the least efficient in scavenging TNFα -induced ROS. However, major differences were observed when using tempol. The most striking result was that tempol increased ROS production *per se* and was unable to decrease oxidative stress following MD and STS treatments. Tempol's antioxidant potential was shown only upon TNFα treatment using concentrations up to 5 mM. At concentrations above 5 mM, tempol lost its antioxidant properties also in TNFα model. However, all the antioxidants were much less efficient in enhancing cell viability as in ROS scavenging. Thereby we have shown not only that different antioxidants have different capacity to scavenge ROS, but also that ROS contribute to different extent to cell death, depending on the pathway. Clarifying the role of antioxidants in ROS production and the correlation with cell death will improve our knowledge of how oxidation modulates molecular signalling and cell faith. Moreover, due to the wide use of antioxidants in

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therapies for diseases from the common influenza to cancer, a complete understanding of how different antioxidants work at the molecular level might be used to improve therapeutic approaches based on antioxidants.

Keywords: Antioxidants, Cell death, Oxidative stress.

TUE-285
Effect of auranofin, N-acetyl cysteine and buthionine sulfoximine on the glutathione status in Taenia crassiceps cysticerci

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In the parasite lineage of Platyhelminthes the redox homeostasis is dependent on thioredoxin-glutathione reductase (TGR), a mixed-function selenoenzyme that acts transfering electrons from NADPH to the oxidized states of both GSSG and thioredoxin. TGR has been proposed as a pharmacological target because in these organisms is the only enzyme involved in the reduction of both GSSG and oxidized Trx. Previously, we studied the effect of TGR inhibition by auranofin (an anti-rheumatic gold compound) on the viability of *Taenia crassiceps* cysticerci. It was demonstrated that micromolar concentrations of auranofin in the culture medium were high enough to fully inhibit TGR and kill the parasites (Parasitol Res. 2010. 107:227-31). In this work we have analyzed the dynamic of changes in the glutathione pool following the addition of auranofin, as well as the effect of N-acetyl cysteine (NAC) and buthionine sulfoximine (BSO).

The results obtained showed that, as result of the presence of the gold compound, the total concentration of glutathione (GSH + GSSG) decrease, concomitant with a diminution in the GSH/GSSG ratio. Such changes were simultaneous with the formation of GSH-protein complexes as well as with the export of GSSG from cysticerci. The incubation of cysticerci in the presence of both auranofin and NAC, a promotor of GSH biosynthesis, prevents all the above changes, mantaining the viability of the parasites. By contrast, the simultaneous addition of auranofin and BSO, an inhibitor of GSH biosynthesis, results in an encourage in the effects of the gold compound.

These results suggests the lethal effect on *T. crassiceps* cysticerci due to auranofin can be explained, at least partially, as a consequence of major changes in the glutathione status resulting in an irreversible damage, probably by an increase in the oxidative stress.

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Keywords: glutathione distribution, redox homeostasis, thioredoxin-glutathione reductase.

TUE-286
Effects of soft argon-plasma jet on the cytoskeleton of fibroblasts: implications to plasma medicine

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Argon-plasma jet is generated by ionizing argon gas, and the resulting argon-plasma jet consists of a mixture of neutral particles, positive and negative electrons, and various reactive species, especially hydroxyl radical. Although argon-plasma jet has been used in various biomedical applications, little is known about the biological effects on cells located near the plasma-exposed region. Here, we investigated the effects of soft argon-plasma jet on actin cytoskeleton of mouse embryonic fibroblasts (MEFs) in response to exposure to argon-plasma jet. This argon-plasma jet was generated at 500 ml/min of flow rate and 100 V electric power with our device. Because actin cytoskeleton is the key cellular machinery involved in cellular movement and is implicated in regulation of cancer metastasis, it seems to be a highly desirable target for cancer therapy. In this study, we examined the actin filament architectures in the argon-plasma jet treated MEFs by staining with a fluorescent actin-specific phalloidin. Interestingly, the argon-plasma jet treatment causes destabilization of actin filament architectures in the regions indirectly exposed to argon-plasma jet, but no changes in MEFs treated with argon gas alone and untreated cell control. The result indicates that this phenomenon is a specific cellular response against argon-plasma jet in the live cells that are indirectly exposed to argon-plasma jet. Collectively, our study provides scientific information that argon-plasma jet may have potential as anti-cancer drug effect through direct destabilization of the actin cytoskeleton. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (NRF-2010-0027963).

Keywords: anti-cancer, destabilization of actin filament, Soft Argon-Plasma Jet.

TUE-287
Epinephrine inhibits the activity of the Na+/K+ atpase in colonic cancer cells via PGE2 and NO

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Stress and stress hormones have been shown to promote tumor growth and angiogenesis. On the other hand a change in the activity of the Na+/K+ ATPase was found to accompany cancer. Whether a cause effect relationship exists between the hormones and the pump is still unclear. This work was undertaken to study the effect of epinephrine on colonic Na+/K+ ATPase using Caco-2 cells, a colorectal cancer cell line, as a model. The activity of the pump was assayed by measuring the amount of inorganic phosphate liberated in presence of ouabain, a specific inhibitor of the ATPase. Epinephrine, added for 20 min, exerted a significant inhibitory effect on the pump. This effect disappeared in presence of yohimbine, a blocker of alpha 2 receptors but persisted in presence of prazocin, propranolol and butoxamine. RpcAMP, an inhibitor of PKA, imitated the effect of epinephrine and confirmed the involvement of alpha 2 receptors which are known to be coupled to Gi proteins. The effect of epinephrine disappeared when cells were pre-treated with indomethacin, PTIO, and calphostin, respective inhibitors of COX-2, NOS, and PKC, indicating the involvement of these mediators in the signaling pathway. Treating the cells with exogenous PGE2, the nitric oxide donor SNAP, and the PKC activator phorbol myristate acetate (PMA), led, similar to epinephrine, to a decrease in the activity of the ATPase, confirming further their involvement. The inhibitory effect of PGE2 disappeared however, when NOS or PKC were inhibited, revealing their presence downstream of PGE2. The data indicate the involvement of alpha 2 receptors in the effect of epinephrine on the pump. This effect is mediated via PGE2 which in turn acts by activating PKC and by generating NO.

Keywords: Epinephrine, Na+/K+ ATPase, PGE2.

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TUE-288

Evaluation of anticancer effects of cetraria islandica extract on huvec and human breast cancer cell lines

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The aim of this study was to assay the anti cancer effects of *Cetraria islandica* on HUVEC and human breast cancer cell lines, MCF-7 and MDA-MB-231. In the experiment, xCELLigence cell index (CI) impedance measurements were performed according to the instructions of the supplier. The cells were resuspended in medium and subsequently adjusted to 50 000 cells/ml. After seeding 200 μl of the cell suspensions into the wells of the E-plate 96, cells were allowed to attached to the bottom of the plate. After 30 min, the cells were exposed to 0, 0 (DMSO), 1, 5, 25 and 125 μg/ml *C. islandica* extracts and monitored every 15 min by the xCELLigence system during 72 h exposure. Half maximum effect concentrations (EC50) were determined based on the dose–response curves derived by xCELLigence measurements. In addition, the effects of *C. islandica* extracts on the proliferation and viability of MCF7 cells were aslo determined by using MTT test. DNA fragmentation and apoptosis by Annexin V/PI double staining assay using FACS were evaluate in MCF-7 cells exposed with *C. islandica* extract during 24 and 48 hour. Following real-time analysis during 72 hour, EC50 values of *C. islandica* on MCF-7 and MDA-MB-231 cells were found to be 9.2047 E^{-5} g/ml and 1.933 E^{4} g/ml, respectively. The MTT test results of MCF-7 cells exposed with *C. islandica* extracts were showed that there was no effect on cell viability and proliferation in MCF-7 cells exposed to 1 and 5 μg/ml *C. islandica* extract. On the other hand, 25 and 125 μg/ml *C. islandica* extract were found to cause a reduction in the survival and proliferation of MCF-7 cells and these results was in accordance with xCELLigence measurements. After 24 hour exposure, there was no stained MCF-7 cells with Annexin V and few cells stained with PI. 2% of MCF-7 cells were found to be in the early or late apoptosis, and 15% of the cells in necrosis. On the other hand, both the apoptotic and necrotic MCF-7 cells decreased in number after 48 hour-exposure.

Keywords: Apoptosis, anticancer, Cetraria islandica, MCF-7, MDA-MB-231 cells, xCELLigence.

TUE-289

Evaluation of cellular dynamics of optogenetically modified cells by electro-optical methods

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A multi-parametric bioanalytical platform is able to reveal dynamic changes in cellular properties like adherence, mobility and morphology (1) in response to extracellular stressors thus responding to basic and applied questions within biomedical and bioevaluation fields. The toolbox introduced by optogenetics (2), a combination of optics, genetics and bioengineering to control different cellular functions within different cell types could be applied for the development of biosensing platforms. To this end, we propose a novel cellular platform integrating electro-optical assessment of optogenetically modified cells via light-sensitive microbial membrane proteins (opsins) (3).

We will present our results regarding dynamic evaluation of cellular processes like spreading, cell-substrate adherence and cell-cell junctions upon light stimulation and application of noxious pharmacological agents on Human Embryonic Kidney (HEK) cells expressing channelrhodopsin-2 (ChR2) in conjunction with voltage dependent calcium channel, $Ca_v1.2$ and inward rectifying ATP dependent potassium channel, ROMK1. Cellular responses at the level of monolayer adherence, mobility, morphology and electrical cell parameters were revealed using sensitive interfacial analytic techniques such as Total Internal Reflection Fluorescence Microscopy (TIRFM) and Electrical Impedance Spectroscopy (EIS) whereas cell membrane parameters were determined based on patch clamp measurements.

The advantages of this bioanalytical platform for real time label-free noninvasive quantitation of the effects of stressor compounds at cellular level will be highlighted.

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Keywords: Biosensing, Cellular dynamics, Electro-optical methods.

TUE-290

Fractional killing in response to TRAIL and therapeutic antibody agonists arises from cell-to-cell variability in overcoming a caspase-8 activity threshold

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Ligands of death receptor (DR) -4 and -5, such as TRAIL and agonistic antibodies, trigger apoptosis in tumor cells. Although promising, drugs targeting this pathway have stalled in Phase II/III of clinical trials because of variable efficacy. Many mechanisms of resistance have been proposed to explain patient-to-patient variability but no quantitative model has been built to evaluate and compare these different hypotheses. In this study, we aim to understand which features of death-inducing signaling complex (DISC) control fate when cells are exposed to DR agonists, and how resistance can be overcome with drug combinations. For this task, we used a quantitative model of DISC dynamics to extract parameters predictive of cell fate.

Using live cell imaging and HeLa cells engineered with a FRET reporter of Bid cleavage, we monitored caspase-8 activity at the single cell level after exposure to DR ligands. For each cell, we derived parameters that characterize DISC dynamics. We find that a simple three parameter model involving the rate and duration of caspase-8 activation by the DISC is sufficient to discriminate between surviving and dying cells across a range of DR4/5 agonist concentrations. In cells in which random variations in these parameters exceeds a threshold value, cells die; otherwise they survive, showing that cell death decisions rely on an activity sensor for caspase-8.

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Using this framework, we also explains the role of c-FLIP and Bcl-2 overexpression in promoting TRAIL resistance and the proteasome inhibitor bortezomib in promoting sensitivity. Finally, our findings help explain why therapeutic antibodies such as Apomab are such poor DR agonists and how new therapeutic molecules should be tested to ensure their potency.

Keywords: apoptosis, drug resistance, single-cell heterogeneity.

TUE-291
Functional specificity of the tail-anchored proteins Mff and Fis1 on peroxisomal and mitochondrial dynamics

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Peroxisomes and mitochondria are ubiquitous, highly dynamic and essential subcellular organelles whose protein composition, morphology and abundance are tightly regulated upon external stimuli in order to maintain cellular homeostasis. Morphology and dynamics of these organelles have been shown to play an important role in cell physiology. Mitochondrial morphology has been shown to influence neurodegeneration, calcium signalling, lifespan, cell death and even the immune response. Similarly, deregulation of peroxisome dynamics has been linked to several disease conditions such as peroxisome biogenesis disorders, carcinogenesis, liver cirrhosis and viral infections.

These organelles exhibit a close interrelationship with metabolic cooperations and cross-talk, as well as an overlap in key components of their division machinery. Final scission depends on the recruitment of the dynamin-like GTPase DLP1 (in mammals, also named Dmn1 in *S.cerevisiae* and Drp1 in *Caenorhabditis elegans*) that forms ring-like structures around constricted membranes to mediate scission through GTP hydrolysis. The search for the adaptor proteins for DLP1 at organelle′s membrane have been subject to many controversial studies. In yeast, the tail-anchored Fis1 has shown to play an essential role in the recruitment of Dnm1 to mitochondrial fission foci through the adaptor proteins Mdv1p and Caf4p, whose mammalian homologues have not yet been identified. There is also evidence that Fis1 is involved in DLP1 recruitment to the peroxisomal and mitochondrial membranes in mammals, however, its absolute requirement remains controversial. Recent reports show that Fis1 may be a bifunctional protein that is also involved on the regulation of apoptosis. More recently, some studies have shown that the novel tail-anchored protein Mff plays a major role in the recruitment of DLP1 to both peroxisomes and mitochondria. Moreover, recent results show that, in peroxisomes, Mff interacts and regulates interaction between DLP1 and Pex11β, which mediates peroxisomal elongation prior to fission.

In order to finally unravel which of the two proteins, Fis1 or Mff, plays the most important role as DLP-adaptor during peroxisomal and mitochondrial division, we have performed a detailed comparison study on the localization and function of these two proteins in the two organelles. Our results surprisingly show that these organelles make use of different DLP-anchors: Mff is the preferential DLP-adaptor at the peroxisomal membrane, whether Fis1 is the protein that assumes this role at the mitochondrial membrane.

Keywords: Membrane fission, Mitochondria, Peroxisomes.

TUE-292
Galectin-3 as new regulator of spindle pole stability

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Mitosis is a highly regulated cellular process, where many internal rearrangements of the microtubule cytoskeleton occur. Through cell division, microtubules are rearranged from a centrosome-centered array to two antiparallel arrays, namely mitotic spindles. Spindle and astral microtubules connect cell poles to kinetochores and to the cell cortex, respectively. Motor proteins and microtubule organizer proteins regulate the assembly of microtubules at spindle poles. This newly formed microtubule network helps control of cell division positioning and orientation, and ensures the correct segregation of sister chromatids in both daughter cells. Any defects in microtubule *organization and dynamics* during mitosis will lead to chromosomal instability and generate aneuploidy.

Recently a new kind of post-translational modification, the *O-linked N-acetylglucosamine* addition, was reported to regulate spindle pole assembly. However no potential molecular mechanism has as yet been described at that level.

One particular protein is interesting in this regard, Galectin-3, a lectin mainly expressed in epithelial cells. Its expression has been frequently correlated with cancer progression. At the cellular level, it is described as a multifunctional intra- and extracellular protein. Most recently, our group described Galectin-3 as a new key player in centrosome biology. We reported during ciliogenesis that Galectin-3 transiently associates with basal bodies in kidney cells as well as in multiciliated tracheal cells; there, Galectin-3 has been shown to be important for the recruitment of g-tubulin and thus the anchorage of microtubules to the basal body MicroTubule Organizing Center.

Here, we investigated the potential participation of Galectin-3 in mitosis and spindle dynamics *in vitro* in different human epithelial cell lines. We show that Galectin-3 is required for correct cell division. Its depletion generates a large panel of mitotic defects (multipolar mitosis, supernumerary centrosomes,...). During mitosis, Galectin-3 is located at the minus-end of spindle microtubules. We demonstrate that this localization is dependent on its interaction with NuMA, a well-known microtubule array organizer. Moreover, this association is glycosylation –dependent. Abrogation of Galectin-3 binding induces defects in NuMA dynamics and in spindle pole structure. This suggests that Galectin-3 strengthens NuMA complexes, helping cohesion of the spindle microtubule array. Altogether, our data demonstrate that Galectin-3 may behave as a stabilization factor for NuMA, and actively take part in the cohesion of spindle microtubule arrays.

Keywords: Cell Division, Microtubule, Spindle Pole.

TUE-293
Grb2 is required for regulation of integrin-mediated podosomal stability in osteoclasts by protein tyrosine phosphatase epsilon

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Protein tyrosine phosphatase epsilon (PTPe) is involved in osteoclast (OCL) adhesion signaling and podosomal stability as it participates in Src kinase activation by dephosphorylating it on its inhibitory tyrosine 527, downstream of integrins. Phosphorylation of tyrosine 638 at the C terminus of PTPe is crucial for the

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ability of PTPe to efficiently activate Src. The adaptor protein Grb2 binds pY638 of PTPe through its SH2 domain; however, the physiological significance for this binding is unknown. We show that Grb2 knockdown (KD) in OCLs results in decreased phosphorylation of Src and Pyk2, as well as decreased Y638 Phosphorylation of PTPe, while Grb2 overexpression increases these parameters. Podosomal stability is also impaired in Grb2 KD OCL; this can be rescued by re-introducing Grb2 into the cells. OCLs from PTPe-knockout (EKO) mice or in which Grb2 was knocked down exhibit similar abnormalities in podosomal organization and Src phosphorylation. Expression of PTPe in EKO OCLs rescues these phenotypes when Grb2 is present, but not in Grb2 KD OCLs; this indicates Grb2 functions downstream of PTPe. Integrin activation induces increase in PTPe phosphorylation at Y638 and Grb2 binding to PTPe, and Grb2 forms a complex with PTPe, Src and Pyk2 in non-stimulated OCLs. We conclude that Grb2 recruits Src to PTPe downstream of activated integrins in OCLs. Upon integrin activation, partially activated Src phosphorylates PTPe at Y638; the Grb2-Src complex binds PTPe at pY638, allowing PTPe to dephosphorylate and fully activate Src. Lack of PTPe, of its Y638 phosphorylation site or of Grb2 disrupts integrin signaling and podosomal organization in these cells.

Keywords: Grb2, Osteoclasts adhesion, Protein Tyrosine Phosphatase Epsilon.

TUE-295

Importance of tissue transglutaminase nitrosylation in fibroblasts migration and MMP regulation

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As an extracellular second messenger, nitric oxide (NO) mediates the modification of proteins through nitrosylation of cysteine and tyrosine residues. Tissue Transglutaminase (TG2) is a Ca^{2+} activated, sulfhydryl rich protein with 18 free cysteine residues, which catalyzes ε-(γ glutamyl) lysine crosslink between extracellular and intracellular proteins. NO can nitrosylate up to 15 of the cysteine residues in TG2, leading to the irreversible inactivation of the enzyme activity. The interplay between these two agents was revealed for the first time by our study showing that NO inhibited the TG2-induced transcriptional activation of TGFβ1 and extracellular matrix (ECM) protein synthesis by nitrosylating TG2 in an inactive confirmation with inert catalytic activity. However, nitrosylated TG2 was still able to serve as a novel cell adhesion protein. In the light of our previous findings, in this study we aim to elucidate the NO modified function of TG2 in cell migration using an *in vitro* model mimicking the tissue matrix remodeling phases of wound healing.

Using transfected fibroblasts expressing TG2 under the control of the tetracycline-off promoter, we demonstrate that upregulation of TG2 expression and activity inhibited the cell migration through the activation of TGFβ1. Increased TG2 activity led to a rise in the biosynthesis and activity of the gelatinases, MMP-2 and MMP-9, while decreasing the biosynthesis and activity of the collagenases MMP-1a and MMP-13. NO donor S-Nitroso-N-acetylpenicillamine (SNAP) treatment relieved the TG2 obstructed-cell migration by blocking the TG2 enzyme activity. In addition, decrease in TG2 activity due to nitrosylation led to an inhibition of TGFβ1, which in turn affected the pattern of MMP activation.

Recent evidence suggests that, once in complex with fibronectin in the ECM, TG2 can interact with syndecan-4 or integrinβ-1 and regulate the cell adhesion. In the other part of this study, the possible role of nitrosylated TG2 on the regulation of cell migration during wound healing was investigated with respect to its interactions with integrin β1 (ITGB1) and syndecan-4 (SDC4). Treatment with TG2 inhibitor Z-DON resulted in a 50% decrease in the TG2 interaction with ITGB1 and SDC4, while increasing concentrations of SNAP firstly led to a substantial decrease and then completely abolished the TG2/ITGB1 and TG2/SDC4 complex formation on the cell surface. Taken together, data obtained from this study suggests that nitrosylation of TG2 leads to a change not only in the binding partners of TG2 on cell surface but also in TGFβ1-dependent MMP activation, which give rise to an increase in the migration potential of fibroblasts.

Keywords: nitric oxide, tissue transglutaminase, matrix metalloproteinases.

TUE-296

In cellulo demonstration of E-cadherin oligomerization and its functional implication on epithelial cell-cell contact formation and maturation

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Oligomerization of Cell Adhesion Molecules could provide the necessary stability and strength to ensure cell and tissue cohesion. Cadherin mediate homophilic cell-cell adhesion by forming adhesive bonds between molecules presented by adjacent cell membranes. These trans interactions are involved in the growth of cadherin clusters which are further stabilized by anchoring of cadherin cytoplasmic tails to actin. Recent structural, biochemical and modeling data suggest that cadherin also interact in cis by bonds formed between EC1 and EC2 domains of an adjacent cadherins. These bonds of weak energy are predicted to stabilize cell-cell junctions by shifting cadherin clusters from a fluid to an ordered (oligomeric) phase. However, no evidence has been provided so far for the existence of such ordered cadherin clusters *in vivo* and for the impact of cadherin oligomerization on cell-cell contact stability. We developed a monovalent nanogold labelling allowing the visualization of single cadherins within cell membrane at a nanometric resolution. Then, we studied the consequences of the disruption of the cis-oligomerization interface on cadherin complexes dynamics, adherens junction formation, cell-cell contact stability and collective cell behavior. E-cadherin molecules arrange in ordered lattices of a few to tens of molecules spaced by less than 10 nm, providing the first demonstration of the existence *in cellulo* of the predicted nanometric arrays of oligomeric E-cadherin at cell-cell contacts. In contrats E-cadherins mutated for the two amino-acids responsible for cis dimerization display a random distribution. We further provided evidence that cadherin cis-oligomerization requires initial trans-adhesive interactions. Cell aggregation, bead-cell adhesion assay and electron microscopy analysis indicated that ci-dimerization was not essential for cell aggregation and adherens junction formation. Analysis of protein dynamics by FRAP revealed that impairment of cis-dimerization slightly increases the mobility of junctional E-cadherin but also α-catenin. This moderate destabilization had a strong effect on E-cadherin anchoring to F-actin, on cell-cell contact dynamics and finally on collective cell migration as evaluated by magnetic tweezers manipulation and wound closure, respectively. Altogether, these data demonstrate that cadherin oligomerization occurs in a cellular context. Unexpectedly, the

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formation of ordered clusters of cadherins at cell-cell junction primarily controls the anchoring of the adhesion complexes to the actin cytoskeleton and finally the fluidity of cell-cell contacts.
Keywords: cadherin, cell-cell contact dynamics, cytoskeleton.

TUE-298
Influence of Snail-1 protein on integrines profile in HMEC-1 cells

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Integrins, which are prevalent cell membrane receptors, actively participate in various cell processes such as adhesion, migration and cell signaling.

It is also known, that EndMT process in endothelial cells is regulated by numerous factors including Snail-1 or Slug transcription factors.

In our experiments we used human microvascular endothelial cells (HMEC-1), transiently transfected with pcDNA3.1 vector containing cDNA for Snail (pcDNA3.1-Snail). The impact of Snail-1 overexpression on integrin receptors has been evaluated by flow cytometry technique. It was found that among investigated proteins, the integrin β1 and α4 subunits expression have significantly decreased. This decrease has been observed also using Western Blotting technique. We have successfully confirmed the impact of Snail-1 protein on different integrin subunits expression regulation.
Keywords: endothelial cell, integrins, Snail-1.

TUE-299
Investigating cell competition between wild-type and scribble mutant cells in tissue culture

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Cell competition is a process whereby defective or less fit cells are selectively recognised and eliminated by their fitter wild-type neighbours. This is thought to act as a cellular quality control mechanism and has additionally been linked to cancer development.

We are investigating the mechanisms involved in competitive interactions using an established *in vitro* cell culture system of cell competition. In this system MDCK cells silenced for the tumour suppressor gene *scribble* are eliminated by their wild-type neighbours (Norman et al., 2012). Our results show that cell contact is required for cell competition and that *scribble*^{KD} cells are compacted in the presence of wild-type cells, before they are outcompeted. We are currently investigating whether this is required for competition. In parallel, in order to home in on potential molecular players and markers of competitive interactions we are using transcriptional profiling to identify genes differentially expressed in *scribble*^{KD} cells. The results from both these approaches will be presented.
Keywords: Cell competition, Cell culture.

TUE-300
Is MVI important for myoblast differentiation?

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Myosin VI (MVI) is the only known myosin walking towards a minus end of actin filament. It acts as a motor transporting a cargo (various particles and vesicles) and/or an anchor positioning various cellular structures within the actin cytoskeleton. MVI seems to play a very important role in striated muscle functioning as mutation in human MYO6 leads to hypertrophic cardiomyopathy. However its function in striated muscles remains poorly understood Our previous studies showed that in skeletal muscles MVI could be involved in postsynaptic trafficking within the muscle fiber, maintenance of and/or transport within the sarcoplasmic reticulum as well as in gene expression. Recently, we have found that MVI could be important for myoblast differentiation. We observed that MVI knockdown leads to aberrations in actin cytoskeleton organization of C2C12 myoblasts, which led to inhibition of their migration and adhesive structure formation. Moreover, we observed impairment of MVI-deficient myoblast differentiation into myotubes. We also found that MVI was engaged in organization of endoplasmic reticulum and Golgi apparatus, and possibly in gene transcription.

Our data indicate that MVI by involvement in myoblast migration and adhesion, key processes during myoblast differentiation could play important role in myogenesis.
Keywords: cell migration, myogenesis, myosin VI.

TUE-301
Lamellipodia and membrane blebs drive electrotactic migration of Walker WC 256 carcinosarcoma cells

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Endogenous electric fields (EF) may provide and important signal for directional cell migration during wound healing, embryonic development and cancer metastasis but the mechanism of cell electrotaxis is poorly understood. Additionally, there is no research addressing the question on the difference in electrotactic motility of cells representing various strategies of cell movement - specifically blebbing vs lamellipodial migration. There is a fundamental difference between bleb formation and mechanisms of lamellipodia expansion. In contrast to bleb formation which is driven by hydrostatic pressure a central role in lamellipodium expansion plays actin polymerization which drives the protrusion of the cell membrane. In the current study we arranged an unique experimental model which allows investigation of the electrotactic movement of cells of the same origin but representing different modes of cell migration. By epigenetic selection we obtained two adherent sublines of Walker carcinosarcoma cells representing different modes of cell migration: weakly adherent, spontaneously blebbing WC256 cells (BC) and lamelliopodia forming WC256 cells (LC). Visualization of-actin in living cells a revealed that in blebbing (BC) WC256 cells, expanded blebs were devoided of actin and the cell membrane detached from the cortex. In contrast, in lamellipodia forming (LC) WC256 cells the protrusion was seen as wide band of fluorescent labeled F-actin. Here, we report that both BC and LC sublines show robust cathodal migration in a physiological EF (1-3 V/cm). The directionality of cell movement was completely reversible upon reversing the field polarity. However, the observed reaction was much faster in the case of BC (5 minutes) than LC cells (30 minutes) after the change of dcEF polarity. We also investigated distinct requirements for: Rac, Cdc42 and Rho pathways and extracellular Ca^{2+} ions in guidance by electric fields of WC256 sublines forming different types of cell protrusions. Significant differences in the reaction were observed after Rac, Cdc42 and ROCK inhibition. It was found that Rac GTPase is required for directional movement of LC but not for BC cells. In contrast Cdc42 and

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ROCK activity was necessary for electrotaxis of BC but not LC cells. The obtained results also showed that Ca^{2+} ions are essential only for the electrotactic reaction of BC cells.

In conclusion, our results reveal that both lamellipodia and membrane blebs can efficiently drive electrotactic migration of WC 256 carcinosarcoma cells but mediated by different signalling pathways.

Keywords: electrotaxis, blebbing and lamellipodial migration.

TUE-302

Late endosomal cholesterol regulates Syntaxin 6 localization and function in cell migration

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Cell migration in wound healing and cancer cell metastasis depends on the cell surface delivery of integrins and the secretion of extracellular matrix (ECM). Both exocytic processes are driven by SNARE proteins, a family of small, membrane-associated proteins that regulate intracellular membrane and cargo trafficking. Cholesterol is an essential membrane component that determines SNARE localization and function and is indispensible for cell migration. This concept is predominantly based on artificial manipulation of cellular cholesterol levels. However, the cellular cholesterol pools that determine SNARE localization and function in cell migration are still unknown. Most cells acquire cholesterol by endocytosis of Low Density Lipoproteins (LDL). In late endosomes (LE)/lysosomes, Niemann Pick Type C 1/2 (NPC1/2) delivers LDL-cholesterol to other cellular sites. We previously implicated LDL-cholesterol from LE to regulate the localization and function of caveolin and t-SNAREs Syntaxin 4 (Stx4) and SNAP23 in the secretory pathway (Cubells et al., Traffic 8, 1568-89, 2007; Reverter et al., Mol Biol Cell 22, 4108-23, 2011). Here, using Chinese Hamster Ovary (CHO) NPC1 mutant cells and human NPC1 mutant fibroblasts, which accumulate LDL-cholesterol in LE, we demonstrate that LDL-cholesterol determines the localization and function of another t-SNARE, Syntaxin 6 (Stx6), in integrin-dependent cell migration. Stx6 is predominantly found in the trans-Golgi-network (TGN) to interact with the v-SNARE VAMP4, and shuttles between the TGN and recycling endosomes (RE). Loss of NPC1 causes diminution of cholesterol at the plasma membrane and TGN, while cholesterol in RE fractions is elevated. This triggers the accumulation of Stx6 into VAMP3, transferrin and Rab11-positive RE to increase Stx6/VAMP3 interaction. Enhanced Stx6/VAMP3 complex formation interferes with Stx6-dependent recycling of $\alpha V\beta 3$ and $\alpha 5\beta 1$ integrins, and is associated with strongly reduced cell migration. Most interestingly, in NPC mutant cell lines, restoration of cholesterol levels in the TGN, but not VAMP3 inhibition or VAMP4 overexpression, restores the steady state localization of Stx6 in the TGN. In contrast, elevation of cholesterol in RE is associated with Stx6 translocation to this compartment. Hence, the fine-tuning of cholesterol levels at the TGN-RE boundaries modulates the localization of a subset of cholesterol-sensitive SNARE proteins to play a novel regulatory role in cell migration and invasion (Reverter et al., Cell Reports 2014, in press).

Keywords: LDL-cholesterol, migration, syntaxin-6.

TUE-303

Lipid droplets as a novel cargo of tunneling nanotubes

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Intercellular communication is one of the fundamental processes in the development and functioning of multicellular organisms. Known mechanisms of cell-to-cell interaction include exocytosis or direct transfer of small cytoplasmic components *via* gap junctions. Recently, an essentially new type of intercellular communication, based on thin membrane channels between mammalian cells, has been reported. These structures, called intercellular or tunneling nanotubes (TNTs) permit direct exchange of various components or signals (ions, proteins, organelles) between non-adjacent cells at distances over 100 μm.

The functional role of this type of intercellular communication between endothelial cells has as yet not been investigated. Therefore, aim of this study was to characterize endothelial TNT morphology, cytoskeletal organization, and cargo.

Our studies show the presence of tunneling nanotubes in microvascular endothelial cells (HMEC-1). The TNTs were studied with live cell imaging, environmental scanning electron microscopy (ESEM), and Coherent anti-Stokes Raman scattering spectroscopy (CARS). Tunneling nanotubes showed distinct persistence: the TNTs could connect cells over long distances (up to 150 μm) for several hours. Even during mitosis dividing cells were connected to other cells by long TNTs. Plasma membrane staining revealed that the whole nanotube always belongs to one "host" cell. We observed at least three types of TNTs: either actin- or tubulin-containing TNTs or nanotubes with both actin filaments and microtubules. Several cellular organelles were present in TNTs, for example lysosomes and mitochondria. Moreover, we could identify lipid droplets as a novel type of cargo in TNTs. Under angiogenic conditions (VEGF treatment) the number of lipid droplets increased significantly. Arachidonic acid application resulted not only in the increased number of lipid droplets, but also in TNT formation.

Taken together, our current study shows for the first time lipid droplets as a cargo of TNTs and thereby opens a new field in intercellular communication research.

Keywords: endothelial cells, lipid droplets, tunneling nanotubes (TNTs).

TUE-304

Lysosomal GTPase Arl8b governs HOPS mediated late endosome-lysosome fusion in mammals

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Eukaryotic cells have an elaborate endo-membrane system consisting of morphologically and functionally distinct compartments that are in constant communication with each other through vesicular carriers. The vesicle fusion machinery has been extensively studied, but what determines the specificity of fusion, is still a question. Our research focuses on deciphering the answer in the context of late endosome-lysosome fusion. Fusion at the lysosome relies on a complex interplay of small GTPases, tethering factors and SNARE proteins. The tethering factors are generally recruited by small GTPases to specific organelles, where they

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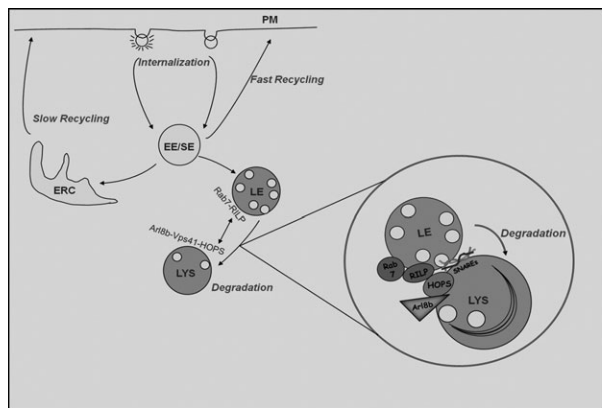

Fig. 1. Late endsome-lysosome fusion orchestrated by small GTPases Rab7 and Arl8b and their effectord. Fusion at the mammalian lysosome is regulated by small GTPase, tethering factors and SNARE proteins. The late endosomal small GTPase Rab7 recruits its effector RILP, which in turn interacts dircetly with HOPS complex, thereby recruiting it to late endosomes. The lysosomal small GTPase Ar18b interacts with HOPS complex, that determines the assembly of HOPS complex on lysosomal membranes. Following its recruitment to lysosomes, HOPS complex tethers the two compartments and mediates SNARE-department fusion of late endosomen with lysosomes.

aid to tether apposing membranes, and in conjunction with SNARE proteins bring about vesicle fusion. One such late endosomal/lysosomal tethering factor is HOmotypic Fusion and vacuole Protein Sorting (HOPS) complex, a six subunit protein complex that is conserved from yeast to humans. Much is known about mechanisms governing HOPS function in yeast, but a similar understanding of the mammalian system is still underway. Here, we present evidence that the recently characterized lysosomal small GTPase Arl8b interacts with Vps41 subunit of mammalian HOPS complex, and promotes its recruitment to lysosomes. Moreover, hVps41 interacts with only the GTP-bound form of Arl8b, suggesting that it is indeed an effector of Arl8b. Small interference RNA (siRNA)-mediated knockdown of Arl8b results in loss of membrane association of hVps41, an effect that can be rescued by expression of siRNA-resistant Arl8b. Using yeast-two-hybrid analysis and immunofluorescence studies we have identified how interaction between subunits of the HOPS complex drives their assembly to lysosomal membranes in an Arl8b-dependent manner. To understand if Vps41 interaction with Arl8b is critical for its function in regulating endocytic traffic to lysosomes, we performed EGF-receptor trafficking assay in Vps41 knockdown cells. Knockdown of Vps41 delays EGF-receptor trafficking to lysosomes that can be rescued by overexpressing Vps41 along with Arl8b in these cells. Together, our data suggests that Arl8b promotes assembly of mammalian HOPS complex on lysosomes, being facilitated by direct interaction of Arl8b with Vps41 subunit of the complex, which in turn directs traffic towards lysosomes.

Keywords: HOPS complex, Lysosomes, Vesicle fusion.

TUE-306
Molecular mechanisms of the CtBP1-S/BARS-dependent membrane fission processes

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Membrane curvature and the membrane fission that follows are essential events in the regulation of intracellular membrane trafficking. They are required for the formation of membranous transport carriers and are controlled by a cooperative contribution of both lipids and proteins. Membrane fission appears to rely on multiple mechanisms, the best-characterised fission process is driven by dynamin, which triggers fission mechanically by GTP-dependent conformational changes. Fission can also be induced by shallow insertion of hydrophobic proteins containing amphipathic alpha-helices, to generate membrane curvature and promote fission (e.g., epsin, amphiphysin, endophilin, Arf1, Sar1). Further, others fission mechanisms have been proposed, although their physiological significance remains unclear; e.g., actin-based fission, or fission induced by phase separation of lipids to create 'line energy' at interfaces, to induce fission, which can be facilitated by actin dynamics. Other fission events are mediated by CtBP1-S/BARS (C-terminal binding protein 1-short form/ brefeldin A ADP-ribosylation substrate; BARS), a dual-function protein, as a transcriptional co-repressor in the nucleus, and a fission-inducer in the cytoplasm. BARS controls fission of post-Golgi and endocytic fluid-phase carriers and COP1-coated vesicles, with roles in mitotic Golgi partitioning and macropinocytosis. We have previously reported that the fission-inducing property of CtBP1-S/BARS is dependent on a lysophosphatidic acid acyltransferase activity, which is responsible for the generation of phosphatidic acid (PA) from lysophosphatidic acid (LPA) and acylCoenzyme A (acylCoA). Lysophosphatidic acid has been proposed to facilitate tubulation when present in the cytosolic leaflet of membranes, through its wedge-like shape; conversely, through its conical shape, PA has been proposed to destabilise tubules, which induces membrane fission. Here we report that BARS specifically binds LPAAT4, a Golgi-resident lipid metabolic enzyme that we have characterized as an LPAAT enzyme able to incorporate AcylCoA into LPA to form PA onto Golgi membranes. The LPAAT4 activity is required during post-Golgi carriers (PGCs) formation, specifically at the fission step as shown by inhibition of this enzyme, which results in long tubular carrier precursor unable to detach from the *trans*-Golgi network. We provide evidence that BARS activates this enzyme to generate a local membrane increase of PA that in turn, induces the fission of post-Golgi tubular carriers.

Keywords: Golgi, Membrane fission, membrane trafficking.

TUE-307
Molecular pathogenesis of low-oxygen enhanced invasion by GBM cells

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Malignant gliomas remain associated with poor prognosis and high morbidity because of their ability to invade the brain; furthermore, human gliomas exhibit a phenotype of accelerated brain invasion in response to anti-angiogenic drugs. Here, we study 8 human glioblastoma cell lines; U251, U87, D54 and LN229 show accelerated motility in low ambient oxygen. Src inhibition by Dasatinib abrogates this phenotype. Molecular discovery and validation studies evaluate 46 molecules related to motility or the src pathway in U251 cells. Demanding that the molecular changes induced by low ambient oxygen are reversed by Dasatinib in U251 cells, identifies neural Wiskott-Aldrich syndrome protein (NWASP), Focal adhesion Kinase (FAK), -Catenin, and Cofilin. However, only Src-mediated NWASP phosphorylation distinguishes the four cell lines that exhibit enhanced motility in low ambient oxygen. Downregulating c-Src or NWASP by RNA interference abrogates the low-oxygen-induced enhancement in motility by *in vitro* assays and in organotypic brain slice cultures. The findings support the idea that c-Src and NWASP play key roles in

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mediating the molecular pathogenesis of low oxygen-induced accelerated brain invasion by gliomas.

Keywords: Glioblastoma Multiforme, invasion, low-oxygen.

TUE-308
Myocardin-related transcription factor B is involved in EndMT in HMEC-1cells as a Snail expression regulator

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Myocardin-related transcription factor A (MRTF-A) and myocardin-related transcription factor B (MRTF-B) are the two members of myocardin family. In contrast to myocardin, which is only restrictedly expressed in smooth and cardiac muscles, MRTFs are broadly distributed in human embryonic and adult tissues but their role in non-muscle cells is largely unclear, except from the regulation of mammary myoepithelial differentiation. Recent data suggested participation of MRTFs in regulation of TGFβ-induced EMT in cancer cells. In our studies we would like to identify the nature of interaction between Snail and MRTFs pathways during EndMT and its effect on HMEC-1 cytoskeleton reorganisation. In our analysis we confirmed the presence of both MRTFs in HMEC-1 on the protein and transcription level. We observed that in endothelial cell line HMEC-1 induced by TGF-β1 the nuclear translocation of MRTF-B appeared. Our analysis based on EMSA and ChIP assays revealed that MRTF-B has the ability to control Snail expression. We also presented that overexpression of MRTF-B provoked changes in cell morphology manifested by more mesenchymal character and increased expression of mesenchymal markers. In conclusion, obtained results suggest that MRTF-B seems to be a mediator of TGFβ by regulation of Snail expression in endothelial HMEC-1 cells.

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Keywords: EndMT, MRTFs, TGF-beta.

TUE-309
Naturally occurring wound electric fields as proposed determinant of bone marrow cells attraction to the site of injury

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Direct current electric fields (DC EFs) were found to occur in living organisms and take part in some physiological processes like embryonic development, wound healing or cancer metastasis. After wounding they are created as a result of the transepithelilal potential (TEP) disruption, when the conductive extracellular pathway is created and ion flux generate electric field with the negative pole located in the site of the wound. These naturally-occurring EFs last during healing and may guide cell migration by process known as an electrotaxis. To date, there was shown that many cell types respond to physiological levels of EFs with accelerated, directed migration mostly toward the cathode. Mounting of evidence suggests that bone marrow derived cells may be involved in optimum healing of cutaneous wound, contributing to skin cells or releasing regulatory cytokines.

In our study, application of DC EF of physiological level (100-300 mV/mm) to the adherent fraction of mouse bone marrow cells results in their accelerated migration parallel to the EF lines. Further analysis lead us to discrimination of two cell subpopulations migrating to the opposite poles. These populations exhibit significantly different morphological and migratory features. The anodal cells are relatively small, elongated and they form a narrow lamellipodium at the anode-facing site of the cell setting their long axis square with the EF lines. At the same time, the cathodal cells are larger, more flattened and they tend to elongate perpendicular to the field lines. Both populations show most directed migration in 300 mV/mm with the directional cosinus γ values of 0.84 ± 0.04 for cathodal, and -0.90 ± 0.02 for anodal cells. Moreover, these two cell types utilize different cellular pathways in their response to DC EF. Phosphatydyloinositol-3 kinase/Akt kinase seem to be more essential for efficient migration of cathodal cells under EF, whereas small GTPases Cdc42 and Rac1 are more involved in electrotactic response of anodal cells. Whether it is cell-type or reaction-type specific has to be established. Interestingly, cell markers analysis lead us to conclusion, that cells migrating toward the anode are of hematopoietic origin, mostly they comprise bone-marrow macrophages, while the cathodal cells contain the fraction of mesenchymal stem cells.

Taken together, our results suggest that response to physiological DC EF may be one of the factors that discriminate bone marrow mesenchymal stem cells attraction to the site of wounding simultaneously excluding other cell types like macrophages from the wound bed.

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Keywords: bone marrow cells, electrotaxis, wound healing.

TUE-310
Nuclear actin isoforms in the human colon adenocarcinoma LS180 cells and their selected sublines with different metastatic potential

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Actin is a multifunctional protein existing in all eukaryotic cells. Six actin isoforms exist in vertebrates: two of them are present in striated muscle (alpha-skeletal actin and alpha-cardiac actin), two isoforms are characteristic for smooth muscles (alpha-smooth and gamma-smooth muscle actin) and two non-muscle isoforms beta and gamma are typically cytoplasmic [Vandekerckhove J and Weber K, 1978 J Biol Chem].

Actin is present in the cytoplasm and in the nucleus. Compared to cytoplasmic actin the organization and functions of nuclear actin are not well recognized. It has been reported that actin in the cell nucleus participates in transcription, chromatin remodeling, nuclear structure organization and signal transduction. However, there is still lack of information about actin isoforms presence in the nucleus, especially about their potential role in cancer metastasis [de Lanerolle P, 2012 J Cell Sci; Migocka-Patrzalek M and Malicka-Blaszkiewicz M, 2009 Postepy Biochem].

The goal of presented research was to identify and analyze the nuclear actin isoforms level in cancer cells with different metastatic potential.

Experiments were performed on human colon adenocarcinoma LS180 cells and their selected sublines exhibiting an increased motility and metastatic potential (EB3, 3LNLN and 5W).

Our studies revealed that actin was shown in the nucleus of investigated cells, using the immunocytochemistry and monoclonal antibodies, directed against total actin and beta and gamma isoforms. We demonstrated that beta- and gamma-actin is present in the nucleus.

Quantitative analysis showed, that the nucleoplasm/cytosol total actin ratio for sublines with different metastatic potential is

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similar when compare to parental line LS180. However the nucleoplasm/cytosol ratio calculated for beta-actin is significantly higher in EB3 and 3LNLN sublines. To highlight the differences in nuclear actin isoforms level in human colon adenocarcinoma sublines we compare the obtained values within each subline. This interpretation let us to conclude, that in the nucleoplasm obtained from EB3 subline the beta- is on the higher level than gamma- actin. In 3LNLN both isoforms are on similar level, whereas in the nucleoplasm from 5W sublines the gamma izoform dominates over beta.

Our study of actin and its beta and gamma isoforms in the nucleoplasm of human colon adenocarcinoma LS180 and its sublines with different metastatic potential shows diverse level of actin isoforms, in spite of the unchanged quantity of total actin. Further investigations focused on nuclear actin isoforms level and its correlation with cancer cells motility and metastatic potential would give more details.

Keywords: nuclear actin cancer.

TUE-311

Occurrence of protein disulfide bonds in different domains of life: a comparison of proteins from the protein data bank

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Disulfide bonds (SS bonds) are important post-translational modifications of proteins. They stabilize a three-dimensional structure (structural SS bonds) and also have the catalytic or regulatory functions (redox-active SS bonds). Although SS bonds are present in all groups of organisms, no comparative analyses of their frequency in proteins from different domains of life have been made to date. Using the Protein Data Bank, the number and subcellular locations of SS bonds in Archaea, Bacteria and Eukarya have been compared. Approximately three times higher frequency of proteins with SS bonds in eukaryotic secretory organelles (e.g. endoplasmic reticulum) than in bacterial periplasmic/secretory pathways was calculated. Protein length also affects the SS bond frequency: the average number of SS bonds is positively correlated with the length for longer proteins (>200 amino acids), while for the shorter and less stable proteins (<200 amino acids) this correlation is negative. Medium-sized proteins (250–350 amino acids) indicated a high number of SS bonds only in Archaea which could be explained by the need for additional protein stabilization in hyperthermophiles. The results emphasize higher capacity for the SS bond formation and isomerization in Eukarya when compared with Archaea and Bacteria.

Keywords: Disulfide bonds, Domains of life, PDB.

TUE-312

Oxysterols-induced cytoskeleton disruption via modifications of lipid raft-associated proteins and cell death receptor activation

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Aims of the Work: Oxysterols (oxidized derivatives of cholesterol) are known to induce important toxic effects and are implicated in some aging-associated diseases. They can be formed de novo by oxidation of cholesterol, but they also come from alimentation (meat, oily fishes...). Molecular mechanisms of oxysterols toxic effects are poorly understood. The purpose of our study was to characterize the effects of two different oxysterols on skin cells.

Methods: The effects of 7-ketocholesterol (7-KC) and 25-hydroxycholesterol (25-OH) were studied on keratinocytes (human HaCaT cell line) and on macrophages (human U937 differentiated cell line). Cytoskeleton disruptions and modifications of lipid raft-associated proteins (P2X7 receptor, caveolin-1 and CD44 receptor) were evaluated using confocal microscopy and in-cell Western technique. P2X7 cell death receptor activation was quantified using microplate cytometry (YOPRO-1/7-AAD assay). Inflammation was studied by quantification of IL-1β and TNFα in cell supernatants using ELISA.

Key Results: After a 48 h-incubation with 7-KC or 25-OH disruptions in actin cytoskeleton were observed particularly at 25 μM (7-KC) and 40 μM (25-OH). Modifications in the expression of some key lipid raft-associated proteins were highlighted on keratinocytes. Activation of P2X7 cell death receptor was observed on keratinocytes and macrophages. IL-1β and TNFα were increased in macrophages supernatants.

Conclusion: Our work shows that toxic effects of oxysterols are associated with cytoskeleton disruption, lipid raft-associated proteins modifications and P2X7 cell death receptor activation on keratinocytes. On macrophages, P2X7 cell death receptor activation is coupled with inflammation. These cellular effects could be considered as interesting targets to prevent some aging-associated diseases.

Keywords: Oxysterol, cytoskeleton, P2X7 receptor.

TUE-314

Phosphorylation-dependent disulphide cross-linking of protein phosphatase-2A and heat shock protein 27: interrelated regulation of activity and localization

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In stressed mammalian cells, the 27-kD heat shock protein (Hsp27) is phosphorylated in the p38 MAP kinase/Hsp27 pathway so that its phosphate-modified (low-size-oligomer) species are involved in cytoprotection. In its turn, protein phosphatase PP2A dephosphorylates a pool of phosphorylated Hsp27 when stressful stimuli disappear. On the other hand, PP2A is known to be extremely sensitive to oxidative stress, while the phosphorylated species of Hsp27 protect oxidatively stressed cells from depletion of reduced glutathione and apoptosis. Taking this into consideration, we hypothesized that, upon oxidative stress conditions, PP2A and Hsp27 interact with each other to regulate their functional activities and intracellular distribution. Using reciprocal immunoprecipitation, native electrophoresis and immunoblotting we found that, in mammalian cells (human endothelium or rat cardiomyoblasts) treated with (pro) oxidants or undergoing oxidative stress in a model of ischemia/reperfusion, a large pool of inactivated PP2A is in complexes with di- or tetramers of phosphorylated Hsp27. These complexes are resistant to a high ionic strength and SDS but dissociate in the presence of mercaptoethanol or dithiothreitol, or other thiol group-containing reagents that implies intermolecular S-S cross-linking. By means of immunofluorescence staining, we revealed analogous S-S-linked PP2A-Hsp27 complexes associated with F-actin bundles which were not extractable with non-ionic detergents; thiol group-containing reagents allowed to extract PP2A from actin filaments and to restore its phosphatase activity that was accompanied by dephosphorylation of Hsp27 and its dissociation from F-actin. Inhibition of the Hsp27 phosphorylation with SB203580 suppressed the S-S-linked PP2A-Hsp27 complex formation in

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oxidatively stressed cells. Non-phosphorylatable mutant forms of Hsp27, being overexpressed in the transfected cells, were never detected in such PP2A-Hsp27 complexes, whereas the pseudo-phosphorylated mutant forms are found in them. We suggest that the PP2A-Hsp27 coupling causally occurs as a response to the oxidative stress-induced Hsp27 phosphorylation and depletion of reduced glutathione and serves for transient inactivation of PP2A: while PP2A remains inactive, phosphorylated Hsp27 stabilizes the actin cytoskeleton and ensures restoration of a pool of reduced glutathione in the cell recovering from oxidative stress. Later, a glutathione circuit-mediated mechanism causes a reduction of the S-S bonds between PP2A and Hsp27 that leads to the PP2A reactivation and subsequent Hsp27 dephosphorylation on the spot. Also, the S-S-linked PP2A-Hsp27 complexes may be a biomarker of some oxidative stress-associated pathological states.

Keywords: None.

TUE-315
Plakophilin3 expression results in an increase in the level of desmosomal components and the formation of calcium independent desmosomes

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Desmosomes are calcium dependent adherens like junctions found in almost all epithelial tissues and in cardiac muscles, that aide in cell-cell adhesion. Our lab has previously reported that loss of the armadillo family member plakophilin 3 (PKP3) results in increased tumour formation and metastasis in vivo. This is characterised by a decrease in desmosome size and cell-cell adhesion and an increase in cell migration and anchorage independent growth. The reduction in desmosome size maybe due to the fact that other desmosomal proteins such as desmoplakin, desmocollins 2/3 and desmoglein 2 fail to localize to the border in the absence of PKP3.

In contrast, our current study shows that, over expression of PKP3 in HCT116 cells results in increased cell-cell adhesion and the formation of calcium independent desmosomes. This was accompanied by the increased localization of other desmosomal proteins such as desmocollins 2/3, desmoglein 2, plakoglobin and desmoplakin to the cell border in the presence and absence of calcium and increased expression of these proteins at the protein level and increase of desmocollin 2 at mRNA level. These results show that PKP3 might regulate the expression of other desmosomal proteins, thereby driving desmosome formation.

These results, taken together with the previously published data about the phenotypes of PKP3 loss, show that PKP3 can play an important role in cancer metastasis by regulating desmosome function.

Keywords: adhesion, desmosome, plakophilin.

TUE-316
Profiling sulfation/epimerization pattern of full length Heparan Sulfate by NMR spectroscopy following 13C-glucose metabolic labeling of cell culture

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Heparan sulfate (HS) is a complex cell surface polysaccharide. Through its ability to bind a large array of proteins, it regulates key functions at the cell-tissue interface, including cell proliferation, migration and development, inflammation and immune response, angiogenesis, matrix assembly, or viral attachment. To do so, HS can either or both provide scaffolds to ensure that proteins mediating specific functions will be presented at the correct site and time, and directly contribute to biological activities or signaling processes.

Consistently with its multiple binding activities, HS features extended structural variability. It consists of glucuronic acid (GlcA)-N-acetyl-glucosamine (GlcNAc) repeats which sulfation and epimerization generate up to 48 different disaccharides, the combination of which creates an extremely high density of information.Therefore, changes in HS structure, which appeared to be cell and development stage specific and is dynamically regulated during physiologic processes or disease states, can alter its protein binding capacity, resulting in change or loss of function.

Here we use NMR spectroscopy to reveal both the sulfation and epimerization pattern of native HS, directly isolated from cell culture so that any modifications induced by stimuli or growth conditions can be determined. For that purpose we took advantage of the presence of 11 C-H bonds in a HS disaccharide unit and thus used ^{13}C-glucose as a precursor of both GlcN and GlcA to metabolically label HS produced by growing cells. To set up the experimental details and demonstrate that quantitative analysis is possible, we first extracted HS from epithelial Hela cells that were maintained in 13C-glucose containing medium. NMR signals assignment of the HS chains enabled to determine the mains characteristics of the polysaccharide, i.e. the percentage of *N*-, 2-*O*- and 6-*O*- sulfation and the IdoA/GlcA ratio.

To support the interest of this approach, we showed that this method permit to follow HS regulatory processes, using human Caco-2 colon carcinoma cells which undergo spontaneous differentiation when maintained in culture post-confluency and WT and KO fibroblasts, the latter being silenced for NDST1 expression, one of the HS biosynthetic enzymes.

In conclusion, these results showed that the main HS characteristics can be profiled using NMR based non destructive analysis, directly from cell culture extracted material. This method should fine ample applications, as it has been well known that HS modifications have a pivotal role in development, physiology and pathology.

Keywords: Cellular regulation, Heparan sulfate, NMR characterization.

TUE-317
Protein/nanoparticules interaction: a case of adsorption

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The understanding of the mechanisms involved in the interaction of proteins with inorganic surfaces is of major interest in both fundamental research and applications such as nanotechnology. Given that nano-sized objects tend to be more toxic than their large scale form it would be unwise to allow the unnecessary build up of nanoparticles within the body until the toxicological effects of that nanoparticle are known.

There are several ways that nanoparticles can enter the body. These include inhalation, ingestion, absorption through the skin and direct injection for medicinal purposes. Once the particles are in the body they undergo surface changes, in particular via coating by a protein corona, which is of primary importance for the biodistribution and the fate of the nanoparticle in the body. Moreover, depending on the composition of the corona, for

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example the blood brain barrier can be crossed by certain nanoparticles, a very interesting property that can be used for future medical applications.

However, despite intense research, the mechanisms and the structural determinants of protein/surface interactions are still unclear. We developed proteomic strategies consisting in identifying, in a mixture of hundreds of soluble proteins, those proteins that are edsorbed on the surface and those that are not and in studying the effect of adsorption on the structure/function of stress proteins. This study was lead with silica nanoparticles on demonstrated that various biological functions can be impaired by nanoparticles.

Keywords: adsorption, Nanopatricle, protein aggregation, misfolding.

TUE-318
Proteomic analyses uncover a new role of mDia2 in p53 regulation

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mDia2 is an auto-inhibited Formin influencing actin dynamics upon conversion to the active conformation. mDia2 regulates actin-based protrusions and cell invasion, cell differentiation, vesicle trafficking and cytokinesis. However, whether mDia2 has additional functions and how its action is functionally specified remain unknown. Here, we draw the interactome of auto-inhibited and constitutively active mDia2 to address these issues. We embed mDia2 in protein networks accounting for its attributed functions and unexpectedly link it to the Ubiquitin Proteasome System. Taking SCFFBXO3 as a test case, we show that mDia2 binds FBXO3 and p53, and regulates p53 transcriptional activity in an actin-nucleation-independent and conformation-insensitive manner. Increased mDia2 and FBXO3 levels elevate p53 activity and expression leading to p53-dependent apoptosis, whereas their decrease produces opposite effects. Thus, we discover a new role of mDia2 in p53 regulation suggesting that the closed conformation is biologically active and an FBXO3-based mechanism to functionally specify mDia2's activity.

Keywords: Formins, mDia2, p53.

TUE-319
Real-time analysis of human breast cancer cell line (MDA-MB-435) proliferation potential

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Purpose: Aim of this study was by continuous monitoring to assay the proliferative capacity of human breast cancer (MDA-MB-435) cell line using the xCELLigence system. These systems utilize an electronic readout called impedance to quantify adherent cell proliferation and viability in real-time.

Methods: The proliferation of human breast cancer (MDA-MB-435) cell line was investigated by a real-time cell analyzer (xCELLigence, ACEA Biosciences, Inc, CA, USA). xCELLigence cell index (CI) impedance measurements were performed according to the instructions of the supplier. MDA-MB-435s were resuspended in DMEM F-12 and subsequently adjusted to 5000, 10 000, 20 000, and 40 000 cells/well. After seeding 100 μL of the cell suspensions into the wells (8 well per each group) of the E-plate 16, MDA-MB-435s were monitored every 15 min for a period of up to 192 h by the xCELLigence system. MDA-MB-435s on the E-plate 16, which has 16 wells, were treated every other day with DMEM F-12 containing 10% FBS. The values of the electrode impedance were represented as the 'cell index'.

Results: To assess dynamic cell proliferation, human breast cancer (MDA-MB-435), were seeded at 5000, 10 000, 20 000 and 40 000 cells per well in 16 E-Plates. The cells were

monitored every 15 minutes for the indicated period of time. Changes in cell status such as cell number, viability, morphology and adherence were monitored and quantified by detecting sensor electrical impedance. Statistical analysis demonstrated that the impedance CI of 5000, 10 000, 20 000, and 40 000 cells/well increased proportionally to cell number.The CI of each cell concentration sharply increased after seeding up to reach its maximum from 15 to 20 h. The CI initial of 5000, 10 000, 20 000 and 40 000 cells/well respectively were determined 0.5, 1, 1.5, 2.5.Thereafter the CI of 5000 and 10 000 cells/well increased to reach a minimum at 15 h to increase again to a maximum at 100 h. The CI of 20 000 cells/well showed a minimum

at 20 h and reached its second maximum at 100 h. In contrast, the CI of 40,000 cells/well made a sharp increase during the first 5 h to reach a plateau value after 50 h, where it remained thereafter. In all, we conclude that the response seen in the 5000–10.000 cells/well experiments reflects cell cycle effects, while the concentration of 20.000-40.000 cells/well were not suited for further experimentation, possibly because of a too high cell density and the resulting contact inhibition.

Conclusion: Our data suggests that the xCELLigence live cell analysis system offers dynamic live cell monitoring and combines high data acquisition rates with ease of handling. Therefore the xCELLigence system can be used as a rapid monitoring tool for cellular proliferation.

Keywords: xCELLigence, MDA-MB-435, Cell Culture.

TUE-321
Relationship between adipocytokines, angiogenesis-related factors and ADP-induced platelet aggregation in patients with preeclampsia

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Preeclampsia and pregnancy loss are considered to result from under perfusion of placenta due to structural and occlusive changes including thrombosis in the placental vessels. The effects of adipocytokines on endothelial function, inflammation and adipocyte metabolism makes them key players in the pathogenesis of metabolic syndrome, a cluster of clinical symptoms including obesity, insulin resistance, hypertension-related diseases. In addition, the anti-angiogenic state in preeclampsia may partially affect of platelet function via changed levels of nitric oxide and prostacyclin. In this study, some of the adipocytokines, angiogenesis-related factors and platelet aggregation levels were determined and correlated in patients with preeclampsia and control pregnant womens.

52 preeclamptic and 27 control pregnant women were included into the scope of this study. Ghrelin, leptin, adiponectin, transforming growth factor β1 (TGFβ1), von willebrand factor (vWF), vascular endothelial growth factor (VEGF), endoglin, placental growth factor (PlGF) and P-selectin levels were determined by ELISA. Colorimetric method was used to determine

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the nitric oxide levels. Platelet aggregation tests with adenosine diphosphate (ADP) were analyzed by using aggregometer.

In patients with preeclampsia, blood leptin, vWF and endoglin levels were found to be significantly higher than controls ($p < 0.001$) Contraversely, blood PIGF levels was lower in pre-eclamptic patients than controls ($p < 0.001$). In addition; we could not found any significant difference for ghrelin, adiponectin, TGF β1, VEGF, nitric oxide, P-selectin and ADP-induced platelet aggregation levels between preeclamptic and control pregnant womens. According to pearson correlation test, there was positive correlations between endoglin and vWF, endoglin and leptin [$r_p = 0.322$, p = 0.005; $r_p = 0.420$, p = 0.000, respectively], and negative correlations between PlGF and vWF, PlGF and Endoglin, PlGF and leptin [$r_p = -0.507$, p = 0.000; $r_p = -0.388$, p = 0.000; $r_p = -0.381$, p = 0.000, respectively]

This study demonstrated that altered blood levels of the angiogenesis-related factors were not related with platelet aggregation in preeclamptic patients. Although insignificant positive correlations, increased leptin and vWF levels may still contribute to platelet activation/aggregation in patients with severe preeclampsia.

Keywords: adipocytokines, angiogenesis-related factors, platelet aggregation.

TUE-322
Retromer complex controls JAK/STAT signaling at the endosome.

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The JAK/STAT pathway is a major intracellular signaling pathway activated by several cytokines including interferons (IFNs) that have strong antiviral and antiproliferative activities. A pioneering approach taken by team in the past proved endocytic trafficking of the IFN receptor (IFN-R) to be a key for the IFN type I induced JAK/STAT activation. Here we propose the endosomal retromer complex – VPS26/29/35 (vesicular sorting protein 26/29/35) to form a direct link between the IFN-R trafficking and the JAK/STAT activation. For the first time we report the interaction between IFN-R and VPS26 or VPS35 (combination of biochemistry and cell imaging approaches). Trafficking assays of the IFN-R in cells depleted from the VPS26 and VPS35 subunits revealed strong block of the IFN-R2 recycling to the plasma membrane (i.e. abnormally prolonged presence in the early endosomes). Study of the IFN type I induced STAT1 phosphorylation and the expression of 96 IFN-stimulated genes revealed prolonged JAK/STAT pathway activation in VPS35 depleted cells when compared to control cells. Therefore VPS26/29/35 trimer may be a good candidate for the spatiotemporal control of the IFN-R sorting at the early endosome and the IFN-R2 endosome-to-plasma membrane trafficking which in turn could regulate proper JAK/STAT activation. In addition, another set of the endosomal proteins - sorting nexins (SNXes) - was identified as the potential IFN-R interacting partner. Whether it contributes to the retromer-dependent IFN-R sorting and possibly JAK/STAT signaling is currently under investigation.

Keywords: Endocytosis, Interferon alpha receptor, JAK/STAT signaling.

TUE-323
Single-cell analysis of heterogeneous cell population in suppression of PC12 neuronal differentiation by direct microinjection of a differentiation inhibitor

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Recent developments in technologies regarding single-cell isolation and small-scale analysis have demonstrated that there exists a large degree of heterogeneity among individual cells growing under the same condition. Especially, genomic and phenotypic diversities of cells going through a seemingly identical differentiation process have been hindering accurate analysis and efficient application of obtained information for clinical purposes. In order to solve the problems regarding efficiency and safety of cell differentiation regulation technologies, it is necessary to investigate the minor changes and characteristics of individual single cells which might in fact be critical.

In our study, to investigate the single-cell changes in PC12 neuronal differentiation which occur when inhibited by U0126, an inhibitor of mitogen-activated protein kinase kinase (MEK), we directly injected the chemical in individual target cells and analyzed the outcomes (neurite outgrowth) at the single-cell level. Direct microinjection of the differentiation inhibiting factor enabled accurate control of the quantity being introduced into each target cell, which was not possible by the common methods of simple addition to culture medium or genetic engineering of target cells. As a result, we were able to elucidate the quantitative relationship between the amount of U0126 in each target cell and the inhibitive effect on neuronal differentiation, in comparison with addition of the inhibitor to the culture medium at certain concentrations. Furthermore, time-course analysis of neurite outgrowth at the single-cell level using fluorescence staining method showed that the changes in neurite length of differentiating PC12 cells were not homogeneous, but were largely variable across individual target cells. Based on these results, heterogeneity in cellular differentiation process and the importance of single-cell analysis on these phenomena will be discussed.

Reference

1. Chung et al, Cell Biology International, in press.

Keywords: microinjection, PC12 neuronal differentiation, single-cell heterogeneity.

TUE-324
Spatiotemporal dynamics of RhoA and Rac1 determine apoptotic cell apoptotic cell removal

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The deletion of unwanted or damaged cells is achieved by finely regulated cleaning process, efferocytosis[1]. It has been established that RhoA and Rac1 GTPases are antagonistically involved in cytoskeleton organization for clearance of apoptotic cells. Over-

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all, Rac1 and its upstream activators facilitate engulfment of apoptotic cells[2], whereas RhoA and its downstream effector Rho kinase has an inhibitory role[3,4]. However, the coordinate actions of Rac1 and RhoA at the individual phagocytic cup would sophisticatedly regulate phagocytic process, which are not fully addressed before. Using FRET biosensors to visualize the spatiotemporal dynamics of Rho family GTPases, here we showed that Rac1 and RhoA coordinately regulate critical points of phagocytic process through turning on/off their activities at precise timing and site. RhoA, a negative regulator, known to be down-regulated during phagocytosis, was unexpectedly up-regulated in broad area upon challenge of apoptotic cells but transiently down-regulated at the phagocytic cup right before the ingestion. Rac1 was up-regulated around phagocytic cup during engulfment then immediately down-regulated ensuing actin disassembly prior to phagosome maturation. Demolition of these dynamic activities of RhoA and Rac1 leaded to uncontrolled engulfment and delayed phagosome maturation, respectively. Our results revealed distinct regulatory roles of Rho family GTPases, RhoA as an engulfment keeper and Rac1 as a maturation keeper during phagocytosis. We believe our findings provide insight how the phagocytes determine when and where to eat apoptotic cells as well as to digest. Furthermore, understanding of regulatory mechanism of phagocytosis could be applied to therapeutic approaches based on phagocytosis of immune cells.

Keywords: Live cell imaging, phagocytosis, smallGTPase.

TUE-325
Structural characterization of human Varp: Rab32 complex involved in melanin production

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VARP (Vps9-domain Ankyrin Repeat Protein) is a Rab32/38 effector and a Rab21 GEF, also able to bind the endosomal R-SNARE VAMP7. VARP expression, as well as its ability to bind Rab32/38 and VAMP7, is required for proper melanosome biogenesis and, more concretely, for the transport of enzymes involved in the synthesis of melanin. The transport of melanogenic enzymes to the melanosome is crucial for synthesis of this pigment and, therefore, for the proper protection of the skin against ultraviolet radiation. Interaction of Rab32/38 with VARP takes place *via* the ankyrin-repeat domain 1 (ANKR1) of the effector, which is the first ankyrin-repeat domain reported to have Rab-effector activity. Here we report the structure of human VARP-ANKR1 domain in complex with human Rab32: GTP, which mechanistically defines a new mode of Rab:Rab-effector binding. Our structural and biochemical studies have allowed us to characterize this novel Rab-effector and its interaction with Rab32/38 GTPases, which also sheds light on the process of melanosome biogenesis.

Keywords: Ankyrin repeat protein, melanosome biogenesis, Rab-effector.

TUE-326
Structural study of whirlin, a crucial PDZ containing protein involved in the mechanotransduction of auditory hair cells

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Mammals perceive sound thanks to mechanosensory hair cells. The sound-induced vibration displaces cilia that are bound together by a network of cadherins and scaffolding proteins. The stretching of the network is directly responsible for the opening of an ion channel that translates the vibration into an electric signal transmissible to the brain. Nearly all proteins involved in the cilia-associated network contain a PDZ binding motif (PBM). PBM are short C-terminal motifs recognized by PDZ domains. PDZ are the most common protein-interaction domains that maintain scaffolding complexes by binding PBM to their target proteins. Only two proteins of the cilia-associated network contain PDZ: Harmonin and Whirlin. They are central for the link between membrane proteins and the cytoskeleton. We study Whirlin, a protein composed of three PDZ domains. The N-terminal part of the protein encompasses two PDZ domains and two HHD domains (Harmonin Homology Domain). HHD is known to interact with the PDZ domain in a highly homologous protein, modulating the PDZ affinity and specificity. Using sequence alignment, we recently identified the second domain HHD downstream to Whirlin second PDZ domain, creating a symmetric organization: HHD_1-PDZ_1-PDZ_2-HHD_2. We show that PDZ_2 and HHD_2 domains are in cis-interaction, likely to modulate the binding of the PDZ domain. We cloned, expressed and purified constructions of each four domains, along with the three tandem constructions to decipher Whirlin interactome and the function of Whirlin modular organization.

Keywords: Auditory mechano-electrical transduction, PDZ domains, protein network.

TUE-327
Targeting of tail-anchored proteins to peroxisomes and mitochondria in mammalian cells

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Tail-anchored (TA) proteins are characterised by a single membrane-spanning helix at the C-terminus which anchors them to the membranes of peroxisomes, mitochondria and the endoplasmic reticulum (ER). They are a diverse group of proteins fulfilling a variety of different cellular processes for which insertion into the membrane of the correct organelle is critical. The position of the membrane-spanning domain at the C-terminus dictates that membrane insertion is post-translational and must involve the action of specific chaperones or systems to guide the TA protein to the correct organelle. The systems involved in targeting TA proteins to the ER are relatively well-understood and involve the GET-protein pathway whereas the mechanisms for mitochondria and peroxisomes are less clear. Further complexity has recently emerged with evidence that there are examples of TA proteins targeted to both peroxisomes and mitochondria in mammalian cells, such as Mff, Fis1 or GDAP1 which are involved in organelle division.

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To assess how extensive sharing of TA proteins between organelles is, we tested a number of mitochondrial TA proteins for peroxisomal association. This revealed a number of candidates which were both peroxisomal and mitochondrial. Additionally, several exclusively peroxisomal proteins were identified which, in the absence of peroxisomes, were targeted to the mitochondria. One of these, ACBD5, was analysed further to dissect the properties which contribute to organelle targeting. ACBD5 targeting to peroxisomes is dependent upon a highly charged region following the membrane-spanning helix. Successive mutations in this region, which replace positively charged residues with hydrophobic residues, led to mis-targeting, initially to the mitochondria, and finally to the ER if sufficient positive residues are replaced. As with GDAP1 targeting to peroxisomes appears to be dependent upon Pex19. In cells lacking Pex19, which do not contain peroxisomes, peroxisomal TA proteins are targeted to mitochondria suggesting a potentially overlapping binding site which is competed for by different shuttling receptors. Subtle changes in this binding site which add or remove polarity may increase affinity for the different receptors resulting in differential targeting. In summary, our findings indicate that the sharing of membrane components between peroxisomes and mitochondria is more prevalent than previously thought and that targeting of TA proteins to either organelle is achieved by subtle changes in the C-terminal tail.

Keywords: mitochondria, Peroxisomes, Tail-anchored proteins.

TUE-328
The CD2 isoform of protocadherin-15 is an essential component of the tip-link complex in mature auditory hair cells

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Protocadherin-15 (Pcdh15) is a component of the tip-links, the extracellular filaments that gate hair cell mechano-electrical transduction channels in the inner ear. There are three Pcdh15 splice isoforms (CD1, CD2, CD3), which only differ by their cytoplasmic domains; they are thought to function redundantly in mechano-electrical transduction during hair-bundle development, but whether any of these isoforms composes the tip-link in mature hair cells remains unknown. By immunolabeling and both morphological and electrophysiological analyses of postnatal hair cell-specific conditional knockout mice ($Pcdh15^{ex38-fl/ex38-fl}$ $Myo15\text{-}cre^{+/-}$) that lose only this isoform after normal hair-bundle development, we show that Pcdh15-CD2 is an essential component of tip-links in mature auditory hair cells. The finding, in the homozygous or compound heterozygous state, of a *PCDH15* frame-shift mutation (p.P1515Tfs*4) that affects only Pcdh15-CD2, in profoundly deaf children from two unrelated families, extends this conclusion to humans. These results provide key information for identification of new components of the mature auditory mechano-electrical transduction machinery. This will also serve as a basis for the development of gene therapy for deafness caused by *PCDH15* defects.

Keywords: Auditory mechano-electrical transduction, Deafness, Protocadherin-15.

TUE-330
The mechanism of aquaporin translocation

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Water is the principal constituent of cells and tissues in the animal kingdoms, and water exchange is essential for life. Water can pass through cell membranes by diffusion, but the rapid control of water flow into and out of cells in continually changing osmotic environments is mediated by a family of membrane proteins called aquaporins (AQPs), which are required to ensure appropriate membrane permeability to water molecules. At least 13 members of this family (named from AQP0 to AQP12) occur in mammals and are subdivided according to their permeability characteristics into three major functional groups: 1) water-channels, 2) aquaglyceroporins, and 3) AQPs of unknown specificity. The wide distribution of AQPs throughout the body and their involvement in many physiologies and pathologies makes them a valuable and important target for drug therapies.

Water homeostasis in the brain is crucial for maintaining the normal function of the central nervous system (CNS), which is considered to be very sensitive to any raise in intra-cranial pressure. Because of the rigid brain encasement, brain oedema could rapidly turn into a serious, life-threatening condition. It has been suggested that AQPs play a key role in maintaining brain homeostasis. At least six AQPs have been identified and characterized in the rodent brain: 1, 3, 4, 5, 8, and 9; 1, 4 and 9 are the best-studied examples.

This project aimed to identify and study the molecular tools that could manipulate the translocation of brain AQPs as promising drug targets. Plasmid DNA encoding AQP4-, AQP1-, or AQP9-GFP fusion protein-was transfected into an immortalised HEK293 cell line and secondly into a more physiologically relevant cell line of U373 MG astrocytes. The responses of these AQPs were visualised following hypotonicity/hypertonicity-mediated translocation using confocal microscopy. The transfection protocol and reagents were optimised for each AQP in the different cell lines.

Western blotting analysis and MALDI were used to determine the membrane localization of AQP4 and AQP1 in astrocytes. In addition, immunostaining was employed to recognize endogenous AQPs in the astrocytes and also to confirm the phenotype of the examined cells.

In order to investigate the effects of mannitol (a proven therapy in the treatment of brain oedema which has been investigated as a model to validate an effective assay for other different molecules) on astrocytes, this project examined the effects of three different mannitol incubation periods on the cross-sectional diameter of U373 MG astrocytes as a function of their response following hypotonic stress through an osmotic swelling assay.

Keywords: Aquaporin, Brain edema, trafficking mechansims.

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TUE-331
The role of [Cl-]i-sensitive kinases on CFTR anion selectivity

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Human pancreas secretes pancreatic juice which contains as much as 140 mM bicarbonate (HCO_3^-). Recently, we have shown that $[Cl^-]_i$-sensitive activation of WNK1-OSR1/SPAK pathway plays a critical role in pancreatic HCO_3^- secretion by increasing the bicarbonate permeability (P_{HCO3}/P_{Cl}) of CFTR. However, how $[Cl^-]_i$-sensitive kinases modulate P_{HCO3}/P_{Cl} of CFTR remains elusive. In the present study, we investigated molecular mechanisms that underlie the WNK1-OSR1/SPAK-induced regulation of CFTR anion selectivity. Overexpression and knockdown of each kinase in HEK 293 and epithelial cells revealed that WNK1 is the key molecule that governs overall effect of $[Cl^-]_i$-sensitive kinases on the CFTR bicarbonate permeability. Furthermore, experiments with truncated WNK1 indicated that N-terminal parts of WNK1 are required to regulate P_{HCO3}/P_{Cl} of CFTR. Interestingly, WNK1 affects permeability of other anions as well as bicarbonate in patch clamp recordings. Especially, the interval of relative permeabilities (P_x/P_{Cl}) between each anion was greatly narrowed by WNK1. Consequently, WNK1 increased the dielectric constant of the hypothetical selectivity filter of CFTR. These findings suggest that WNK1 increases the bicarbonate permeability of CFTR by modulating the polarizability of anion selectivity filter and provide insight into the fundamental question of how ion selectivity of anion channels can be regulated by cytosolic signaling at the molecular level.

Keywords: CFTR, WNK1, anion selectivity.

TUE-332
The role of Galectin-7 in collective cell migration

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Galectins are a family of lectins characterized by a conserved carbohydrate recognition domain and a high affinity for b-galactosides. These proteins are involved in multiple biological processes and exhibit unique expression patterns. Galectin-7 is expressed in stratified epithelia such as the epidermis. We have previously shown that galectin-7 null mice present healing retardation and defective migration properties of the keratinocytes at the wound edge. Moreover, electron microscopy analysis of epidermis from galectin-7 null mice revealed altered cell to cell junctions. In order to decipher the role of galectin-7 in cell-cell contacts during cell migration, we set up an *in vitro* model of collective cell migration using immortalized human keratinocytes (HaCaT). Removal of a Plexiglas insert generates a free edge that initiate collective cell migration and mimics gap closure during wound healing. Preliminary results show defects in cell migration in keratinocytes with reduced galectin-7 expression (HaCaT cells expressing a Galectin-7 specific shRNA) compared to control HaCaT cells. Consistently, proteomic analysis suggested that galectin-7 directly or indirectly interacts with components of epithelial cell junctions. Our model is that galectin-7 is involved in the establishment and/or the maintenance of cell-cell contacts in the epidermis and thus, the absence of Galectin-7 leads to altered cell-cell junctions that impairs collective cell migration. By relying on the proteomic results, our aim is to further characterize the role of galectin-7 at the molecular level in the keratinocytes intercellular contacts during cell migration.

Keywords: Cell migration, Galectins, Keratinocytes.

TUE-333
The search of nucleic ACID-binders and cell membrane – directed molecules as antineoplastic drugs: the use of boron clusters as reagents to measure and manipulate cell functions

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Boron-containing agents have been studied for the purpose of both boron neutron capture therapy of brain tumours and as anticancer agents not involving boron neutron capture. These efforts led to searches for synthesis of novel isostructural boron compounds with remarkable antineoplastic and cytotoxic effects. Having considered their yet unknown cellular targets, our study focuses on their nucleic acid and biomembrane lipid binding mechanisms in terms of exerting cellular therapeutic effects. Basic physicochemical issues related to preparation and use of organometallic self-assemblies formed between boron agents and cell membrane phospholipids, and nucleic acids with various conformation and size are presented. The boron agents interact with DNA as seen by shifts of T_m value from microcalorimetric measurements and UV melting curve analysis, as well as by the lack of hyperchromic shift in the UV absorption from 220 to 340 nm. This suggests that possibly the agents intercalate between the polynucleotide base pairs, as supported by Circular Dichroism spectroscopic measurements. The model is based on the mechanisms of DNA strand scission and reduced DNA viscosity. Subsequently, the resulting DNA fragmentation is deduced from agarose gel electrophoresis. Data is interpreted in terms of previous models suggesting that the major target of the boron agents appeared to be the purine synthetic pathway and nucleoside enzymes along with considerable DNA fragmentation and cytotoxic effects on nucleic acid methabolism, including inhibition of DNA topoisomerase activities. Alternatively, the surface recognition and interfacial behaviour of such complexes with lipids modeling biomembranes are studied by Langmuir-Blodgett lipid monolayers and lipid vesicles, coupled to Transmission Electron Microscopy. Size analysis and morphology of these complexes are determined by z-potential measurements and by Dynamic Light Scattering. Isothermal Titration Calorimetric measurements of boron-biomolecule structures are used to build the thermodynamic and kinetic models of their structural transitions. Such biophysical approaches are used to follow their secondary structural changes upon recognition with the organometallic compounds. This work forms the base for our further efforts directed towards studying promising metal-based nucleic acid and cell membrane pharmaceuticals.

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Keywords: boron agents, boron-lipid vesicles' associations, DNA binding.

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TUE-334

The temperature dependence of cell division is shifted between *C. elegans* N2 and the high temperature tolerant *C. briggsae* AF16

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Accurate timing of early embryogenesis is key for the development of an organism. Temperature fluctuations challenge the coordination of cell division and development in ectothermic organisms, where molecular rates depend on ambient temperature. We compared *C. elegans* N2 with the high temperature tolerant *C. briggsae* AF16 to address the following questions: How is cell division affected by changes in temperature and what sets the thermal limits of the viable range? We could show that the asymmetric first cell division of *Caenorhabditis* embryos is most sensitive to high temperature. In *C. elegans* N2, timing of the first embryonic cell division is exponentially proportional to temperature - it follows the Arrhenius law. However, it does so only within certain limits. Close to the upper temperature limit of reproduction, the cell cycle rates slow down, so that the temperature at which they reach the maximum rate of cell division coincides with the maximum temperature at which they are fertile. In *C. briggsae* AF16, the range over which the speed of cell division follows the Arrhenius equations is shifted upwards. The gain in fitness at high temperature comes at a loss of fitness at low temperatures. Interestingly, the maximum speed of cell division is the same for these two organisms. Our results indicate that timing of cell division is controlled by one universal mechanism that can respond to evolutionary pressure. We found that hatching success and fecundity of *C. briggsae* are also shifted to higher temperatures compared to *C. elegans*. Understanding the link between embryonic fitness and survival of an organism will help to recognize the adaptive potential of species to changing temperature, and thus how they cope with current and future climatic change.

Keywords: Cell dynamics, evolution, temperature.

TUE-335

Time dependent phosphorylation in eNOS linked signal transduction pathways reveals a complex feedback network in endothelial cells

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A complex network of signaling pathways regulates developmental and metabolic states in mammalian cells. Negative feedback systems are prone to oscillation, are inherently stable only for some parameter sets, and may be subject to bifurcation.

S600 in eNOS is phosphorylated by MAP kinases including ERK and p38. S600 phosphorylation inhibits NO synthesis through effects on the reductase conformational manifold that produce changes in FMN fluorescence. S1177 and S617 phosphorylation by kinases including Akt and PKA conformationally activate eNOS. Other covalent modifications also act via effects on protein-protein interactions with regulators such as calmodulin.

We examined the phosphorylation of eNOS and multiple signal transduction components of cultured endothelial cells in response to stimuli (e.g., EGF, bradykinin, insulin) as a function of time. After stimulation, rapid changes in phosphorylation of eNOS (e.g., S1177 and S600) and associated kinases (including Akt and ERK2) are observed; oscillatory behavior at several sites is obvious, establishing the presence of feedback loops that include delays (e.g., from diffusion, unobserved intermediates, complex formation/dissociation, etc.). Minimal models allowing simulation of the results with delay differential equations will be presented that reproduce the observed time courses, establishing the existence of several linked negative feedback loops incorporating specific kinases and phosphatases.

The experimentally observed oscillations can be simulated using the minimal feedback model shown below, or with models including extended cascades incorporating additional protein kinase components. Such cascades are described by analogous systems of differential equations, but at least some of the delay may be accounted for by additional intermediates.

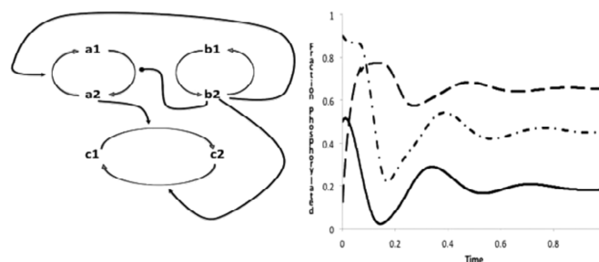

Fig. 1.

Figure: Feedback network model for oscillation of phosphorylation state in response to external signals. A represents kinase arm, B phosphatase arm, and C passive target; traces at left show a time course for phosphorylated states a2 (dash-dot), b2 (dashed), and c2 (solid) with oscillations induced by moderate delays.

Keywords: endothelial nitric oxide synthase, Kinases, Signaling pathway.

TUE-336

Towards understanding the structure-function relationship of tubulin modifying enzymes

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Microtubule filaments are dynamically assembled from tubulin subunits, and play fundamental roles in diverse cellular processes including cell division, cell migration and intracellular transport. Tubulin is posttranslationally modified by a range of different modifications, however two particularly modifications are polyglutamylation and polyglycylation that generate glutamate or glycine side chains on tubulin. Specific patterns of these two polymodifications were shown to be involved in the regulation of microtubule functions and behavior in vitro and in vivo, most likely by affecting the activity of microtubule-associated proteins and molecular motors. Tubulin Tyrosine Ligase-like (TTLL) proteins and Cytosolic Carboxypeptidases (CCP) belong to two families of enzymes that are implicated in the generation of polyglutamylation and polyglycylation.

Characterization of these enzymes has shown that they have a specific reaction and substrate preference, allowing them to generate differentially modified tubulin variants.

The aim of our study is to elucidate the molecular mechanisms by which TTLLs and CCPs assure the specificity of the modifications they catalyze. For that purpose, we are using recombinant protein technologies, biophysical methods and X-ray crystallography to analyze these enzymes alone or in complex with their substrates. In addition, we are developing biochemical assays for the

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quantitative measurement of the enzymatic and binding properties of TTLLs and CCPs towards both tubulin and microtubules. Together, this work will lead to a detailed description of mechanisms underlying these two key families of tubulin modifying enzymes. As alterations in the activity of TTLLs and CCPs have been suggested to be linked to human pathologies, including neurodegenerative diseases and cancer, our work should also be valuable to the development of TTLL and CCP inhibitors capable to block enzyme activities in the purpose to develop therapeutic interventions.

Keywords: Tubulin posttranslational modification.

TUE-338
Transfer of mitochondria through tunneling nanotubes rescue apoptosis-stressed cells

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Tunnelling nanotubes (TNTs) are membrane tubes that form between cells in culture and tissue. They are reported to mediate intercellular communication ranging from electrical signalling to the transfer of organelles. Here, we studied the role of TNTs in the interaction between apoptotic and healthy cells. We found that PC12 cells treated with ultraviolet light (UV) were rescued when co-cultured with healthy PC12 cells. UV-treated cells formed a different type of TNT with healthy cells, which was characterised by continuous microtubule localized inside these TNTs. Observation of movement of end-binding protein 3 (EB3) and accumulation of detyrosinated tubulin in these TNTs indicate that they are highly regulated structures. In addition, these TNTs show different biophysical properties such as increased diameter allowing dye entry, prolonged lifetime and decreased membrane fluidity. Further study demonstrated that microtubule-containing TNTs were formed by stressed cells which had lost cytochrome C but did not enter into the execution phase of apoptosis characterised by caspase-3 activation. Moreover, mitochondria were found to be co-localized with microtubules and pass through TNTs from the healthy to the stressed cells. Importantly, cells lacking TNT-connections and cells with defective mitochondria were unable to rescue stressed cells in the co-culture. We conclude that the delivery of functional mitochondria via TNTs reverse stressed cells in the early stages of apoptosis. This provides new insights into the survival mechanisms of damaged cells.

Keywords: apoptosis, microtubule, tunneling nanotubes (TNTs).

TUE-339
Heavy metal induced physiological changes in oregano (*Origanum vulgare* L.)

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Evaluation of heavy metal toxicity on oregano (*Origanum vulgare* L.) was done by application of Cobalt and Copper (50, 100 and 200 mg per kg of air dried soil) at duration of three months under 16 h photo regimes. Decreasing of germination rate was recorded when oregano was exposed to higher Co and Cu concentrations, and no seedling development on 200 mg of Co per kg of dried soil was detected, while for the same treatment with Cu number of seedlings was drastically lower comparing to control. Increasing of protein content was noticeable for every treatment in comparison to control. Peroxidase activity was statistically increased when plants were exposed to higher Co or Cu concentrations. Lower concentrations of Cu had some stimulating effect on chlorophyll *b* production, while production of carotenoids was inhibited. Reduction in chlorophyll content was obtained for oregano exposure to 100 mg of Co per kg of dried soil. This study showed that higher concentrations of Co, as well as Cu, induce both, reduction in carotenoids content and noticeable increase in protein content and peroxidase activity.

Keywords: heavy metal stress, Peroxidase activity, Protein content.

TUE-340
Unraveling the molecular basis of cardiotoxicity in immunoglobulin light chain amyloidosis

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Amyloidoses are a set of heterogeneous pathological conditions that range from cerebral forms such as Alzheimer's disease, to systemic disorders such as immunoglobulin light chain (AL) amyloidosis. Their common trait is the extracellular deposition of misfolded proteins as amyloid fibrils, whose presence is associated with cell and organ dysfunction. In AL amyloidosis, which is the most common systemic form, whose prognosis is dictated by cardiac involvement, fibrils originate from monoclonal immunoglobulin light chains (LC). A growing body of experimental evidence suggests that soluble amyloidogenic LC proteins are the principal mediators of cardiotoxicity. However, the mechanisms underlying AL cardiomyopathy remain obscure and no human cell models have yet been tested in this field, although studies conducted using animal cell cultures have shed light on human LC toxicity. Human cardiac fibroblasts (hCFs) are a promising cellular model since they play a critical role in the homeostasis of the extracellular matrix and are involved in cardiac tissue remodeling in response to several pathogenic stimuli. Therefore, using a differential proteomic approach, we evaluated the cellular and molecular effects induced by amyloidogenic cardiotoxic LC in hCF. We purified soluble LC, collected from the urine of a well characterized patient with cardiac AL amyloidosis. Non-cardiac AL amyloidosis LC, namely LC from a patient affected by multiple myeloma, served as control. First, our confocal microscopy study confirmed that all the analyzed LCs were internalized and directed to the endo-lysosomal compartment. Furthermore, conventional cell viability assays demonstrated increased apoptosis and impaired viability in hCF cultured in presence of cardiotoxic LC. We next investigated protein expression profiles of hCF exposed to cardiotoxic LC and control LC versus untreated hCF using proteomic 2D-DIGE technology. The quantitative step of this analysis showed statistically relevant protein spots related to species whose expression profiles differ significantly in the three conditions. In conclusion, the identification of proteins with altered expression by mass spectrometry may shed light on the molecular basis of cardiac damage in AL amyloidosis.

Acknowledgements: This work was supported by grant GR-2010-2317596 from the Italian Ministry of Health.
Keywords: AL amyloidosis, cardiotoxic light chain, differential proteomics.

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TUE-341
Visualizing cell state transition during myogenesis using Raman microscope

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Background: We have visualized the cell state transition during C2C12 differentiation using Raman microscope. C2C12 differentiation takes multiple steps where cells first become confluent and elongate, then fuse to form multi-nucleated myotube, and mature to form sarcomere structure with contractility. This process is well documented in biochemical methods, however, how each cell change their state is poorly understood due to the difficulty in analyzing the cell state at a single cell level. Because Raman spectrum arises from molecular vibration of the constituents, Raman spectrum from the cell contains information such as molecular species, their amount and location, it is expected to be capable of reporting the cell state.

Observations: Large differences were observed in Raman spectrum from C2C12 cell nucleus at various stages of differentiation. By calculating and comparing the size of the Raman peaks, we were able to distinguish the extent of C2C12 differentiation. Furthermore, using Raman microscope in combination with principal component analysis (PCA), we have visualized the cell state transition during C2C12 differentiation. Myoblasts which were in homogeneous cell state before induction of differentiation became heterogeneous when differentiation was induced. After forming myotubes, cells state became homogeneous again, being in different cell state compared with myoblasts. These results illustrate the disappearance and reappearance of attractor in the differentiation landscape during myogenesis.

Conclusions: Raman microscope is capable of distinguishing the cell state in non-invasive manner without the need of any labeling. Because Raman microscope can investigate the cell state at a single cell level, it is especially useful in analyzing the population dynamics of the cell state. Raman spectral imaging combined with PCA can be used to distinguish the cell state, where Raman spectrum can be used as a fingerprint of the cell state.

Keywords: C2C12, muscle, Raman.

TUE-342
Zn(II)-binding diversity of human metallothioneins isoforms – insights into protein stability and cellular functions

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Human metallothioneins are encoded by 17 genes located on chromosome 16q13. They consist of 61–68 amino acid residues, with 25–30% of the cysteine content of the sequence. MTs are divided into four isoforms: MT1, MT2, MT3 and MT4, whereof the MT1 is present in seven sub-isoforms: MT1A,B,E,F,G,H,X. Differences between isoforms arise mainly from changes in the amino acid sequence, despite the sequence homology reaches about 90%. MT1 and MT2 are present in every type of tissue but MT3 and MT4 occur specifically in nervous system and squamous epithelium, respectively. Metallothioneins are involved in numerous cellular processes, they are important regulators of metal ion homeostasis and protectors against oxidative damage [1]. Their altered mRNA expression has been correlated with variety of cancers [2]. The understanding of metallothionein functions is not completed due to its high diversity. Our previous studies on MT2A showed that metallothionein differs in Zn(II) affinity (10^{-8}–10^{-12} M) and demonstrated high coordination dynamic of metal clusters [3]. So far, not all metallothionein isoforms were characterized in terms of metal ion binding and their thermodynamic stability. Here, we present the procedure of expression and purification of ten metallothionein isoforms MT1-MT4 with special attention on their apo- and holo-forms preparation. All purified apo-isoforms were characterized in terms of Zn(II) and Cd(II) binding using UV-Vis and CD spectroscopy. Zn(II)-reconstituted proteins were analyzed in details in terms of a number of metal ions bound per protein molecule using UV-Vis spectroscopy, fluorimetry and ICP-MS. We found that all metallothionein isoforms bind seven Zn(II) per molecule (Zn_7T) in a tetrathiolate coordination environment. Our studies uncovered that all metallothionein isoforms demonstrate similar to MT2A affinities for Zn(II) that vary from nanomolar to low picomolar range. This unusual properties of metallothioneins are responsible for free Zn(II) ions control under cellular conditions.

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Keywords: metallothionein, zinc homeostasis.

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CSIV-03 – Metabolism

TUE-344
1H-NMR urinalysis of healthy subjects and patients with complications of Type 2 diabetes mellitus

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In the first study, we obtained the global profiles of biochemical composition in urine samples from healthy individuals living in Romania and we evaluated the gender-related and age-related urinalysis differences by proton nuclear magnetic resonance spectroscopy method (^{1}H-NMR). The metabolic profiles were compared with ^{1}H-NMR urine profiles of Italian, Greek and Swedish healthy subjects.

In the second study, we compared the NMR urine profile between type 2 diabetes mellitus patients (T2DM) with hepatic steatosis and hepatic chirrosis and we investigated the potential relationship between diabetic retinopathy (DR), diabetic neuropathy (DN) and urinary T2DM metabolites concentrations trying to obtain a reliable expression of the progression of diabetic complications.

Serial urine samples of 159 healthy subjects and 173 T2DM patients with a history of type 2 diabetes mellitus less than 5 years were investigated by ^{1}H-NMR. The ^{1}H-NMR spectra have been recorded on a Bruker Avance DRX 400 MHz spectrometer. To 0.9 ml urine, 0.1 ml of a stock solution of 5 mM sodium 3-(trimethylsilyl)-[2, 2, 3, 3-d_4]-1-propionate (TSP) in D_2O has been added. The results are evaluated in mmol/mol of creatinine. $p < 0.05$ was taken as significant.

There are gender-related differences in the excretion of citrate, lactate, 3-hydroxyisovaleric acid, alanine, TMAO, glycine and hippurate between males and females in healthy Romanians. The healthy subjects above 35 years old tended to have higher urinary concentrations of TMAO, dimethylamine and 3-hydroxyisovaleric acid compared to subjects below 35 years old. There are significantly decreased differences in the excretion of citrate, hippurate, glycine in Romanian healthy subjects vs. Italian normal group. Romanian volunteers men excreted lower concentrations of TMAO than women, whereas the excretion of this metabolite in Greek men was higher.

A significant difference between the excretion of 3-hydroxyisovaleric acid, lactic acid and dimethylamine in the T2DM patients with hepatic steatosis and T2DM patients with chirrosis was found. The values for citrate, 3-hydroxyisolateric acid and gamma-aminobuthyrate increase in T2DM patients with retinopathy vs. without retinopathy. There was no correlation between DN and urinary metabolite picture in T2DM patients.

^{1}H-NMR spectroscopy could explore the metabolites and their concentrations in healthy subjects and T2DM patients for early detection of associated diseases and complications in diabetes.

Keywords: diabetes mellitus, Healthy Subjects, Proton Nuclear Magnetic Resonance Spectroscopy Method.

TUE-345
The effects of ceranib-2 on cancer and non-cancer cell morphology

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Inhibitors of acid lysosomal ceramidase can induce apoptosis causing an increase in ceramide in the cell. Acid ceramidase inhibitors found to be effective on radiotherapy and chemotherapy causing cell death on *in vivo* and *in vitro* cancer models. A number of acid sphingomyelinase inhibitors are developed. Ceranib-1 and ceranib-2 are developed as inhibitors of human ceramidase activity. In our study we aimed to investigate the effects of ceranib-2 on 5RP7 cancer cells and 3T3 non cancer cells morphology via confocal microscopy. For this manner, 3T3 and 5RP7 cells threated with ceranib-2 (IC_{50} value for each cell line) were incubated for 24 hours.At the end of incubation period, growth medium was removed and cells were washed with PBS, fixed washed again with PBS and finally double-stained with acridine orange and Alexa fluor-488 phalloidine. Stained cells were imaged on confocal microscopy. On our results condenced nuclei, frangmented DNA and cytoskeleton and cell membrane disorders are showed. In conclusion, ceranib-2 was found to be effective in changing cell morphology in low concentrations and cause apopotosis in cancer cells. This may be an inspiration for developing new anti-cancer drugs.

Keywords: Cancer treatment, Ceramidase, Confocal Microscopy.

TUE-346
A new type of nonribosomal peptide synthase is involved in the biosynthesis of marine toxins

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Polyketide synthases (PKSs) and nonribosomal peptide synthases (NRPSs) are multienzyme complexes of bacterial and fungal secondary metabolism. They comprise various enzymatic activities and are often organised in so-called modules, each of which inserts one building block. The minimal NRPS module consists of an adenylation domain, which selects and activates the substrate, a condensation domain, which catalyses the formation of the new peptide bond, and a thiolation domain for binding intermediates. In the last decade, PKS and NRPS genes have been identified in various protists, including some dinoflagellate species. A strain of the dinoflagellate *Ostreopsis ovata* that was sampled in Okinawa, Japan, produces several ovatoxin variants. Ovatoxin belongs to the same structural family as the extremely poisonous compound palytoxin. Sequencing of the *O. ovata* transcriptome by means of RNA-Seq revealed a wide range of genes encoding PKSs and NRPSs. Most interestingly, *O. ovata* possesses a new type of NRPS, which is characterised by an unusual monofunctional architecture. These enzymes form a dinoflagellate-specific clade in phylogenetic reconstructions. Expression of those genes on the RNA level was confirmed by RT-PCR, and the sequences of the full-length transcripts could be obtained. Heterologous expression of identified adenylation domains in *Escherichia coli* followed by biochemical determination of sub-

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strate specificities showed that the new type of NRPS found in *O. ovata* is probably involved in the biosynthesis of ovatoxin and related compounds.

Disclosure of Interest: None Declared.
Keywords: Dinoflagellate, Marine toxin, Nonribosomal peptide synthase.

TUE-347
A pollutant mixture strengthens the metabolic shift in colon cancer cells

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During tumorigenesis, most cancer cells exhibit an altered metabolism that is characterized by an elevated uptake of glucose and an increased glycolytic rate (Warburg effect).This alteration of cellular metabolism is critical during cancer development and constitutes a major feature of aggressive tumors conferring selective advantage to cancer cells. Considering the recent observations on the impact of persistent organic pollutants (POPs) on cell metabolism, we hypothesized that POPs could exert their carcinogenic effects by promoting metabolic alterations that could converge to a metabolic shift favoring a tumoral phenotype.

Colon cancer cells were treated with dioxin and/or α-endosulfan, two environmental pollutants mainly produced by human activities and designated by the International Agency for Research on Cancer as probable or well-established carcinogens for humans. A significant decrease of glucose oxidation associated with an increase in lactate production was observed after a 48 hr-treatment with the two pollutants whereas each pollutant alone had no significant effect. The pollutant mixture induced a drop in respiration suggesting a deregulation of the mitochondrial respiratory chain. These changes were associated with a significant modulation of cell impedance, suggesting a modification of cell proliferation or an epithelial- mesenchymal transition. We have shown that calcium, a key regulator of mitochondrial metabolism, is a mediator of these changes since EGTA, a calcium chelator, completely abolished the POP-induced decrease in glucose oxidation. Moreover, the reduction of oxidative capacity by the POPs was totally abolished after the blockade of mitochondrial calcium uniport by ruthenium red.

Taken together, these results suggest that the POPs mixture strengthens the Warburg metabolic phenotype of colon cancer cells and deregulates mitochondria homeostasis. Further studies are in progress to elucidate the signalling pathways involved in these effects.

Keywords: Pollutant, metabolism, cancer.

TUE-348
A role for peroxisomes in prostate cancer development

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Peroxisomes are highly versatile subcellular organelles whose composition, morphology and abundance are tightly regulated upon external stimuli in order to maintain cellular homeostasis. Defects on their morphology and dynamics lead to significant implications in health and disease. However, information on the role of peroxisomes in cancer is still limited. Prostate cancer (PCa) is the second most common malignancy in men, involving challenging diagnostics with at times insufficient sensitivity and specificity. Several proteins, among which monocarboxylate transporter 2 (MCT2), have been identified as PCa biomarkers. Although MCTs mainly localize at the plasma membrane, the localization MCT2 at peroxisomes has initially been shown in non-tumor liver fraction.

The main aim of this study was the evaluation of MCT2 localization at subcellular level in several prostate cell lines PNT1A (non-tumor), 22Rv1 (localized tumor) and PC3 (bone metastasis). Our results show that MCT2 localizes at peroxisomes in all the tested PCa tumor cell lines, whether in non-tumor prostate cells its localization is mainly cytoplasmic. We have also observed that MCT2 travels to peroxisomes through an interaction with Pex19, the protein that is responsible for the transport of peroxisomal membrane proteins to this organelle.

Our results suggest a possible role for peroxisomes in PCa that may further be exploited for the study of this organelle's dynamics as target for cancer therapy.

Keywords: MCT2, Peroxisomes, Prostate cancer.

TUE-349
A study of embryonic type of copper metabolism in rats and its switch to adult type

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Copper in mammals is essential as a catalytic and structural cofactor for copper-requiring enzymes and it also acts as a second messenger. But free copper ions can initiate Fenton type reactions and catalyze formation of free radicals. So, in cuproenzymes copper is tightly bound in active centers and the safe cell traffic of copper is provided by dedicated cellular metabolic system. Copper level in circulation is controlled by liver – the central organ that distributes copper throughout the body. Absorbed copper enters hepatic cells. A portion of this copper is incorporated into cuproenzymes, such as ceruloplasmin (Cp), and secreted to bloodstream. Nowadays there appears an increasing amount of data indicating that copper secretion from liver is stimulated by organs requiring copper. Moreover, sparse data show that liver copper secretion is performed under the control of adrenal glands (AG). Nevertheless, current knowledge on the copper metabolism in AG is almost absent so far. The current study is focused on the ontogenetic changes of copper metabolism in liver and AG.

Rats with embryonic type of copper metabolism (ETCM, before 13th day postnatal) and adult type of copper metabolism (ATCM) were taken for this study. We used AAS to measure copper concentration, RT-PCR analysis to assess gene expression level and Western blot to evaluate content of immunoreactive polypeptides. Our results provide several significant insights into the turnover of copper and the profiles of expression of genes related to copper metabolism. We have shown the primary accumulation of copper in nuclei of hepatocytes in early ontogenesis and then relocation of copper to mitochondria. The second peculiarity of ECTM consists in the fact that copper import into hepatocytes of newborns does not involve CTR1 protein, but, supposedly, CTR2 plays an important role. Expression of CTR1 gene increases rapidly in liver after the ETCM to ATCM switch. We showed that SOD1-mRNA content and translation level do

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not change significantly in liver, but SOD1 activity rises in adult rats. The system of copper metabolism was studied in AG for the first time. We revealed that from two Cu(I)-ATPases (ATP7A and ATP7B) only ATP7A is expressed in AG cells during both ETCM and ATCM. The expression level differs for two genes encoding cuproenzymes. The PAM-mRNA content falls, but the concentration of mRNA of GPI-Cp rises. Besides, Cp gene is active in AG of newborn rats and its activity increases, but only splice isoform encoding GPI-Cp is synthesized.

Our results allows us to suppose the existence of inter-organ interaction between copper metabolic systems of liver and AG.

Keywords: adrenal glands, copper, liver.

TUE-350

Altered vasoactive peptide composition in the tissues of Cathepsin A deficient mice

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Cathepsin A (lysosomal carboxypeptidase A), belonging to serine proteases family, has deamidase/esterase as well as carboxypeptidase activity. Besides its protease activity, it has a protective role in lysosomes. It forms a multienzyme complex with sialidase Neu1 and b-galactosidase enzyme in lysosome to protect them against proteolytic degradation. Since it is secreted to the blood plasma by platelets and lymphocytes, it has been suggested that Cathepsin A play an extra-lysosomal regulatory role for a variety of bioactive peptide hormones by hydrolyzing them. However, the physiological function of Cathepsin A outside the lysosome is not explored fully. Previously generated knock-in mouse model with inactive Cathepsin A had a significantly induced level of the vasoactive peptide Endothelin-I resulting increased arterial blood pressure (Seyrantepe *et al.*, 2008). The aim of our study is to analyze serum and brain of mouse model with Cathepsin A enzyme deficiency and understand whether Cathepsin A has hydrolyzing function on Endothelin-I, Angiotensin-I, Oxytocin, Substance P and Bradykinin in physiological states by immunohistochemistry and ELISA. In immunohistochemistry we showed that there is significantly increased level of Endothelin-I in deficient mice brain. We also confirmed the increase in level of the Endothelin-I in serum of deficient mice by ELISA but not in brain. In addition, increased concentration of Angiotensin-I in serum was determined in Cathepsin A deficient mice but not in brain. It has been shown in those mice 4 fold increased oxytocin in serum and 14 fold increased in brain indicating that Cathepsin A play important role on oxytocin cleavage. However, this difference has not been shown in immunohistochemistry analysis of brain tissues. Although the level of Substance P in serum is not increased significantly, 5 fold higher level of accumulated Substance P was detected in the brain of Cathepsin A deficient mice. Although the level of Bradykinin did not appear significant changed in the Cathepsin A deficient mice brain, there was 2 fold increase in serum. Our study suggests that Cathepsin A enzyme in vivo contributes hydrolysis of varies vasoactive peptides in serum and brain.

Keywords: None.

TUE-351

An enzymatic analysis of serum esterases of pesticide toxicological concern in the Mediterranean spur-thighed tortoise, Testudo graeca, from the Cappadocia Region (Turkey)

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Free-ranging vertebrates inhabiting agricultural areas may be at risk from pesticide exposure. Nevertheless, there is a lack of field studies concerning pesticide toxicity on terrestrial tortoises. As the preliminary part of a broader project aimed to investigate blood biomarkers for monitoring pesticide exposure on the Mediterranean spur–thighed tortoise (*Testudo graeca*), serum esterases were enzymatically characterized and basal levels were determined in individuals sampled from an agricultural and a reference (uncontaminated) area. Blood samples were collected from adult tortoises caught from rangelands (n = 54 individuals) and agricultural (n = 34) sites in Cappadocia Region (Turkey) during their active period (April to October).

Cholinesterase (ChE, EC 3.1.1.7/8) and carboxylesterase (CbE, EC 3.1.1.1) activities were examined in vitro using multiple substrates, specific inhibitors and pesticides. Serum ChE activity cleaved the substrate acetyltiocholine at a higher rate (V_{max} = 1.70 ± 0.14 U/ml serum, mean ± SE) than butyryltiocholine (V_{max} = 0.32 ± 0.03 U/ml serum), but the hydrolysis of both substrates decreased dose-dependently with tetraisopropyl pyrophosphoramide (a specific inhibitor of butyrylcholinesterase activity). Carboxylesterase activity was significantly higher towards naphthyl- and nitrophenyl acetate (V_{max} = 10.5–15.8 U/ml serum) than when naphthyl- and nitrophenyl butyrate were used as substrates (V_{max} = 4.3–5.2 U/ml serum). In vitro inhibition of ChE activity by chlorpyrifos-oxon or carbaryl resulted in IC50 (i.e., median inhibitor concentrations) values in the range of 10^{-7} M, whereas the sensitivity of CbE activities to these pesticides was higher (IC50s = 10^{-8}–10^{-9} M). The occurrence of multiple esterase isozymes in the tortoise serum was corroborated by native polyacrylamide electrophoresis; a proteomic method that enabled to identify sensitive esterases to pesticides in an isozyme-specific basis.

No significant differences were found in the serum esterase activities between both field populations suggesting no pesticide exposure, at least, during the sampling campaigns. However, there was a significant seasonal variation for all esterase activities with a maximum enzyme activity in September-October.

These preliminary results indicated: (1) the occurrence of a ChE activity with hydrolytic properties close to mammalian-type butyrylcholinesterase activity, (2) a hihger sensibility of CbE activity to pesticide inhibition than ChE activity, and (3) a marked seasonal pattern of serum esterase activities in natural populations of tortoise.

Keywords: Mediterranean tortoise, Pesticides, Serum esterases

TUE-352

Analysis of cytochrome *c* modifications induced by reactive aldehydes

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Several lines of evidence associate pathological states and diseases, such as cancer, atherosclerosis, Alzheimer's, amyotrophic lateral

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sclerosis (ALS), and prion diseases to oxidative stress. Nucleophilic attacks to phospholipids by reactive oxygen species could lead to the breakdown of these molecules, producing reactive aldehydes. Unsaturated aldehydes have been shown to promote protein and DNA damage, to induce mutagenesis, and to be involved in cell signaling cascades. Proteins are major targets of reactive aldehydes by means of reactions, mainly with nucleophilic amino acids. A great deal of attention has focused on modifications promoted in cytochrome *c* by reactive aldehydes. This protein has a high content of lysine residues that make it a target to covalent modifications. It is important to note that mitochondria are major producers of reactive oxygen species, which makes their proteins targets of oxidative injuries. Here, the modification of cytochrome *c* promoted by 4-hydroxy-2-nonenal (HNE) and 4-hydroxy-2-hexenal (HHE) was investigated. Matrix-assisted laser desorption/ionization time offlight experiments indicated increases in the molecular weight of cytochrome *c* (2.5 mM), consistent with the formation of two Michael additions by HNE (2 mM) and six Michael additions by HHE (2 mM). *Nano LC-ESI-TOF*-MS analysis of tryptic digests indicated that His-33 was modified by HNE. Lys-13, Lys-22, Lys 53, Lys-79, Lys-87 and His-33 were modified by HHE. Today, it is generally accepted that cytochrome *c* release from mitochondria into cytosol is a determining event in apoptosis signaling through the activation of the caspase family proteins. The covalent modifications in cytochrome *c* could play a role in mitochondrial dysfunction associated with oxidative stress. Supported by FAPESP: 2011/10048-5, CAPES, INCT Redoxoma: 573530/2008-4, NAP Redoxoma: 2011.1.9352.1.8, CEPID Redoxoma: 2013/07937-8.
Keywords : Cytochrome C, Reactive Aldehydes

TUE-353
Analysis of thiopurine *S*-methyltransferase genotypes in children with acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer. The thiopurines, 6-mercaptopurine (6MP) and thioguanine (TG), are the backbone of current therapy for childhood ALL. Since their introduction to leukemia treatment in the 1950s, they have played an essential role in treatment protocols for ALL. Thiopurine S-methyltransferase (TPMT) polymorphism represents a determinant of 6-MP response and ALL outcome and is well characterized in most populations. Four common polymorphic alleles are associated with impaired activity of the enzyme. These are TPMT*2 (238G>C), TPMT*3A (460G>A, 719A>G), TPMT*3B (460G>A) and TPMT*3C (719A>G).

Objective: The aim of the present study was to determine the frequency of the functional TPMT polymorphisms and their association with the occurrence of adverse events, in pediatric patients with standard risk ALL who are subjected to 6-Mercatopurine therapy for consolidation.

Patients and methods: TPMT polymorphism was analyzed in 40 children diagnosed with acute lymphoblastic leukemia and 40 age and sex matched healthy controls. The frequency of TPMT genotypes was examined by PXG-TPMT StripAssay based on Polymerase Chain Reaction (PCR) and reverse hybridization using blood samples. Clinical follow up using complete blood picture and liver transaminases following 6-MP therapy for consolidation were then performed for patients in order to access drug toxicity.

Results: In the study sample, none had homozygous mutant TPMT genotypes (e.g. TPMT*3A/*3A, TPMT*2/*2, TPMT*3A/3C, etc.). Also neither the cases nor the controls in the study sample had TPMT*1/*2 and TPMT*1/*3B genotypes. In patients group, 39 (97.5%) were of the wild-type homozygous TPMT*1/*1 genotype, 1 (2.5%) patient only was of the heterozygous TPMT*1/*3A genotype and no patient had TPMT*1/*3C genotype. In the control group, we identified 36 subjects (90%) with wild-type homozygous TPMT*1/*1 genotype, 3 (7.5%) with heterozygous TPMT*1/*3A genotype and 1 (2.5%) heterozygous TPMT*1/*3C genotype. TPMT*3A was the most prevalent variant allele followed by TPMT*3C detected in the studied sample with an allelic frequency of 2.5% and 0.6%, respectively. The only patient with variant TPMT*1/*3A genotype did not show any evidence of thiopurine intolerance (hematotoxicity and hepatotoxicity).

Conclusions: Cases of myelosuppression in ALL pediatric patients treated with 6-MP cannot be all explained by the existence of TPMT alleles (*2, *3A, *3B and *3C). Other polymorphic alleles in TPMT gene, or factors other than TPMT polymorphisms may be responsible for the development of toxicity.

Keywords: pediatric ALL, thiopurine toxicity, TPMT polymorphism

TUE-354
Antioxidant activities of purine and pyrimidine bases

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Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. There are two basic categories of antioxidants, namely, synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of variousdegrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds (tocopherols, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivates, amino acids, and amines), or carotenoids as well as ascorbic acid. The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Free radical are responsible for causing large number of diseases including cancer, cardiovascular disease, ulcerative colitis, aging and atherosclerosis. Protection against free radicals can be enhanced by ample intake of dietary antioxidants. Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In the present study, the antioxidant properties of purin and pyrimidin bases were investigated by using different antioxidant assays such as 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH•) scavenging, N,N-dimethyl-p-phenylenediamine (DMPD•+) scavenging, and reducing power. Trolox and ascorbic acid were used as the reference antioxidant compounds. Antioxidant activity was increased with increasing bases concentration. All purine and pyrimidine bases showed a potential antioxidant activity. According to these results purine and pyrimidine bases are important compounds in pharmaceutical industries due to their antioxidant activity.

Keywords: Purine, primidine, antioxidant activity

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TUE-355
Antioxidant activitis of *Salvia fruticosa* and its inhibitory effects on glutathione-*S*-transferase

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In recent years, increased consumption of fruits and vegetables has been consistently recommended since many epidemiological studies have showed the relation between consumption of polyphenol-rich foods or beverages and the prevention of certain diseases such as cancers, cardiovascular diseases and aging. Phenolic compounds are abundant in all plant organs therefore they form an integral part of the human diet. Salvia species, commonly known as sage, have been used since ancient times for more than 60 different ailments ranging from aches to epilepsy. There are around 900 species of Salvia, 95 of which are represented in Turkey. Rosmarinic acid, reported to be a powerful antioxidant, is the main phenolic component in Salvia species. Other phenolics and flavonoids in Salvia species include catechin, caffeic acid, vanillic acid, ferulic acid, rutin, apigenin, quercetin, and luteolin. Glutathione S-transferases (GSTs) are multifunctional detoxification enzymes that protect the cell from the damage of electrophilic compounds. Overexpression of GSTs in cancer results in resistance to chemotherapeutic agents and inhibition of the over expressed GSTs has been suggested as an approach to combat GST-induced resistance.

In this study, rosmarinic acid content of water extract of *Salvia fruticosa* was determined by using RP- HPLC. DPPH● and ABTS● radicals scavenging activities, total phenolic and flavanoid contents as well as bovine liver cytosolic Glutathione S-transferases (GSTs) inhibitory potentials of the extract were investigated. Total phenolic content and the total flavanoid content of the plant extract were determined as 425, 1 mg GAE/g extract and 94 mg CE/g extract, respectively. IC_{50} value for DPPH radical scavenging percent inhibition was evaluated as 0.0348 mg/ml and TEAC value of the extract was evaluated as 1297 mmol TE/g extract. Rosmarinic acid content of the extract was determined as 59.3 mg/g extract. IC_{50} value for GST inhibiton activity was determined as 6.8 μg/ml.

Turkish endemic sage *Salvia fruticosa* is reported to be a potantial GST inhibitor and as a promising medicinal plant, it has the potential to be used as adjuvant with chemoterapeutic agents to overcome the drug resistance occuring during chemotherapy. Further investigations are ungoing to reveal its bioactive components and their beneficial activities in biological systems.

Keywords: GST, Antioxidants, Salvia

TUE-356
Antioxidant and anticholinesterase activity of extracted from wild beet (*Beta maritima* L. var. pilosa Del.)

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Chenopodiaceae family contains about 103 genera and 1400 species worldwide. In Turkey there are about 129 species from 33 genera. The members of the family are annual or perennial herbaceous plants that are spreaded out in many places all over the world but especially on dry regions, gravel soil, sea coasts, sides of the roads, sides and insides of fields and soils that are rich in nitrogen and potassium nitrate.

Beta genus belonging to Chenopodiaceae family, has 6 species in Turkey: *Beta trigyna*, *Beta lomatogona*, *Beta macrorhiza*, *Beta maritima* and *Beta trojana*. In this Ph. D. thesis *Beta maritima* L. var. *pilosa* Del., will be investigated. The spread zone of this variety in Turkey is the coastal and inner parts of Western and Northern Anatolia.

In this study, after the aerial parts and roots of "*Beta maritima* L. var. *pilosa* Del." were dried and ground. After exracting with petroleum ether, dichloromethane and ethanol the total phenolic and flavonoid contents and total phenolic and total flavonoid contents of the extracts from the aerial parts of Wild Beet (*Beta maritima* L. var. pilosa Del.) were determined as pyrocatechol and quercetin equivalents, respectively. The antioxidant activity was carried out by using DPPH free radical scavenging activity methods, Cuprac method and the anticholinesterase activity by the Ellman assay. In this study, anticholinesterase and antioxidant activity of the *Beta maritima* L. var. *pilosa* Del. will be carried out for the first time.

Keywords: anticholinesterase activity, antioxidant activity, *Beta maritima* L. var. pilosa Del.

TUE-357
Antioxidant efficiency of seabuckthorn extract in ehrlich ascites carcinoma of Balb/c mice Model

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Background: Seabuckthorn (SBT; *Hippophae rhamnoides* L.), a unique and valuable plant has recently gained worldwide attention, mainly for its medicinal and nutritional potential. The aim of the present study was to evaluate the antioxidant activity of SBT against Ehrlich Ascites Carsinoma (EAC) cells by measuring primer antioxidant enzyme superoxide dismutase (SOD) and lipid peroxidation products malondialdehyde (MDA).
Methods: Mice were divided into three groups. First group was control. Second group was injected EAC interperitoneally. Third group was received both EAC and SBT extract daily 10 U for 3 weeks. All groups received diet and water *ad-libitum*. Both control and experimental groups SOD activities, MDA levels and EAC cells protein contents were determined by the methods of Sun, Buege JA and Lowry, respectively.
Results: Both in ascetic fluid and EAC cells, EAC group MDA levels (93.27 ± 5.63, 220.31 ± 13.91 nmol/mg protein, respectively) were significantly high when compared with EAC treated with SBT group (26.61 ± 4.18, 121.59 ± 5.24 nmol/mg protein, respectively) ($p < 0.001$). Although, SOD activity was found decreased in ascetic fluid ($p < 0.001$), same enzyme activity was found increased in EAC cells ($p < 0.001$). When plasma EAC group compared with control, high levels of EAC plasma MDA levels were found ($p = 0.001$). But, SBT application on EAC showed that plasma oxidative stress were found decreased by MDA levels ($p < 0.001$).
Conclusions: According to these results, increased SOD activity by SBT implementation in EAC cells shows that radical metabolism is still active in EAC cells. SBT has antioxidant effect in ascetic fluid when significantly decreased SOD activity is considered. It is the case that plasma MDA levels significantly deplete and also SBT indicates antioxidant effect to whole body by circulation.

Keywords: Ehrlich ascites carcinoma, Radical metabolism, Seabuckthorn

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TUE-358
Antioxidant response to titanium dioxide nanoparticles by *Saccharomyces cerevisiae* grown in different carbon sources and heat-shock conditions

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The physicochemical properties that make nanomaterials unique, also equip them with potential for affect environment adversely, causing oxidative injuries in the living beings. However, organisms also had to develop antioxidant defences to protect their cells from reactive oxygen species (ROS). Failure in the cell antioxidant defences, due to the contact with xenobiotic, results in stress causing oxidatives damages leading to loss of cell viability. Yeasts can contribute to understand the toxicity of titanium dioxide nanoparticles (TiO2-NP), because its cell structure and functional organization, share similarities with mammalians. Since the response of yeast to NPs can be influenced by temperature and available carbon source, the aim of this study was to evaluate the antioxidant response of *Saccharomyces cerevisiae*, grown in presence of glycerol with addition of 2% glucose and 5 µg/mlTiO_2-NP, in heat-shock conditions. TiO_2-NP (size <100 nm) stock suspensions were prepared by sonication. Bioassays were performed in YEPG medium (1% yeast extract, 2% peptone, 3% glycerol). Culture flasks were inoculated with wild-type *Saccharomyces cerevisiae* UE-ME_3 and shaken 150 rpm, at 28°C. At exponential phase was added glucose and TiO_2-NP stock solution (YEPGD-NP) to obtain a final concentration of 2% and 5 µg/ml. Yeasts grown 200 min at 28 or 40°C (heat-shock, HS). Flasks lacking glucose (YEPG) or NPs served as controls. Biomass was quantified by dry weight. Post-12000 *g* supernatants were used for determination of GSH, GSSG and ROS contents by fluorescence as well as glutathione reductase (GR), glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G6PD), catalase (CTT1) activity by spectrophotometry. Post-12000 *g* pellets were used for determination of catalase (CTA1) activity. Statistical analysis by ANOVA I and Duncan test. The results showed that biomass, ROS level and GR activity in the cells grown in YEPGD were higher than those detected in cells grown in YEPG. Furthermore, cells grown in YEPGD exhibited lower levels of GSH and MDA and CTT1activity comparatively with yeasts grown in YEPG. *S. cerevisiae* grown in YEPGD-NP in HS showed growth inhibition to levels near of cells which used glycerol as carbon source. Additionally, it was also detected a decrease in the GSH contents, GSH/GSSG ratio, GPx, CTT1 and CTA1 activities as well as an increase in ROS content and GR activity, relatively to the cells growing only in glycerol. It was also observed an increase in ROS level and GR activity in the yeast grown in YEPGD-NP, relatively to *S. cerevisiae* grown in YEPGD. TiO_2-NP in HS caused oxidative stress in yeast grown in presence of glycerol and glucose, decreasing GSH/GSSG ratio, increasing ROS content and GR activity.

Keywords: metal nanoparticles, stress oxidative, yeast

TUE-359
Assessment of urinary epidermal growth factor level in patients with chronic renal failure

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Purpose: Epidermal growth factor (EGF) is a peptide expressed in various tissues. It has mitogenic effect on epithelial and mesothelial cells. EGF can be measured in all body fluids. It has been suggested by previous studies that a large part of the urinary EGF is originated from kidney, a small amount of EGF is derived from other organs. If urinary EGF has renal origin, EGF concentration in the urine may reflect the number of functional nephrons and it may be a good marker for assessment of renal function. In the present study, determination of the urinary EGF concentration was purposed in diabetic and non-diabetic patients with chronic renal failure.
Method: Urinary EGF levels were measured in 24-hour urine samples obtained from patients with chronic renal failure (n = 57) and age matched controls (n = 20). EGF measurements were performed with commercial ELISA kit and EGF concentration is calculated as ng/mg creatinine. Statistical analysis was performed with SPSS 10 software package.
Results: In the control group, urinary EGF level in women was higher than those in men. Urinary EGF level was found to be lower in the total patient group than those in the control group when gender differences were not taken into account (p = 0.010). Urinary EGF level was lower in the women of the total patient group as compared to women of the control group (p = 0.025), but there was no significant difference for men. No significant difference was found for urinary EGF concentration between diabetic and non-diabetic chronic renal failure groups with regard to gender. While urinary EGF level was lower in the non-diabetic women with chronic renal failure compared to women of the control group (p = 0.025), there was no significant difference between diabetic women with chronic renal failure and women of the control group.
Conclusion : It was concluded that the urinary EGF excretion may be a reliable marker for assessment of renal function in non-diabetic women with chronic renal failure.
Keywords: Diabetic nephropathy, Renal function, Urinary epidermal growth factor

TUE-360
Association between blood Pb and Fe levels in Turkish metallurgy workers and a polymorphism of DMT1 gene

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Lead is a heavy metal that used for years and still used for various industrial purposes. Lead exposure can cause many biological effects depending upon the level and duration. In adults, lead toxicity is most commonly caused by occupation in workplace. Especially after oral exposure, toxic metals are derived from gastrointestinal tract. In the duodenum Divalent Metal Transporter-1 (DMT-1) protein plays a crucial role for dietary Fe uptake but also recognizes nonessential metals such as Pb. Aim of this study

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was to determine whether DMT1 IVS4 + 44C/A single nucleotide polymorphism (SNP) has an effect on Pb, Fe and Erythrocyte protoporphyrin (EP) levels in blood and Aminolevulinic Acid (ALA) and total porfirin levels in urine of Turkish metallurgy workers. For this purpose blood samples were obtained from 82 male and studied by standard PCR-RFLP technique. Pb and Fe were measured Atomic Absorption Spectrometry. EP was measured with molecular fluorimeter, and ALA and Total Porphyrin were measured with spectrofotometer. Genotype frequencies of IVS4 + 44 C/A polymorphism were determined as; 42.68% homozygote typical, 45.12% heterozygote and 12.20% homozygote atypical. Consequently, Pb and Fe levels were measured as; 372.37 ± 145, 20 ppb; 463.91 ± 83.45 ppm. When Pb levels were evaluated with groups, Pb levels were determined as 317.02 ± 122.39 ppb, 399.22 ± 121.85 ppb and 466.79 ± 221.34 ppb in homozygote typical (CC), heterozygote (CA) and homozygote atypical (AA) genotypes, respectively. Statistically significant association ($p < 0.005$) was found between the IVS4 + 44 C/A and Pb, but statistically no association were found between the IVS4 + 44 C/A polymorphism and Fe, EP, ALA and Total Porfirin levels ($p > 0.05$).

Keywords: Divalent Metal Transporter 1, single nucleotide polymorphisms (SNP), Turkish Metallurgy Workers

TUE-361
Association of adiponectin with acute myocardial infarction

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Backgrounds: Adiponectin is an adipose tissue-derived mediator with significant anti-atherogenic properties. A few studies were done in acute phase of myocardial infarction especially in non obese patients. We design a study to investigate the association between adiponectin concentration and acute phase of myocardial infarction in non obese patients.
Methods: This case-control study was done in Paymaneah Hospital (Jahrom, Iran) from Feb 2007 to May 2008. Plasma adiponectin levels were measured in 43 patients with acute myocardial infarction (mean age: 62.7 +/– 13.3 years, male: 67.4%) at the first 24 h of admission and 43 normal controls (mean age: 62.1 +/– 12.3 years, male: 55.8%) matched for age, sex and other coronary artery disease risk factors.
Results: Adiponectin levels in patients with acute myocardial infarction (3.36 μg/ml) were significantly lower than that of the control group (5.03 μg/ml) ($p < 0.0001$). Lower adiponectin were independently associated with higher risk of acute myocardial infarction (odds ratio= 8.97; 95% CIs: 2.3–34.5; $p = 0.001$). Adiponectin levels negatively correlated with triglyceride (r: -0.46, $p = 0.002$) and total cholesterol ($r = -0.32$, $p = 0.03$) in the case group and with body mass index in control subjects.
Conclusion: The present study showed that adiponectin was associated with acute myocardial infarction in non obese patients but it is not related to sex, age and other coronary artery disease risk factors.

Keywords: Adiponectin, infarction

TUE-363
Atrazine herbicide cause cell damages in *Saccharomyces cerevisiae*, probably due a slowdown of glutathione redox cycle

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Atrazine (ATZ) has been used extensively as an herbicide, mainly due to its relatively low cost and ease of application. Previous studies have shown that many pollutants are redox active being able to enter microorganisms, causing a univalent reduction of dioxygen with reactive oxygen species (ROS) formation. These products can severely attack cell membranes causing lipid peroxidation. Many cells have developed antioxidative defence system, consisting of ROS-scavenging enzymes, e.g. glutathione peroxidase (GPx) or catalases (CTT1, CTA1), and antioxidants, e.g. glutathione (GSH). Catalases and GPx can catalyze H_2O_2 reduction to H_2O and GPx can also scavenge lipid hydroperoxides, converting them in correspondent alcohols. ROS can be produced in cells not only as by-products of normal cellular metabolism but also under stress situations as contact with xenobiotics. So far, the oxidative stress responses to several pollutants as atrazine have been examined in bacteria plants and animals, but few studies have shown the response of antioxidant enzymes in *S. cerevisiae* to herbicides stress. So, the purpose of the present work was to evaluate the antioxidant response by yeast to atrazine exposure. *Saccharomyces cerevisiae* UE-ME_3 a wild-type yeast deposited in the collection of laboratory of Enology, University of Évora, at mid-exponential phase were inoculated in YEPD medium, 2% (w/v) glucose, at 28°C, and shaken 150 rpm for 72 h in presence of 5 or 50 μM ATZ and compared with control (YEPD). Yeasts were harvested by centrifugation at 3000 *g* for 10 min and washed with ultra-pure sterile water. The obtained cells were suspended in 10 mM phosphate buffer pH 7.0, and disrupted by sonication. The post-12 000 *g* supernatants were used for ROS, malondialdheyde (MDA), glutathione (GSH) and glutathione disulfide (GSSG) determination by fluorescence as well as alkaline phosphatase (ALP), CTT1, CTA1, glutathione reductase (GR), GPx and glucose 6-phosphate dehydrogenase (G6PD) activities by molecular absorption spectrometry. The statistical analyses were performed by ANOVA I and Duncan test ($p < 0.01$), using SPSS for Windows, version 22. The results showed a decrease in biomass, GSH/GSSG ratio, GR and GPx activities in the cells grown in presence of 5 or 50 μM atrazine. Additionally, it was also detected an increase in ROS and MDA contents as well as in CTT1, G6PD and ALP activities of cells exposed to 50 μM atrazine. In conclusion, the exposure to 50 μM atrazine, a triazine herbicide, caused oxidative stress and cell damages in wild-type *S. cerevisiae* UE-ME_3, probably due a slowdown of glutathione redox cycle, despite a protection resulting from an increase of cytoplasmic activities catalase and ALP.

Keywords: malondialdheyde, Triazines, yeast

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TUE-364
Beta-blocker timolol has important beneficial action on diabetes-induced kidney tissue damage by enhancing the activities of some antioxidant enzymes

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Together with other risk factors, increasing diabetes-prevalence result in significant increases in chronic kidney disease, globally. Since hyperglycemia generates more reactive oxygen species and reduce the efficiency of antioxidative mechanisms, numerous studies demonstrated that, hyperglycemia-induced oxidative stress played a major role in extracellular matrix expansion. The eventual objective of the present study was to determine whether timolol treatment of streptozotocin-induced diabetic rats (timolol for 12-week with 5 mg/kg daily following diabetes-induction) has advantage to prevent hyperglycemia-induced renal damage by enhancing the depressed antioxidant defence in the kidney. Light microscopy data and their quantification demonstrated that timolol treatment prevented basically glomerular hypertrophy, mesangium expansion, thickening of glomerular basement membrane, fibrosis, and accumulation of glucogen in epithelial cells of tubules. Additionally, electron microscopy data demonstrated that timolol treatment also prevented hypertrophy of podocytes, disappearing of filtration gaps and slit-diaphragms, mesangium cell increase, and vacuolization in distal tubular cells. Biochemical analysis of kidney tissue, related with antioxidant defence system enzymes such as glutathione-S-transferase, glutathione reductase, and glucose-6-phosphate dehydrogenase, further supported that diabetes-induced damage in kidney is mostly dependent on increased oxidative stress and timolol, having an antioxidant-like action, can prevent kidney against hyperglycemia-induced damage though it does not alter high blood glucose levels of diabetic rats. Consequently, it can be suggested that, although β-blockers are widely used for the treatment of cardiovascular diseases, β-blocker therapy of diabetics seem to be a new therapeutic approach against hyperglycemia-induced organ damage.

Keywords: Antioxidants, Beta-blockers, Diabetes

TUE-365
Biochemical modifications induced in mouse liver post-exposure to micelle coated iron oxide nanoparticles

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Iron oxide nanoparticles have a great variety of biological and medical applications including MRI usage as contrast agents.

The aim of our study was to evaluate the biochemical effects induced by micelle coated iron oxide nanoparticles in male CD1 mice liver. The individuals were injected with the suspensions of nanoparticles in 0.7% sodium chloride in the tail vein in 3 animal groups of 10 individuals each: one control, and the other ones treated with solution of 5 mg Fe/Kg body weight, respectively 15 mg Fe/Kg body weight. The level of some biomarkers of oxidative stress such as: reduced glutathione (GSH), advanced oxidation protein products (AOPP), protein thiol groups (PTG), glutathione reductase (GR) activity as well as glutathione-S-transferase (GST) and glutathione peroxidase (GPX) ones were analyzed after one, two, three, seven and fourteen days after administration.

For both doses, the AOPP level slowly decreased towards 14 days, but it always stayed above the control, while the concentration of PTG and GSH always stayed below the control, decreasing from 24 h to 72 h and then increasing to 14 day exposure. The GR activity decreased until 72 h exposure below the control one and then settles above the control after 7 days, whereas GST activity gradually increased up to 14 days. On the other hand, the level of GPX gradually decreased in a time-depndent manner.

Taking into acount all these data it appears that, the CD1 mouse liver antioxidant defense system counteracts to a certain extent, the oxidative stress induced by the exposure to micelle coated iron oxide nanoparticles.

Keywords: Liver, Micelle coated iron oxide nanoparticles, Oxidative stress

TUE-366
Biological activities of some Lamiaceae species

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Daily dietary herbal teas are important part of the food industry. In addition, they have gained greater importance in treatment of some diseases because of their flavonoids, total phenolic contents and antioxidant capacity. In this study, some of the Lamiaceae species (*Salvia tomentosa* Miller, *Sideritis perfoliata* L. subsp. *athoa* (Papanikolaov & Kokkini), *Sideritis trojana* Bornm, *Stachys tmolea* Boiss, *Stachys cretica L. Smyrnaea* Rech fil) were obtained from Kazdagi (Ida mount). The antioxidant capacity, antimicrobial activity, total phenolic, flavonoid contents, total protein content and cytotoxic activity of these species were studied. Their antioxidant capacity, total phenolic and flavonoid contents were determined by 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH) method, Folin-Ciocalteu method and by the method of Ramful et. al (2011), respectively. Moreover, by the disc diffusion method a gram-positive and a gram-negative bacteria (*Staphylococcus aureus* ATCC-6538, *Escherichia coli* ATCC-8739) were studied to determine antimicrobial activity of plant extracts. Cytotoxic activity is determined by the method of Smitha et. al. and the total protein content is determined by the DUMAS nitrogen analyser.

As a result, among all these samples the highest antioxidant capacity, total phenolic content and total flavonoid amount were observed in *Sideritis perfoliata* (94.7%), *Sideritis trojana* (30.8 mg/g) and *Salvia tomentosa* (0.1 m/g), respectively. In addition, highest cytotoxic activity and total protein amount were seen in *Sideritis trojana*. On the other hand, *Stachys cretica* showed lowest total phenolic content, flavonoid amount and antioxidant capacity (13.57 mg/g, 0.065 mg/g, 92.79%, respectively). As a result of antimicrobial analysis the highest activity was observed in *Sideritis athoa* (1.934 mm) with *E. Coli and Sideritis trojana* (1.972 mm) with *S. aureus*.

Keywords: antioxidant activity, cytotoxicity, Lamiaceae

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TUE-367
Biosynthesis of brassinosteroids, a plant hormones, in *Scenedesmus obliquus* cultures

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Brassinosteroids (BRs) occur ubiquitously in angiosperms, gymnosperms and lower plants. The occurrence of BRs has been demonstrated in almost every part of higher plants, such as pollen, flower buds, fruits, seeds, vascular cambium, leaves, shoots and roots. In this study, BRs have been isolated in microalga *Scenedesumus obliquus (Turpin) Kützing (Chlorophyceae) (SAG Strain No.: 276-6)*. BRs, including teasterone, typhasterol, 6-deoxoteasterone, 6-deoxotyphasterol, 6-deoxocastasterone, castasterone and brassinolide, were identified by UPLC–MS/MS. All compounds belong to the BR biosynthetic pathway. The results suggest that early and late C6 oxidation pathways are operating in *Scenedesmus obliquus*. Elucidation of BR biosynthesis is fundamental to understanding how plants regulate the endogenous level of active BRs for their proper growth and development.

This project has been financed from the funds of the National Science Centre allocated on the basis of the decision number DEC-2012/05/B/NZ8/00958.
Keywords: biosynthesis, brassinosteroids, microalgae

TUE-368
Bisphosphogliceratemutase: a key player in cancer cells metabolic reprogramming

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Many kind of cancer cells exploit glycolysis rather than oxidative phosphorylation for energy production even in the presence of oxygen. This kind of metabolism, although less efficient in terms of ATP production, generates high levels of glycolytic intermediates necessary to support the high biosynthetic flux of rapidly proliferating cells.This mechanism is further enhanced in cancer cells by the expression of a particular form of pyruvate kinase (PKM2) which promote a low efficiency glycolysis (in terms or ATP production) and consequently an increase in the formation of biosynthetic metabolites. In this work we investigate the role of Bisphosphogliceratemutase (BPGM) an enzyme involved in the metabolic reprogramming of highly proliferating cancer cells. BPGM acts both as a mutase, converting the glycolytic intermediate 1,3-bisphosphoglycerate (1,3-BPG) to 2,3-bisphosphoglycerate (2,3-BPG) and as a phosphatase, converting the 1,3-bisphosphoglycerate to 3-phosphoglycerate. BPGM is an erythrocyte-specific enzyme but our real time PCR and western blotting experiments show its expression in many cancer cell lines and in proliferating primary human fibroblasts. BPGM silencing lead to a strong decrease of cell proliferation rate.BPGM activity in cancer cell lead to the skipping of the first ATP production in the glycolytic pathway of glycolysis, causing an increase of glycolytic flux necessary to sustain the high rate of intermediates production needed for support cancer cells growth.

Keywords: BPGM, Cancer metabolism, PKM2

TUE-369
Carbonic anhydrase activity of isolated coupling factor CF_1 from spinach chloroplasts

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ATPsynthase (EC 3.6.3.14) is a membrane enzymatic complex carried out synthesis and hydrolysis of ATP coupled with the transmembrane proton transfer in mitochondria, chloroplasts and bacteria. It consists of a hydrophobic portion – Fo functioning as a proton channel and hydrophilic part – coupling factor F_1 which contains several nucleotide binding sites and performs catalytic function. The catalyst portion of all ATPsynthases consists of five types of polypeptides in stoichiometric ratio $\alpha 3\beta 3\gamma\delta\varepsilon$. Unlike other F_1ATPases, ATPase activity of isolated chloroplast F_1 (CF_1) is latent and increases due to heating or the treatment with thiol compounds, trypsin, alcohols, oxyanions or detergents. Thylakoid membranes of chloroplasts were prepared from fresh spinach leaves. Soluble CF_1 was isolated from thylakoid membranes by 1 mM EDTA. The purity of the isolated CF_1 was tested by electrophoresis in native conditions, and its subunit composition was analysed by SDS electrophoresis. Latent isolated CF_1-ATPase was activated by heating at 60°C for 2 min or by trypsin treatment. A solution of trypsin in 1 mM HCI was used at a concentration of 100 μg/ml and added to 30–40 μg of purified enzyme. The action of trypsin was stopped by adding double amount of soybean trypsin inhibitor. Ca -dependent ATPase activity of the enzyme was assayed by the release of inorganic phosphate. Latent enzyme activity was 0.9 μmol $Pi \cdot min^{-1} \cdot mg^{-1}$ (protein).

Carbonic anhydrase activity was determined in PAAG using pH -dependent color reaction in the presence of a pH indicator (bromothymol blue) or in solution using an infrared gas analyzer (IFGA S151, Qubit Systems Inc. Canada). The rate of HCO_3^-dehydration was determined by an increase in CO_2 concentration in the gas phase of IFGA in response to the addition of 15 mM sodium bicarbonate.

Bromothymol blue analysis of the isolated CF_1 after separation in native gel showed that protein zones exhibiting ATPase activity accelerated also a conversion of carbon dioxide with bicarbonate and protons formation. Water-soluble carbonic anhydrase inhibitor acetazolamide inhibited also ATPase activity of isolated CF_1.

Thus, an isolated catalytic part of the ATPsynthase – multisubunit coupling factor CF_1 along with ATPase has also carbonic anhydrase activity. Ability of CF_1 to catalyze the interconversion carbonic acid forms may have functional significance for ensuring the efficient operation of ATPsynthase promoting a proton exchange associated with the ADP phosphorylation and ATP hydrolysis.

Keywords: ATPase activity, Carbonic Anhydrase, Coupling Factor

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TUE-370
Cardiovascular risk assessment in obese children treated with Omega-3 fatty acids supplements

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Childhood obesity is associated with increased risk of cardiovascular disease in young adulthood. It was demonstrated that during adulthood, Omega-3 fatty acids reduce the cardiovascular disease risk.

This study aimed to determine the effects of Omega-3 fatty acids supplements on the cardiovascular system, in obese children. The most important cardiovascular risk factors were assessed before and after the treatment.

Sixty obese children (9–16 years old) and thirty lean children were involved. Each day, for three months, obese children took Omega-3 fatty acids (DHA 130 mg and EPA 25 mg) and vitamins (A 200 μg, D 1.25 μg, E 2.5 mg and C 30 mg). The measured variables were: fasting lipid profile (total cholesterol, HDL-C, triglycerides, LDL-C), inflammatory markers as fibrinogen, CRP (C reactive protein), WBC (white blood cells), ESR (erythrocyte sedimentation ratio), adiponectin/leptin ratio, proteinuria, CIMT (carotid intimal media thickness), IVS (interventricular septum thickness) and HOMA-IR (homeostatic model assessment-insulin resistance). Spectrophotometric and ELISA methods were used for plasma variables and ultrasounds for IVS.

All the measured parameters were modified in obese children versus lean subjects. In the obese children, the treatment lowered the rise of CIMT ($p < 0.001$), IVS ($p < 0.001$), inflammatory markers ($p < 0.01$) with the exception of WBC, HOMA-IR ($p < 0.001$), proteinuria ($p < 0.001$), blood pressure values ($p < 0.001$), total cholesterol ($p < 0.001$), triglycerides ($p < 0.01$) and LDL-C ($p < 0.001$) and increased the adiponectin/leptin ratio ($p < 0.001$) and the level of HDL-C ($p < 0.001$). Proteinuria was positively correlated with HOMA-IR ($r = 0.34$, $p < 0.05$), IVS with CIMT ($r = 0.31$, $p < 0.05$) and CIMT with adiponectin/leptin ratio ($r = -0.30$, $p < 0.05$) and with CRP ($r = 0.29$, $p < 0.05$).

In conclusion, in childhood obesity, Omega-3 fatty acids supplements reduced the increased values of the interventricular septum, lowered the atherosclerotic risk and decreased inflammation and insulin resistance.

Keywords: cardiovascular risk, childhood obesity, Omega-3 fatty acids

TUE-371
Change peroxidation cascade in blood of women with helminthiasis invasion

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Introduction: In recent years, there is an active study of pathogenesis in helminthiasis, it is known that during parasitism, especially during the migration of the larvae, the body receives a huge amount of metabolic products of helminths damaging hereditary apparatus of somatic cells of the host, causing parasite antigens intoxication and violation of free radical processes in helminthiasis.

Aim: To evaluate the condition of the products lipoperoxide cascade in the blood of women with helminthiasis invasion.

Materials and methods: To assess the performance oxidative metabolism was studied content of primary, secondary and final products of lipid peroxidation.

Determination of conjugated dienes (CD), ketodienes (KD), total of the primary product (TPP), total of secondary product (TSP). Shiff bases was performed by standardized methods of Ushkalova V.N. and Kadochnikova G.D. (1987). Determination of malonic dialdehyde (MDA) was performed according to the method of Korobeynikova Ye.N (1989).

Statistical analysis of the results was performed using the standard package of statistical program Microsoft Excel.

Results: The results showed that in blood of women with ascaridiasis invasion observed activation of lipid peroxidation. In blood of women with ascaridiasis invasion CD content exceeds 1.4 times as compared with control. MDA levels in blood of persons surveyed was significantly higher than control values – in 4.0 times ($p < 0.05$).

From the obtained results it is evident, aside from CD, MDA at different stages of peroxide cascade formed a number of other toxic metabolites LPO: ketodien, conjugated trienes, 4-hydroxy alkenes, alkenes, aldehydes therefore the content in blood not only individual, but also total of primary (TPP) and total of secondary (TSP) products the LPO was defined.

Mean levels of TPP and TSP in women with ascaridiasis invasion exceed the value of control in 2.0 times ($p < 0.05$).

Conclusion: In our view, the preferential growth of the TPP and the TSP is a testament to the whole spectrum of education toxic catabolites LPO, both at the stage of initiation and lipo peroxide cascade the propagation stage, as well as increasing the concentration of the contents of primary and secondary products can be attributed to the lipoperoxide cascade pronounced cytotoxicity of MDA, which contribute to the formation of other deep oxidation products of lipid aldehydes, ketones and acids.

Keywords: blood, helminthiasis invasion, lipid peroxidation

TUE-372
Characterization of the squalene synthase from *Methylococcus capsulatus* that requires no detergent

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Owing to the dearth of information regarding squalene synthases (SQSs) from prokaryotes, we set out to characterize the SQS from *Methylococcus capsulatus* (mcSQS). We also studied its reaction mechanism by kinetic analysis and evaluated the structure of the substrate/inhibitor-binding sites by homology modeling. To this end, we transfected the gene for mcSQS to *Escherichia coli* for its expression and purification by Ni-NTA column chromatography. We also produced mcSQS mutants by site-directed mutagenesis directed at various amino acids and potential active-site residues in the two binding-site motifs $^{58}DXX^{61}E^{62}D$ (S1 site) and $^{213}DXX^{216}D^{217}D$ (S2 site), which have been thought to be involved in the binding of the substrate farnesyl diphosphate (FPP) through the Mg^{2+} ion. Very interestingly, mcSQS was water-soluble and did not require any detergent for its activity, unlike other SQSs hitherto studied; in fact, supplementation of any type of detergent inhibited the enzyme's activity. Under catalytic optimal conditions, the specific activity of the wild-type mcSQS was estimated to be 800 ± 36 nmol·min^{-1}·mg^{-1}. The kinetic constant values for the substrate FPP were as follows: $K_m = 13.6 \pm 0.92$ μM, $k_{cat} = 0.549 \pm 0.01$ s^{-1}, and $k_{cat}/K_m = 0.04$ μM^{-1}·s^{-1}. For NADPH, the parameters were as follows: $K_m = 75.4 \pm 1.02$ μM,

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k_{cat} = 0.33 ± 0.0015 s^{-1}, and k_{cat}/K_m = 0.004 $\mu M^{-1} \cdot s^{-1}$. The substrate analog farnesylmethylenediphosphate was a potent inhibitor of the enzyme, with a K_i^{FPP} value of 0.1 μM. From the site-directed mutagenesis studies, we first demonstrated the importance of the S1 site. In addition, two basic residues (R55 and K212) were revealed to be responsible for the binding of the substrate FPP.

Keywords: farnesyl diphosphate, *Methylococcus capsulatus*, squalene

TUE-373
Charge-tagging approach in corticosteroid detection

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Corticosteroids belong to a group of steroid hormones that are produced by adrenal cortex of vertebrates and involved in a wide range of regulatory processes, including electrolyte homeostasis, carbohydrate metabolism, stress and immune response. Corticosteroid level detection is a valuable instrument for understanding mechanisms of physiological processes. Although corticosteroids are rather polar molecules, its detection limit by HPLC-MS in APESI ionization mode is too high to detect minor quantities in biological samples. To improve detection limit of oxysterol metabolites, charge-tagging derivatization was proposed and now it is widely used for oxysterol identification and quantitation in biological samples [1]. Up to date charge-tagging of corticosteroids for MS-analysis was not a subject of specific investigation, although the interest in this derivatization approach is growing [2, 3]. In our work we tested three variants of charge-tagging reagents, viz Girard reagent P and T, hydroxylamine to introduce a positive charge or to enhance proton binding capability of cortisol, corticosterone and aldosterone. Presence of two (or three in case of aldosterone) carboxyl group in molecules of tested steroids suppose few reaction products, but we show that mainly reaction occurs at the carbonyl group of C3. We obtain and interpret SID as well as CID fragmentation patterns for derivatization products, so this information may be very useful for identification of minor unknown metabolites of the stated hormones. Optimized technique was applied for comparative analysis of corticosteroid level in brain sections, obtained from intact and stressed rats.

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[2] D.F. Cobice, C.L. Mackay, R.J. Goodwin et al. Mass spectrometry imaging for dissecting steroid intracrinology within target tissues. *Analytical chemistry*. 2013, **85**, 11576–11584.

[3] D.W. Johnson. Ketosteroid profiling using Girard T derivatives and electrospray ionization tandem mass spectrometry: direct plasma analysis of androstenedione, 17-hydroxyprogesterone and cortisol. *Rapid communications in mass spectrometry*. 2005, **19**, 193–200.

Keywords: charge-tagging, corticosteroids, Girard's reagents

TUE-374
CHO-MT58: a model system for functionality studies of *Plasmodium falciparum* CTP: phosphocholine cytidylyltransferase

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Malaria – caused by the protozoan *Plasmodium falciparum* – is still one of the most threatening infectious diseases in the 21th century. In *Plasmodium falciparum* as in all eukaryotic cells phospholipid biosynthesis has a key role in synthesis of cell membrane and intracellular membrane elements. The most prevalent way of *de novo* biosynthesis is Kennedy-pathway, where the reaction catalyzed by CTP: phosphocholine cytidylyltransferase[1] (CCT) (CTP + choline-phosphate à CDP-choline) is rate-limiting. Because of the persistent need of Plasmodium for membrane synthesis during its life cycle, de novo phospholipid biosynthesis emerges as a target for new generation antimalarial drugs.[2]

CHO-MT58 cell line was proved to be an appropriate tool for investigating intracellular function of CCT harboring a point mutation in its endogenous CCT, causing thermo-sensitivity.[3] At non-permissive temperature (40°C) the endogenous CCT activity decreases dramatically, which blocks membrane synthesis and ultimately leads to apoptosis.[3] It was demonstrated that supply of external phosphatidylcholine or the heterologous expression of the rat CCT can successfully complement the mutant strain. Our aim was to develop a model system using this inducible CCT "knock out" cell line, where we can study the functionality of heterologously expressed *Pf*CCT constructs in cellular environment, excluding the effect of endogenous CCT.

In this study we transfected CHO-K1 and CHO-MT58 cells with CCT constructs and incubated them at permissive and non-permissive temperatures. To verify the rescue we performed FACS experiment with these and with cells transfected with catalytically inactive *Pf*CCT as control. Having obtained a higher ratio of active *Pf*CCT expressing cell population at non-perimissive 40°C than at permissive temperature 37°C, we have demonstrated for the first time that heterologously expressed Plasmodium CCT is able to complement the respective endogenous cytidylyltransferase activity in mammalian cells. Thus, a suitable test system has been established for the functional investigation of different protozoan-specific structural elements of *Pf*CCT. Furthermore, the possibility to assess the functionality of either endogeneous mammal CCT or Plasmodium CCT enables testing the selectivity of novel antimalarials acting on *de novo* phospholipid metabolic pathways.

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[2] Vial et al. *PNAS* 2004

[3] Sweitzer & Kent *Archives of Biochemistry and Biophysics* 1994

Keywords: FACS, Lipid biosynthesis, Malaria

TUE-375
Circulating CTRP1 levels in patients with nonalcoholic steatohepatitis

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Introduction: Non-alcoholic steatohepatitis (NASH) is assumed to be developed by two-hit process, the first hit is lipid accumulation in the liver cells and the second hit includes Oxidative stress,

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proinflamatory cytokines and adipokines. Regarding their metabolic and inflammatory activity, adipokines have been shown to play key role in the pathogenesis of NASH. C1q/TNF-related protein-1(CTRP-1) is a member of CTRP superfamily which has been demonstrated to play important roles in energy homeostasis, insulin resistance and inflammation. This study was conducted to measure the plasma concentrations of CTRP1 in four groups including NASH, type 2 diabetes mellitus (T2DM), NASH-T2DM and healthy controls.

Methods: A total of 86 subjects including 22 with NASH, 21 with T2DM, 22 with NASH-T2DM and 21 healthy controls were participated in this study. Plasma concentration of CTRP1 was measured with ELISA method. Anthropometric assessment, ultrasonographic and biochemical evaluation were performed for all study subjects. Liver stiffness scores were measured via transient elastography. The homeostasis model assessment (HOMA) was used as a surrogate model for assessmant of Insulin resistance (IR)

Results: Plasma concentration of CTRP1 in NASH, T2DM and NASH-T2DM was significantly elevated compared to healthy subjects ($P < 0.001$). Moreover, we observed the a significant correlation ($P < 0.001$) between plasma level of CTRP1 and conventional diabetes and fatty liver risk factors including the HOMA, fasting blood suger (FBS),liver stiffness scores,alanine aminotransferase (ALT) and aspartate amino transferase (AST) in the whole study population.

Conclusion: Our study, uncovered,for the first time, changes of CTRP1 levels as a possible tool for NASH and T2DM detection. Our data also suggest that serum CTRP1 level may be associated with more advanced form of NASH.

Keywords: None.

TUE-376

Citrulline counters hepatic lipid metabolism disorders in fructose-induced non-alcoholic fatty liver disease

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Rationale: A high fructose (HF) intake has been shown to induce insulin-resistance, changes in liver metabolism and gut barrier function ultimately leading to non-alcoholic fatty liver disease (NAFLD). Citrulline (Cit), Glutamine (Gln) and Arginine (Arg) may improve insulin sensitivity and may have beneficial effects on gut trophicity. The aim of our study was to evaluate the effects of these amino acids (AA) on liver and gut functions in a rat model of fructose-induced NAFLD.

Methods: 58 male Sprague-Dawley rats (225–250 g) received for 4 weeks a fructose-enriched (60%) diet or standard chow either alone or supplemented with Cit (0.15 g/d) or an isomolar amount of Arg or Gln. All diets were made isonitrogenous by addition of a mixture of non-essential amino acids (NEAA). On week 4, nutritional and metabolic status (plasma glucose, insulin, cholesterol, triglycerides (TG) and AA, and net intestinal absorption) were determined. Steatosis (hepatic TG content (HTG), histological examination), hepatic function (plasma AST, ALT, ALP, and bilirubin) and hepatic inflammation (TLR4 mRNA and protein expression) were assessed. Gut barrier integrity (myeloperoxydase activity, portal endotoxemia, tight junction protein expression and localization) was evaluated. We also assessed the influence of the different diets on caecal microbiota by quantifying total bacteria and main bacterial groups of caecal microbiota.

Résultats: In our experimental conditions of HF isonitrogenous feeding, fructose led to significantly increased liver weight, HTG and plasma TG. However, there was no alterations in glucose homeostasis, liver function and gut permeability. Fructose diet tended to increase endotoxemia and decreased significantly Bifidobacterium and Lactobacillus intestinal contents. While Arg and Gln supplementations were ineffective, Cit supplementation prevents hypertriglyceridemia and attenuates liver fat accumulation.

Conclusion: While nitrogen supply by itself notably attenuate fructose-induced NAFLD, Cit seems to act directly at hepatic level on lipid metabolism partially preventing hypertriglyceridemia and steatosis. This occurs in the absence of noticeable changes in gut microbiota, intestinal barrier function, and insulin sensitivity suggesting a specific regulatory role of this AA on lipid metabolism.

Disclosure of Interest: L.CYNOBER and J.P DE BANDT are shareholders of CITRAGE.

Keywords: amino acids, non alcoholic fatty liver disease

TUE-377

Citrulline reduces lipid stores in adipose tissue from overweight rats by altering glyceroneogenesis and fatty acid re-esterification

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Obesity is a major cause of increased risk of atherosclerosis and diabetes. Visceral adipose tissue (AT) development plays an important role in the occurrence of metabolic syndrome, observed with excessive weight gain. The metabolic dysregulations linked to obesity is the result at least in part of the unbalance between fatty acid (FA) storage in AT and their release determined by lipolysis, beta-oxidation and re-esterification. Our previous results showed that CIT exerted a direct effect on FA metabolism in explants of retroperitoneal (RET) AT from old rats, hence favoring FA output. We postulated that CIT could also directly affect FA metabolism in RET AT explants from young Sprague Dawley male rats (2 month-old) fed a high-fat diet (HFD) for 8 weeks compared to a standard diet (CD). HFD rats were overweight and RET AT mass doubled compared with their CD counterparts. We first evaluated the effects of a 24 h incubation with 2.5 mM CIT on RET CD (n = 5) and HFD (n = 5) AT explants on FA and glycerol release in the incubation medium. In the presence of CIT, no significant release of glycerol was detected, whereas FA output was significantly increased about twofold selectively in HFD explants. Concomitantly, CIT significantly up-regulated the level of phosphorylated hormone-sensitive lipase (HSL) in CD (60%) and HFD (100%) explants. The absence of glycerol release can be explained by the observed twofold increase in glycerol kinase gene expression in both CD and HFD explants. To start deciphering the mechanism by which FA output was augmented, we monitored beta-oxidation capacity, by measuring the production of $^{3}H_2O$ from (9,10-^{3}H) palmitate, and the glyceroneogenic flux, through the incorporation of [1-^{14}C]-pyruvate into lipids. We observed a threefold increase in oxidative capacity in both CD and HFD explants

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in agreement with a 68% (CD) and 42% (HFD) up-regulation of very long-chain acyl-CoA dehydrogenase. The glyceroneogenic flux was attenuated in CD explants (50%) but drastically decreased (80%) in HFD explants. This was the result of a significant down-regulation (40–48%) by CIT of the cytosolic phosphoenolpyruvate carboxykinase, the key enzyme of glyceroneogenesis. Therefore, CIT alters FA metabolism in RET AT from overweight rats predominantly by reducing glyceroneogenesis and FA reesterification.
Keywords: Adipose tissue, Citrulline, Glyceroneogenesis

TUE-378
Connective tissue metabolism markers for increase of ovarian cancer efficiency treatment

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As known glycosaminoglycans (GAG) are components of connective tissue (CT) and biochemical markers of its metabolism. We used total GAG and GAG-spectrum as part of a diagnostic complex evaluation of treatment efficiency in ovarian cancer (OC) stages III-IV. It is well known that 65–85% of patients having OC start the first course of treatment at the III-IV stages of tumor process. The above mentioned fact makes difficult the possibility of doing citoreductive operations at the first stage of treatment and it requires the use of neoadjuvant chemotherapy (NPChT), the optimal course number of which has not been determined yet. After the NPChT it becomes possible to make an operation to delete the original tumor in (OC) patients.Structural degradation of extracellular matrix (ECM) appears in the epithelial tissues of patients during the OC III-IV stages- development. It effects the GAG spectrum in blood serum and evidently precedes invasion and tumor metastasis.

GAG fractions and total GAG in blood serum of 82 patients having OC at III-IV stages before treatment and after NPChT were determined by M. Shtern et al. method. ELISA method were used to determine CA125 content-famous marker of OC.

It was found that the changes in level of total GAG in blood serum up to 75–70%, chondroitinsulphate up to 80–65%, II GAG- fraction (contained Ch-4-S and dermatansulfate) up to 75–65% of the original, I (contained mainly Ch-6-S) and III (contained heparansulfates, keratansulfates and heparin) fractions, ratios of total GAG and their fractions, to normal data, as well as the content of CA 125 to 9.6–3.6% of its initial value are markers of making operation possibility in patient having OC stages III-IV. These findings correlated with changes of clinical, ultrasound and morphological parameters and became the basis of diagnostic complex of treatment efficiency evaluation.
Keywords: None.

TUE-379
Covalent immobilization of benzoylformate decarboxylase on magnetic solid support and its carboligation reactivity

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In the present work, HIS-tagged benzoylformate decarboxylase (BFD E.C. 4.1.1.7) from *Pseudomonas putida* was covalently attached onto the magnetic epoxy support. In order to obtain an immobilized biocatalyst with high amount of enzyme loading and activity recovery, the effects on the immobilization efficiency of immobilization conditions including enzyme concentration, pH, ionic strength and coupling time were investigated. The maximal enzyme loading and enzyme activity were investigated under the optimal immobilization parameters. A three-step immobilization/stabilization procedure is applied. The enzyme is primarily covalently immobilized under appropriate experimental conditions (e.g. pH 7.0, no added $MgSO_4$ and 20°C). Secondly, the enzyme is immobilized under more drastic conditions (higher pH values, higher ionic strengths etc.) to facilitate an increase in effective concentration of the enzyme on the support near the epoxide reactive sites. Thirdly, the remaining epoxy groups are blocked to stop any additional interaction between the enzyme and the support. With more drastic conditions, the loading of enzyme can be increased from 1.25 to 6.70 mg enzyme per gram of support. The covalently bounded enzyme was characterized in terms of its activity and stability for the formation of (*S*)-2-hydroxypropiophenone (2-HPP). The activity of the immobilized BFD was determined to be 53.0% related to the activity of the free enzyme. The immobilized biocatalyst retained 95% of its original activity after five reaction cycles.
Keywords: Benzoylformate Decarboxylase, Covalent Immobilization, magnetic epoxy support

TUE-380
CTRP-5 levels and non-alcoholic steatohepatitis: is it through insulin resistance?

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Introduction: A growing body of evidence indicates that type 2 diabetes, obesity and insulin resistance are major risk factors for non-alcoholic steatohepatitis (NASH). Recently, it has been suggested that C1q/TNF-related protein-5(CTRP-5), as a novel adipokine of adiponectin paralogs, may play an important role in lipid and glucose metabolism. The aim of present study was to investigate,for the first time, plasma levels of CTRP-5 in NASH patients with type 2 diabetes,NASH patients without type 2 diabetes and also patients with type 2 diabetes in comparison with healthy subjects and also examine the association between circulating CTRP-5 levels and clinical parameters of NASH and type 2 diabetes and liver stiffness scores in all participants.
Methods: A total of 86 subjects, all males were recruited for this study. The study participants were categorized into healthy subjects (n = 21), NASH with type 2 diabetes (n = 22), NASH without type 2 diabetes (n = 22) and type 2 diabetic patients (n = 21). All participants underwent extensive physical examination, anthropometric assessment, ultrasonographic and biochemical evaluation. Plasma CTRP-5 levels were analyzed by enzyme-linked immunosorbent assay. Liver stiffness scores were examined by transient elastography .Insulin resistance (IR) was determined by the homeostasis model assessment (HOMA).
Results: The plasma concentration of CTRP-5 was found to be significantly decreased in patients with type 2 diabetic NASH as compared with controls (122.52 ± 22.24 ng/ml and 164.96 ± 12.15 ng/ml, respectively). In addition, the plasma level of this adipokine were significantly lower in non-diabetic NASH patients (124.7 ± 19.55 ng/ml) and type 2 diabetic subjects (118.31 ± 19.27 ng/ml) as compared with healthy individuals .

Additionally,there was a significant correlation between the plasma levels of CTRP-5 with HOMA-IR (r = –0.496),fasting

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blood sugar (FBS)(r = −0.445) and insulin (r = −0.338) .The circulating CTRP-5 levels also showed an inverse and significant correlation with body mass index (BMI) (r = −0.337),alanine aminotransferase (ALT)(r = −0.243) and liver stiffness scores (r = −0.544).

Conclusion: It appears that the assessment of CTRP-5 with other biochemical and metabolic parameters could be partially useful to elucidate the pathways contributing to NASH and other diabetes –related diseases.

Keywords: None.

TUE-381
Cytotoxic effect of two endemic subspecies of *Tanacetum praeteritum* (Horw.) Hey-wood (Asteraceae) from Turkey on cervical carcinoma

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Background: Cervical cancer is the second most common form of cancer in women aged 15–44 years in developing countries. Approximately 530 000 new cases are diagnosed and 275 000 deaths occur each year. Up to 60% of the approved chemotherapeutic drugs are derived from natural compounds. *Tanacetum* is a genus which is composed of nearly 200 species in the aster family (Asteraceae). Several species of *Tanacetum* genus are traditionally used in a variety of health conditions including pain, fever, inflammation, arthritis, migraine, respiratory and gastrointestinal disorders. Sesquiterpenoids and sesquiterpene lactones as the typical constituents of these plants might be partially or wholly responsible for these effects. Additionally, earlier investigations showed that sesquiterpene lactones isolated from *Tanacetum praeteritum* ssp. *praeteritum* have cytotoxic activity against the human lung carcinoma cell line GLC_4 and the colorectal cancer cell line COLO 320. Therefore, in the present study we investigated the cytotoxic and anticancer activities of the methanol extracts of the aerial parts of *Tanacetum praeteritum* ssp. *praeteritum* and *T. praeteritum* ssp. *massicyticum* which are endemic subspecies from Turkey on human cervical carcinoma.

Methods: Plant materials collected in flowering time from Antalya/TURKEY have been extracted by the method described in the European Pharmacopoeia. Air-dried and powdered aerial parts of the plant materials (20 g) were extracted with methanol (3 × 500 ml) in a water-bath at 60°C, concentrated to dryness under reduced pressure and lyophilized in vacuo. Cervix cancer cell line, HeLa was grown in EMEM medium supplemented with 10% fetal bovine serum and 2 mM glutamine. Cytotoxicity of methanol extract of *T. praeteritum* ssp. *praeteritum* and *T. praeteritum* ssp. *massicyticum* on HeLa cell lines were determined with Alamar blue Assay and IC_{50} value was calculated.

Results: The yield of extract obtained from *T. praeteritum* ssp. *praeteritum* was 14.1% and obtained from *T. praeteritum* ssp. *massicyticum* was 12.6%. In this study, we found both *T. praeteritum* ssp. *praeteritum* and *T. praeteritum* ssp. *massicyticum* have the potential to inhibit the proliferation of HeLa cell line in a concentration-dependent manner with an IC_{50} of 0.25 mg/ml and 0.21 mg/ml, respectively.

Conclusions: The results showed that the methanol extracts of both *T. praeteritum* ssp. *praeteritum* and *T. praeteritum* ssp. *massicyticum* were found to be effective inhibitor against cervical carcinoma at low concentration, which indicates that these plants might have potential for cervix cancer treatment.

Keywords: cervix cancer, cytotoxicity, Tanacetum

TUE-382
Development of low-temperatures adaptation mechanisms in winter wheat cells under conditions of a heterotrophic suspension culture

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Cells are exposed to various chemical and physical influences *in vitro* environment of a heterotrophic culture, so the conditions more effective forming the low-temperature adaptation mechanisms in cells of a suspension culture and intact plants may differ. In this connection, the purpose of this study was to investigate the metabolic changes of cells in a plant suspension culture under the influence of low positive temperatures, namely their effects on fatty acid composition, stress proteins accumulation and changes of a respiratory metabolism. The work was carried out with 8 day old winter wheat suspension culture (*Triticum aestivum* L.), using the treatment with 4 and 8°C for 7 days. It has been shown that the exposure of the culture to 8°C led to decreasing of saturated fatty acids contents in cells: pentadecylic acid – by 35%, palmitic acid – by 19.9%, stearic acid – by 65.4%, and to increasing the content of unsaturated linolenic acid nearly in two times. The treatment of the culture with 4°C caused no significant changes in the cells fatty acid composition. Then the exposure of the culture to low positive temperatures also affected the dehydrins synthesis in the winter wheat cells: their content increased with the treatment with both studied temperatures, but during the action of temperature 8°C this process occurred more intensely. It is known that an important component of plant adaptation is change of a respiratory metabolism, in particular, the ratio of the alternative cyanide-resistant and the cytochrome cyanide-sensitive pathways contributions in respiration. Alternative oxidase (AOX) functioning in a plant cell is one of the mechanisms preventing the generation of reactive oxygen species. In our study an increase of the AOX contribution in the respiration is shown to occur only in the treatment of the culture with 8°C, while the effect of 4°C treatment resulted in a significant increase of the respiration rate, but almost no influenced on the AOX functioning. Thus, our results indicate that the treatment of the winter wheat suspension culture with 4°C had a stress impact to the cells, and adaptation mechanisms forming more effective occurred at the temperature of 8°C. This work was supported by RFBR grant (■ 14-04-32126).

Keywords: fatty acid composition, respiratory metabolism, suspension culture

TUE-383
Disruption of carbohydrate metabolism by xenobiotic mixtures

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Epidemiological studies have shown that exposure to certain xenobiotics is associated with an increased prevalence of metabolic

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diseases. Since humans are exposed to mixtures of xenobiotics detoxified by the liver, we used two xenobiotics, both endocrine disruptors and persistent organic pollutants, which use different signaling pathways, to study hepatic energy metabolism: 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which uses the AhR, and α-endosulfan, an organochlorine pesticide, which acts via the PXR and/or the estrogen receptor.

Differentiated HepaRG cells, which resemble human hepatocytes, were exposed for 30 h to 25 nM TCDD, 10 μM α-endosulfan, or the mixture. Gene expression was measured by transcriptomics and RT-qPCR. Oxidation of glucose to CO2 (radioactive incorporation) and production of lactate and glucose (colorimetry) were measured.

The expression of two genes of hepatic gluconeogenesis critical for hepatic energy metabolism, glucose transporter 2 (GLUT2) and glucose-6-phosphatase (G6Pc), were reduced 80% by the mixture (more than either xenobiotic alone). The expression of other glucose metabolism genes (pyruvate kinase, glycogen synthase, glycogen phosphorylase, pyruvate dehydrogenase 2) also was decreased suggesting that the mixture may markedly impact carbohydrate metabolism. Glucose production decreased 40% with the mixture under gluconeogenic conditions. Under glycolytic conditions, although the oxidation of glucose into CO_2 decreased 30% after 72 h exposure to the mixture, lactate production was not affected. Long-term treatment (8 days) with lower doses (0.2–5 nM TCDD, 3 μM α-endosulfan) similarly decreased G6Pc and Glut2 expression.

Thus, the mixture of TCDD and α-endosulfan decreases, *in vitro*, the expression of hepatic glucose metabolism genes even at low doses and decreases hepatic gluconeogenesis and glucose oxidation. Chronic exposure of individuals to low doses of xenobiotics might significantly affect hepatic carbohydrate metabolism and be a contributing factor in the development of the metabolic syndrome.

Keywords: Metabolism, Mixture, Pollutant

TUE-384
Donepezil analogs as acetylcholinesterase inhibitors

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To explore novel effective drugs for the treatment of Alzheimer's disease (AD), a series of acetylcholinesterase (AChE) inhibitors were designed based on a molecular docking strategy.

AD, a progressive neurodegenerative disorder, is characterized by cholinergic system deficits leading to deposition of beta amyloid in the form of neurofibrillary tangles and amyloid plaques, a process based on the modulation of the cholinergic system. Tacrine, donepezil, rivastigmine and galantamine are clinically employed for the treatment of AD as they enhance cholinergic transmission directly by inhibiting the activity of AChE. In addition, also butyrylcholinesterase (BuChE) activity play an important role in Ab-aggregation during the early stages of senile plaque formation.

In this study, two series of new Donepezil-like derivatives were developed, with chemical replacements on indanone or benzilpiperdine ring systems. Firstly, a double bond between these two moieties, leading to a more rigid structure, was inserted. On this scaffold a metoxilic 5-components series, characterized by the presence of metoxyl groups in different structural positions, was developed and synthesized (GP9, GP10, GP11, GP12, GP13). On the basis of molecular docking analysis these modifications should ensure a better interaction with the AChE catalytic site. Furthermore, with the aim at improving compound solubilisation, a different phenolic 4-components series was designed and synthesized (DEMG3, DEMG7, DEMG5, DEMG6).

The inhibitory effect of the new synthesized compounds was assessed on AChE from *Electrophorus electricus*, using Ellman assay with acetyl-thiocholine as substrate, and the results were compared to that of Donepezil. For all the compounds tested the IC_{50} of AChE activity was within micro-molar range except for GP9 (IC_{50} = 162.8 nM) which showed an inhibitory efficacy much close to that of Donepezil (IC_{50} = 30 nM). Dilution experiments showed that the inhibition is completely reversible for all the compounds tested and kinetic analysis highlighted a non-competitive mechanism of action. To assess the specificity of these compounds, their effect was also checked on BuChE from horse serum, using butyrylthiocholine as substrate, and the results indicated that all tested compounds exhibited a higher specificity towards AChE.

Experiments are in progress to evaluate the cytotoxicity of synthesized compounds on neuronal cell lines as well as in other cell lines.

Keywords: acetylcholinesterase inhibitors, Alzheimer's disease, donepezil

TUE-385
Early laboratory diagnosis and bone marrow transplantation in MPS type I

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Mucopolysaccharidosis (MPS) Type I is a rare lysosomal storage disorder in which heparan and dermatan sulphates accumulate in different tissues and organs due to a defect of IDUA gene which encode the α-L iduronidase. The purpose of presenting this case is to highlight the importance of the establishment of laboratory diagnostic tests and early laboratory diagnosis in the populations that have higher incidence of MPS. A 16-months-old girl was admitted with complaints of waist curvature and nasal obstruction realized at 6 months by her mother. On examination, her head circumference, weight and height were found in 95, 3–10 and 3–10 percentiles, respectively. She had coarse facial features, a depressed nasal bridge, large tongue, rough and though hair, thick eyebrows and hypertrichosis. Corneal clouding, kyphosis in the lumbar spine and minimal limitation in knee extension were observed. Radiologic X-ray images were compatible with dysostosis multiplex. Ultrasonography of abdomen showed minimal hepatomegaly, increased liver parenchymal echogenicity and coarsening. Recently, MPS urinary GAG analysis with electrophoresis and specific enzyme activities from leukocytes for all types of MPS were established in our laboratory. Increased dermatan and heparan sulphate bands in electrophoresis were found in urinary GAG analysis. Alpha-L-iduronidase specific activity measurement in leukocytes was found 0.008 μmol/gram protein/hour (reference range: 10–50). The marked deficiency of alpha-L-iduronidase suggested a diagnosis of MPS type I. Molecular analysis revealed that the patient was heterozygous for IVS11 + 5G>C and exon 14 1893 del C mutations of IDUA gene. Enzyme replacement therapy (ERT) was started at 20 months of age. Eight months after (28-months-old) bone marrow transplantation (BMT) was performed from a non-relative, tissue group 4/6 compatible donor. Chimerism in first month was

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found 85% and second, third and sixth months were found 100%. Rashes occured in the second month. She received treatment for graft-versus-host-disease (GVHD). At fifth month alpha-L-iduronidase specific activity was found normal. Meanwhile, she is 6 years of age and going to preschool, except little hearing loss life quality is fine and being followed up for the progress. If patients are to be diagnosed as early as possible they could be treated safely and effectively. Due to the high ratio of consanguineous marriages (21%) in Turkey, the incidence is expected to be more and it is important to establish the laboratory diagnostic tests in the populations that have higher incidence of MPS.

Keywords: bone marrow transplantation, early laboratory diagnosis of MPS, Mucopolysaccharidosis

TUE-386
Effect of co-administration of endosulfan and morin on biomarker enzyme activies in rat liver

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Endosulfan is an organochlorine pesticide, used extensively in the agricultural areas against insects. It may exist in air, freshwater, sea water, soil samples and foods. Endosulfan causes damage in the immune system, central nervous system and reproductive system. Morin is one of the flavonoids obtained from plants. The role of morin in the treatment and prevention of toxic effects of chemicals have not been clarified yet. The aim of this study is to determine the effect of morin in the presence of endosulfan. For this purpose, twenty eight male Wistar rats (6–7 weeks old and weighing 170–245 g) were randomly selected and divided into four groups. The rats in control group were treated with corn oil three times in a week. Morin-treated groups (morin and endosulfan + morin) were gavaged with 25 mg/kg body weight morin three times in a week. Endosulfan treatment was started at the 12th day of the administration period at a dose of 5.0 mg/kg body weight endosulfan in endosulfan and endosulfan + morin groups. This treatment was continued through the administration period with the frequency of three times in a week. Rats were killed by cervical dislocation on the 54th day of the administration period. Microsomes and cytosols were prepared for each liver tissue by differential centrifugation. Cytochrome P4501A (CYP1A) associated 7-ethoxyresorufin O-deethylase (EROD) activities of control, endosulfan, morin, and endosulfan + morin groups were 71 ± 7, 121 ± 5, 112 ± 6, and 121 ± 9 pmol/min/mg protein, respectively. Glutathione S-transferase (GST) activities of control, endosulfan, morin, and endosulfan + morin groups were 365 ± 14, 365 ± 24, 430 ± 27, and 460 ± 10 nmol/min/mg protein, respectively. Catalase activities of control, endosulfan, morin, and endosulfan + morin groups were 618 ± 29, 540 ± 38, 569 ± 34, and 612 ± 48 nmol/min/mg protein, respectively. Glutathione reductase activities of control, endosulfan, morin, and endosulfan + morin groups were 46.7 ± 2.2, 46.3 ± 1.7, 49.7 ± 1.6, and 46.3 ± 1.2 nmol/min/mg protein, respectively. In conclusion, morin, endosulfan and endosulfan + morin administrations increased EROD activities in rats. EROD activities measured in treatment groups were significantly different from EROD activities measured in control group ($p < 0.05$). Catalase and glutathione reductase activities were not altered with co-administration of morin and endosulfan. GST activities increased in morin and endosulfan + morin groups. The results of this study indicate that co-administration of morin and endosulfan significantly increases CYP1A and GST activities.

Keywords: Cytochrome P4501A, Endosulfan, Morin

TUE-387
Effect of high glucose and AGEs on lysosomal proteases of cultured kidney cells

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Diabetic nephropathy is one of the most prevalent consequences of diabetes mellitus (DM), and microalbuminuria is one of its early symptoms. It was previously reported that, while urinary excretion of albumin increased, excretion of sulfated polysaccharides decreased in DM rats. Exogenous dextran sulfate accumulated in liver and kidney, suggesting cell internalization, and higher amounts accumulated in DM. Recently, we have reported decreased lysosomal enzymes (proteases and glycosidases) in DM rat kidney. Excretion of less degraded or intact albumin explained observed albuminuria. Concerning sulfated polysaccharides, decreased activities of glycosidases led to intracellular deposition of partially digested macromolecules, and this could explain their decreased urinary excretion and tissue buildup. Concerning kidney morphology, the main changes observed were proximal convoluted tubules with thinner walls and thinner brush border, and immunohistochemistry revealed that most of cathepsin B was located in the brushborder of proximal tubular cells, highlighting the involvement of these cells in diabetic nephropathy. The aim of the present study was to investigate the possible mechanisms leading to these effects on lysosomal enzymes. As persistent hyperglycemia contribute to the formation of advanced glycation end products (AGEs), the first issues to be investigated were the effects of sustained high-glucose and of AGEs on lysosomal protease activities of immortalized lineages of kidney cells: human mesangial cells (IHMC), porcine proximal tubular cells (LLCPK) and canine distal tubular cells (MDCK) were used. These cells were cultured under normal (5.5 mM) or high (30 mM) glucose, for 15 days. They were also exposed to 0.5 mg/ml albumin (BSA) or ribose-derived albumin (AGE-BSA), and were cultured for either 48 or 72 h. Cell proliferation curves revealed that only mesangial cells behaved differently under high glucose, doubling 13% faster. Nevertheless, all cells were affected by AGE-BSA, proliferating less than controls, while BSA by itself did not affect them. Cysteine protease activities were measured in cell extracts with two different fluorimetric substrates, in presence or absence of inhibitors. The main cysteine protease was cathepsin B, and its activity was differently modulated by AGE-BSA depending on cell lineage: it increased in mesangial and distal tubular cells (IHMC: 1.2–1.5; MDCK: 2.0–2.5), while decreased in proximal tubular cells (0.6–0.75). These data show that different kidney cells are not affected in the same way by sustained high-glucose and AGEs, and is in agreement with our previous report on proximal tubules. Grants from FAPESP, CNPq and CAPES.

Keywords: diabetes mellitus, diabetic nephropathy, lysosomal proteases

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TUE-388
Effect of morin on cytochrome P450-dependent monooxygenase activities in 7,12-dimethylbenz[a]anthracene treated rats

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7,12-Dimethylbenz[a]anthracene (DMBA) is a lipid soluble molecule, producing toxic and carcinogenic effects within the body. It is possible to expose to DMBA with smoking cigarette, breathing car exhaust and furnace gases in daily life. Morin is a dietary flavonoid having chemoprotective and antioxidant properties in living organisms. The effects of morin have not been well defined in the presence of toxic chemicals. Therefore, this study is aimed to determine the effect of morin on cytochrome P4501A2 (CYP1A2), cytochrome P4502B (CYP2B) and cytochrome P4503A (CYP3A) in DMBA-treated rats. For this purpose, twenty eight male Wistar rats (6–7 weeks old and weighing 170–255 g) were randomly selected and divided into four groups. The rats in control group were treated with corn oil three times in a week. 25 mg/kg body weight morin was given to morin and DMBA + morin groups three times in a week. DMBA-treated groups were gavaged with 30.0 mg/kg body weight DMBA at 12th, 19th and 26th days of the administration period. Rats were killed by cervical dislocation on the 54th day of the administration period. Microsomes were prepared for each liver tissue by differential centrifugation. CYP1A2 associated 7-methoxyresorufin O-demethylase (MROD) activities of control, morin, DMBA, and DMBA + morin groups were 34.0 ± 0.7, 41.4 ± 1.2, 52.1 ± 1.9 and 58.8 ± 4.1 pmol/min/mg protein, respectively. CYP2B associated 7-pentoxyresorufin O-depentylase (PROD) activities of control, morin, DMBA, and DMBA + morin groups were 18.0 ± 1.0, 17.6 ± 0.8, 16.9 ± 0.7 and 18.5 ± 1.0 pmol/min/mg protein, respectively. CYP3A associated erythromycin N-demethylase (ERND) activities of control, morin, DMBA, and DMBA + morin groups were 0.38 ± 0.01, 0.44 ± 0.03, 0.48 ± 0.03 and 0.44 ± 0.04 nmol/min/mg protein, respectively. In conclusion, DMBA exposure increased the activities of CYP1A2, CYP3A, but not CYP2B in rats. CYP3A related ERND activities decreased in DMBA + morin group compared to DMBA group. CYP1A2 related MROD activities increased in DMBA + morin group compared to DMBA group.

Keywords: 7,12-Dimethylbenz[a]anthracene (DMBA), Cytochrome P450, Morin

TUE-389
Effects of chard (*Beta vulgaris* L. var. cicla) on spleen damage in valproic acid induced toxicity

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Valproic acid (VPA) has been used for more than 30 years for treating various medical disorders in adults and children, including migraine headaches, seizures and psychiatric disorders. In animal studies, it was effective in shrinking both lymph nodes and spleen in animals with conditions similar to autoimmune lymphoproliferative syndrome. Moreover in recent years VPA has been shown to be effective in various cancer types and Alzheimer disease. Chard (*Beta vulgaris* L. var. cicla) is a herbaceous biennial leafy vegetable cultivated in many parts of the world. It is a low cost plant and has a widespread use in many traditional dishes. It has been demonstrated that chard has antioxidant, anti-acetylcholinesterase, antidiabetic, antitumor and hepatoprotective effects. The aim of this study is to evaluate whether VPA and/or chard might interfere with oxidative metabolism in spleen. Female rats were divided into four groups as intact control animals, VPA (0.5 g/kg/day,i.p.), chard (100 mg/kg/day, oral) and VPA + chard (in same dose and time) given groups for seven days. Chard extract was given 1 h prior to the administration of VPA. On the 8th day the animals were sacrificed under anesthesia and spleen samples were homogenized in saline. Oxidant-antioxidant biochemical parameters were determined in homogenized spleen samples. Results were evaluated statistically and discussed.

Keywords: spleen, valproic acid, chard, oxidant-antioxidant parameters

TUE-390
Effects of combination treatment with amiodarone and white cabbage extract on rat aorta

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Amiodarone which is used for the treatment of arrhythmias, causes many side effects in all organ systems. Cabbage (*Brassica oleracea* L. var. capitata) is one of the most important vegetables grown worldwide. It provides significant amounts of vitamins (A,C,E,K) and other photochemicals, such as glucosinates and other sulfur containing compounds which are beneficial for human health. Numerous studies report protective effects of cabbage against many chronic diseases, several cancer types, cardiovascular, cerebrovascular, ocular and many neurological diseases and peptic ulcers. It may protect from the side effects of amiodarone. In literature there is no study which focuses on the effects of amidorone and cabbage on aort. In this study, we aimed to investigate the effects of amiodarone and cabbage extract on rat aorta. Female Sprague-Dawley rats were randomly divided into four groups as follows; control group receiving corn oil; cabbage extract (500 mg/kg/day) given group; amiodarone (100 mg/kg/day) given group; amiadaron + cabbage extract (in same dose) given group. Cabbage extract and amiodarone were given by gavage to rats for 7 days. Amiodarone was given to the animals one hour after cabbage extract administration during the experimental period. All animals were fasted overnight and on the 8th day they were sacrificed under anesthesia. Aorta samples were taken from animals and homogenized in saline. Oxidant-antioxidant biochemical parameters were determined in homogenized aorta samples. Results were evaluated statistically and discussed.

Keywords: Aorta, White Cabbage, Amiodarone, antioxidant-oxidant parameters

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TUE-391

Effects of Cr(VI) on the production of reactive oxygen species, and the ethylene response in *Arabidopsis thaliana* L. (Magnoliophyta: Brassicaceae)

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Chromium in its hexavalent [Cr(IV)] form is a toxic element that has increased its concentration in the environment due to human activities, representing a danger to organisms, including plants (1). The responses of plants to Cr(VI) are similar to those induced by other heavy metals, so it is proposed that there are common physiological mediators that help plants adapt to stress conditions that generate these metals, mainly reactive oxygen species (ROS) and some signaling pathways of hormones like etileno (2). In this work, the production of ROS in *A. thaliana* seedlings exposed to various concentrations of Cr(VI) was evaluated. Plants with wild phenotype (Col0) were used, as well as a mutant of a key gene in the signaling pathway of ethylene (*ein2-1*), which were grown at concentrations of 20, 40, 60, 80, 100 μM Cr(VI). We found that at concentrations of 20 and 40 μM, the seedlings showed an increase in primary root length and in the number of lateral roots, both in the wild line as in the *ein2-1* mutant. However, from 60 μM, the root length decreased, with a maximum reduction at 100 μM Cr (VI), 87.6% and 28.04% for wild seedlings and *ein2-1*, respectively. Additionally, in the case of the wild line, this coincides with an increase in the number and length of root hairs near to the root apex region, and the formation of adventitious roots. Also, the mitotic activity of the root meristem was lost in the wild line, but not in *ein2-1,* at a concentration of 100 μM Cr(VI). To assess whether the effects of Cr(VI) on the modification of root architecture were associated with increased levels of reactive oxygen species (ROS), an analysis was performed to detect the levels of H_2O_2 using the Hyper reporter line (4) or using the fluorochrome (H2DCF-DA) for wild and mutant line *ein2-1*, respectively. H_2O_2 levels were increased significantly in the wild line in a concentration-dependent manner, peaking at 100 μM Cr(VI) (Figure 1A). In line *ein2-1*, H_2O_2 levels were increased at a lower rate relative to the wild (Figure 1B). Our results suggest that Cr(VI) modulates the interaction of ROS and root architecture possibly through an ethylene-dependent signaling pathway.

Key words: Chromium, ROS, Arabidopsis.

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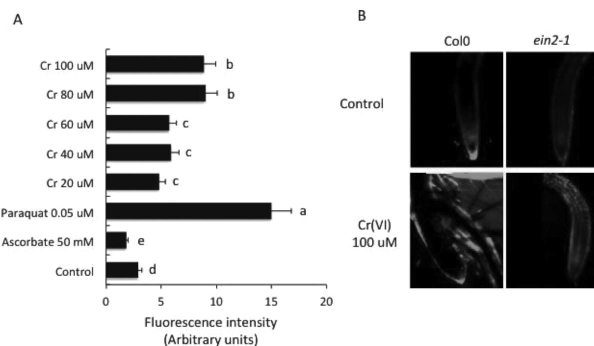

Fig. 1. Endogenous H_2O_2 detection by fluorescence. A. Fluorescence in Hyper Col0 line, with different treatments. B) Fluorescence in Col0 line (wild) and the *ein2-1* mutant line, with and without Cr(VI).

Keywords
Arabidopsis, Chromium, ROS

TUE-393

Effects of hormone replacement therapy on plasma and tissue fibrinolytic activity in a rat model of surgically induced menopause

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Purpose : The purpose of this study was to analyze the effects of estrogen deficiency and hormone replacement therapy (HRT) on fibrinolytic activity in a rat model of surgically induced menopause.

Methods: Twelve-week-old, sexually mature female Sprague-Dawley rats, each weighing 200–250 g, were randomly divided into four groups: (1) sham-operated group, (2) ovariectomy group, (3) ovariectomy group followed by oral administration of daily 17β-estradiol (0.02 mg/kg/day) (E2) + norethisterone acetate (0.01 mg/kg/day), and (4) ovariectomy group followed by oral administration of daily 17β-estradiol (0.01 mg/kg/day) + drospirenone (0.02 mg/kg/day). Tissue plasminogen activator (tPA) antigen, plasminogen activator inhibitor-1 (PAI-1) antigen, and PAI-1/tPA levels were measured as markers of fibrinolysis in plasma and liver and brain tissue.

Results: Compared with sham-operated rats, ovariectomized rats showed higher levels of fibrinolytic activity; however, the increased fibrinolytic activity in plasma and liver tissue was significantly reduced by HRT regimens. No change was observed in the levels of fibrinolytic activity in brain tissue.

Conclusions: HRT showed beneficial effects by decreasing fibrinolytic activity related to surgically-induced menopause. Short-term HRT treatment was associated with a shift in the procoagulant-anticoagulant balance toward a procoagulant state.

Keywords: Hormone replacement therapy, tissue plasminogen activator antigen, plasminogen activator inhibitor-1 antigen, fibrinolytic activity

TUE-394

Effects of resveratrol and melatonin on metabolic disturbances induced by a high-fructose diet in rats

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Metabolic syndrome is a disease characterized by hypertension, dyslipidemia, hyperinsulinemia, glucose intolerance, insulin resistance, and is associated with increased risk for development of both cardiovascular diseases and type 2 diabetes. It was determined that a close relationship between incidence of metabolic syndrome and increased fructose consumption in human and animal studies. In the present study, investigation of possible protective effects of resveratrol and melatonin treatment on metabolic changes caused by high-fructose diet in rats were aimed. For this purpose, 48 male adult Sprague-Dawley rats were randomly divided into six groups (n = 8); control, fructose, resveratrol, mel-

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atonin, fructose + resveratrol and fructose + melatonin. Metabolic syndrome was induced in rats by 20% (w/v) fructose solution in tap water for 8 weeks. Melatonin (20 mg/kg) or trans-resveratrol (10 mg/kg) were administered by oral gavage. Systolic blood pressures were measured by tail-cuff method, and fluid/food intakes were measured daily. At the end of 8th week, the animals were sacrificed under anesthesia, and blood samples obtained by intracardiac puncture. Serum lipids, glucose and insulin levels were evaluated using appropriate enzymatic analysis kits. Serum asymmetric dimethylarginine (ADMA) and homocysteine (Hcy) levels, which are known to be reliable markers of especially cardiovascular diseases as well as other numerous metabolic disorders, were quantified by HPLC. In comparison with control group, fructose consumption increased systolic blood pressure, serum triglyceride and insulin levels and insulin resistance significantly, and metabolic syndrome model was successfully demonstrated. Moreover, important changes, including increase of the serum ADMA and Hcy levels were observed following fructose treatment. Resveratrol administration showed a protective/therapeutic effect on hypertension which is an important criteria of metabolic syndrome, by preventing the systolic blood pressure rise caused by fructose. However, it did not show a protective effect on atherogenic lipid profile, hyperinsulinemia and increased ADMA levels induced by high-fructose diet. Melatonin administration prevented the increase in systolic blood pressure, insulin resistance, serum insulin, ADMA and Hcy levels. These results show that melatonin administration compared with resveratrol treatment can be useful for the prevention/treatment of the cardiovascular complications of metabolic syndrome by its beneficial effects on not only the well-known criteria of the disease, but also on the reduction of plasma ADMA and Hcy levels.

Disclosure of Interest: None Declared.
Keywords: Fructose, Melatonin, Resveratrol

TUE-395
Effects of testosterone replacement therapy on impaired redox homeostasis of aged brain

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Production of endogenous testosterone gradually decreases in aging males. Not only spermatogenesis but also androgen synthesis in testicle adversely affected by impaired redox homeostasis. Thus, optimal regulation of antioxidant enzyme activity is crucially important in the aging process. Our aim to study effects of testosterone replacement therapy on neuronal redox homeostasis in aging.

We analyzed various oxidative stress biomarkers including protein carbonyl groups (PCOs), lipid hydroperoxides (LHPs), malondialdehyde (MDA), advanced glycation end products (AGEs) and antioxidant status parameters such as total thiol groups (T-SH) and Cu-Zn superoxide dismutase (Cu,Zn-SOD) in different anatomical parts of rat brain Experimental animals divided into three groups: naturally aged rats (NA:% 0.9 saline), testosterone administrated naturally aged rats (TNA:25 mg/kg testosteron) and their corresponding young controls (YC:% 0.9 saline).

PCO, LHP, MDA concentrations in hippocampus tissue of NA rats were significantly higher than TNA and YC rats (NA vs YC and NA vs TNA $p < 0.001$ for each parameters). Additionally, TSH levels in NA rats were significantly lower than TNA and YC groups ($p < 0.001$ and $p < 0.01$ respectively). Cu, Zn-SOD activities in TNA group were significantly higher than NA group ($p < 0.01$). LHP and AGEs concentrations in parietal lobe of NA rats were significantly higher than TNA and YC groups ($p < 0.001$; YC and NA for both parameters and $p < 0.05$; $p < 0.01$ NA vs TNA respectively). MDA levels in young controls were significantly lower than NA rats. Although, there was a trend toward higher MDA levels in NA rats, MDA levels were not found to be significantly higher than TNA. TSH levels in NA rats were lower than TNA and YC groups ($p < 0.05$). Both MDA and LHP levels in peduncle of NA rats were significantly higher than other groups ($p < 0.001$YC vs NA for both parameters and $p < 0.001$ and $p < 0.01$ NA vs TNA). Additionally LHP concentrations in TNA group were significantly higher than corresponding young controls ($p < 0, 001$). MDA, LHP and AGEs concentrations in cerebellum of NA rats were significantly higher than TNA and YC rats ($p < 0.001$ NA vs TNA and YC vs NA for each parameters and $p < 0.001$; $p < 0.001$ and $p < 0.05$ YC vs NA respectively).

Our results related to TNA rats show that testosterone replacement therapy has a positive impact on establishment of redox homeostasis in different anatomical parts of aged brain.

Keywords: Testosterone administration, aging, brain

TUE-396
Enhancement of bioethanol production from peanut shells with soy flour supplementation

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Today, fossil fuels are exhausting with a high rate according to the energy demand. Therefore, alternative energy sources like biofuels are very important to being explored to replace fossil fuels. Among biofuels, bioethanol is considerable according to the usage of it as petrol additive or substitute. In the current study, bioethanol production from peanut shells was investigated and the efficiency optimization of the production was induced by nitrogen rich additive. In Turkey, peanut is produced in abundance; therefore the waste of it (peanut shell) can be used for ethanol production which would be a very cheap way to produce biofuels. In this study, peanut shells were pretreated with acid hydrolysis and autoclave. Then, soy flour was added into this pretreatment solution. Bioethanol production experiments were carried out with *Saccharomyces cerevisiae* at pH 5, 30°C in a rotary shaker (100 rpm). Ethanol production was determined by Gas Chromatography (GC; Shimadzu Japan). Reducing sugar content was determined by spectrophotometrically. Optical density and dry biomass of the yeast were also investigated. The data obtained from the trials showed that with an increase in peanut shell, yeast growth and usage of sugar were also increased. In media with 10% (w/v) shell and 0.1% (w/v) soy flour, usage of sugar was 40% and alcohol production were two times higher than in media with 10% (w/v) shell and without soy flour. This data indicated that soy flour increased bioethanol production.

Keywords: Bioethanol, Peanut shells, Yeast.

TUE-398
Expression and activity of extracellular ectoenzymes (CD39 and CD73) in aortic valves cells and in endothelial cells under the shear stress conditions

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Extracellular nucleotides have major impact on various pathological processes like thrombosis or inflammation. Ectonucleoside triphosphate diphosphohydrolase 1 (CD39) and ecto-5′-nucleotidase (CD73) are ectoenzymes involved in extracellular nucleotide

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breakdown cascade. Changes in activity of those enzymes affect the concentrations of adenosine which mediates anti-inflammatory response as well as influence the levels of ATP which is known pro- inflammatory mediator. The deregulation of ratio between adenosine and ATP might be involved pathological valve calcification.

Therefore, we characterise extracellular nucleotide metabolism in valve cells and the area of expression of ectoenzymes in healthy and calcified valves was determined. Aortic valves were analyzed for CD39 and CD73 expression. In order to verify presence and activity of CD39 and CD73, we characterised nucleotide metabolism by incubation of aortic valve endothelial (AVEC) and interstitial cells (AVIC) – human and porcine origin – with AMP and ATP *in vitro* and conversion of substrates into products was analysed by HPLC. Moreover, we hypothesised that expression and activity of enzymes might be regulated by blood flow and therefore we investigated the effect of flow on different types of endothelial cells in bioreactor mimicking ventricular and aortic flow.

Our results prove that CD39 and CD73 are highly expressed in healthy valves and expression of both enzymes is downregulated in calcified valve, an exception is calcified regions of human valve where CD73 is upregulated. Enzyme activity assay showed a high activity of CD39 and CD73 in both types of cells. We found upregulation of CD73 expression (pic.) and high concentration of adenosine in valve interstitial cells, what might be of great importance as this enzyme is typically absent in fibroblast-like cells. In response to shear stress significant upregulation of both enzymes was found in endothelial cells in comparison to static culture conditions.

In conclusion, these findings highlight a novel role of CD39 and CD73 in heart valve physiology. High activity of those enzymes and in consequence higher levels of anti-inflammatory adenosine could be adaptive and protect heart valves from inflammation and calcification. Ventricular and aortic flow could play an important role in regulation of ectoenzymes expression in endothelial cells what induces increase in nucleotide metabolism. Our data from shear stress system indicate that disturbance of flow has impact on the expression and activity of ectoenzymes in endothelial cells.

Keywords: adenosine, ATP, valve.

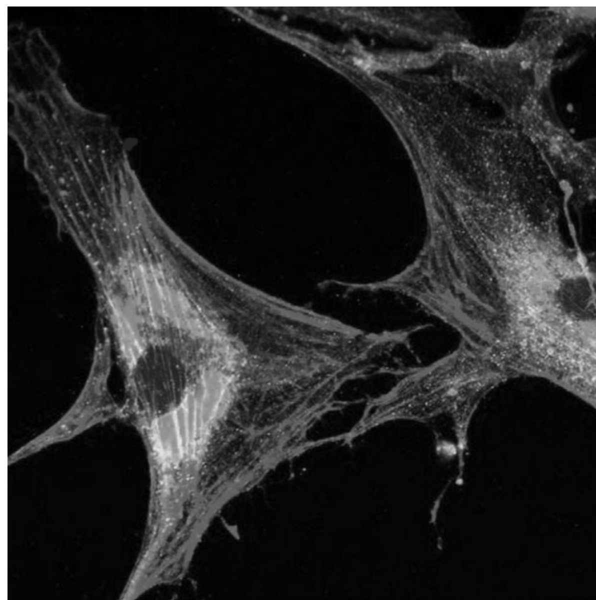

Fig. 1.

TUE-399

Expression of genes responsible for the synthesis of nucleic acids precursors, methionine and cysteine metabolism in human placenta of the first and the third trimesters of gestation

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Introduction: The folate-mediated interdependent reactions responsible for the synthesis of nucleic acids precursors, mitochondrial translation, the utmost majority of methylation reaction, the oxystatus and the maintainance of the balance of methionine and common amino acides play an essential role in placental physiology and in ethiology and pathogenesis of pregnancy complications and birth defects. However, the physiological characteristics of folate-mediated players in human placenta and their pathological counterparts are not still well known.

The aim of the study: was to examine the expression of the genes encoding the enzymes catalysing the synthesis of nucleic acids precursors (*GART, ATIC)*, methionine *(MTR)* and cysteine metabolism viz. its catabolism to taurine (*CDO, CSAD)* and the synthesis of glutathione (*GCL)*. The object of the study was human placenta from the first and the third trimester of uncomplicated pregnancies.

Methods: Gene expression was evaluated by the amount of individual RNAs detected with RT-PCR in real-time using standard curves for absolute quantification of the values.

Results: The analysis of gene expression at mRNA level has revealed that in the first trimester the abundance of examined RNAs is in the range between the minimal values of 7.0 copies per ng of total RNA and the maximal one – 320.0 copies.The abundance of all examined RNAs but *CDO* decrease with the rate of changes characteristic for each RNA: the levels of *GART* and *ATIC* mRNAs responsible for the two sequentional steps of purine ring synthesis, are 3 times less though the ratio *ATIC*/*GAR*≈3/1 remains constant at both periods of gestation; the level of *MTR* RNA decreases two times thus being the precursor of SAM synthesis it points to the potential diminished requirements for SAM activity at term; the nearly two times down-regulated *GCLC* mRNA raises the question on the oxystatus in the term placenta; the opposite changes in the abundance of CDO and CSAD RNAs (CDO/CSAD ≈3 in the first trimester and 70 at term due to the 10-fold increase of CDO and 2-fold decrease of CSAD levels) attract the attention and requires the further studies to explain the role of these unexpected enormous changes during development of placenta.

Conclusions: The results have given a new insight into the regulation of gene expression in the developing placentae. They may be used as reference marks for the study of placental metabolism in complicated pregnancies.

Keywords: folate-mediated 1-carbon metabolism, human placenta.

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TUE-400
Expression of pro-proliferative factors in subcutaneous adipose tissue of obese men with and without glucose intolerance

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Obesity, metabolic syndrome, and type 2 diabetes result from interactions between genes and environmental factors and are the most profound public health problems. In obese individuals adipose tissue is at the center of metabolic syndrome. We sought therefore to clarify the molecular mechanisms of subcutaneous adipose tissue growth and development of impaired glucose tolerance in men through investigation of pro-proliferative gene expressions. The expression of *SPARC, CYR61, MYLK, HSPA6, CTGF, UBD,* and *ALCAM* genes was studied in subcutaneous adipose tissue from 18 adult males divided into three equal groups: lean controls and obese men with normal glucose tolerance (NGT) and impaired glucose tolerance (IGT). We have shown that the expression levels of *CYR61, MYLK, CTGF, UBD,* and *ALCAM* genes were increased in subcutaneous adipose tissue of obese (NGT) men versus lean controls and these changes positively correlated with increased body mass index (BMI). More robust effect was shown on *CTGF, UBD,* and *ALCAM* gene expressions. At the same time, no significant changes were observed in *SPARC* and *HSPA6* gene expressions in adipose tissue of this group of patients. We also demonstrated that the expression of *SPARC, HSPA6,* and *ALCAM* genes in subcutaneous adipose tissue of obese men with glucose intolerance is increased versus obese (NGT) individuals, with more robust effect on *HSPA6* gene. However, in this group of patients the expression of *CYR61, MYLK, CTGF,* and *UBD* genes is decreased versus obese (NGT) individuals.

Present study demonstrates that increased expression of *CYR61, MYLK, CTGF, UBD,* and *ALCAM* genes in subcutaneous adipose tissue of obese men with NGT is associated with obesity because positively correlated with increased BMI and possibly participate in development of obesity. At the same time, glucose intolerance in obese men is associated with strong increase of *SPARC, HSPA6,* and *ALCAM* gene expressions as well as with suppression of *CYR61, MYLK, CTGF,* and *UBD* gene expressions in subcutaneous adipose tissue of obese men.

Keywords: Metabolic syndrome, Obesity, Pro-proliferative factors.

TUE-401
Extramitochondrial oxidative phosphorylation: characterization and quantification of cardiolipin in myelin sheath

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Cardiolipin is a phospholipid with unique properties, usually associated with the inner mitochondrial membrane. It was demonstrated that Cardiolipin plays an important role in the membrane structure and function. In particular, the presence of cardiolipin is essential for the energy metabolism, since it is associated with many complexes of the respiratory chain, which are involved in the transfer of electrons and protons for the production of ATP in the mitochondrial inner membrane. Our laboratory recently speculated on a role for cardiolipin as H^+ shuttle between F_oF_1-ATPsynthase and the electron transport complexes [1]. Furthermore, cardiolipin is necessary for the maintenance of oxidative phosphorylation (OXPHOS) supercomplex, which consist of multiple OXPHOS complexes resulting in efficient clustering of this metabolic pathway. In this work, we searched for cardiolipin in myelin, the sheath which surrounds axons in central and peripheral nervous system. In fact, our studies showed that, besides mitochondria, also myelin expresses functional OXPHOS complexes [2]. Thin layer chromatography analyses were performed on lipid-enriched fractions extracted from myelin. Also immunohistochemistry and spectrophotometric analyses on optic nerve sections and isolated myelin, respectively, were carried out, employing the specific cardiolipin probe 10-N-Nonyl acridine orange (NAO). Native electrophoresis analyses were preformed on isolated myelin to assess the presence of the OXPHOS supercomplex. The positive preliminary data as well as our previous observations, reinforce the hypothesis of a specific energetic role played by myelin toward the axon.

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Keywords: Cardiolipin, Myelin sheath, oxidative phosphorylation.

TUE-402
Fatty acid composition and functional state of mitochondria from hardened and unhardened winter wheat seedlings after a cold shock

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The relation among the oxidative stress development, fatty acid composition, membranes intactness and functional activity of mitochondria in winter wheat seedlings under cold hardening and freezing temperatures has been studied in this work. Mitochondria were isolated from control and hardened at 2°C for 7 days (the first phase of cold hardening) and 2°C for 7 days with subsequent −2°C for 2 days treatment (two phases of cold hardening) etiolated winter wheat seedlings subjected to the action of the freezing temperature −8°C. Duration of the exposure temperature −8°C for 6 h was lethal to unhardened seedlings, while hardened seedlings successfully resisted the destructive action of the freezing temperature. Hardening of winter wheat seedlings, accompanied by increased frost hardiness, prevented the development of oxidative stress in the tissues of shoots during subsequent cold shock. The content of reactive oxygen species (ROS) and products of lipid peroxidation (LP) in mitochondria of hardened winter wheat seedlings, subjected the cold shock was less than in the mitochondria from the unhardened seedlings. Analysis of fatty acid composition displayed the highest content in winter wheat mitochondria of the palmitic (14.6%), linoleic (46%) and α-linolenic (26.1%) acids. The content of α-linolenic acid was maintained at a high level in the mitochondria of hardened seedlings subjected the cold shock, while it decreased in mitochondria from the unhardened seedlings after the cold shock. Higher content of α-linolenic acid in mitochondria corresponded to higher intactness of the outer membrane. The freezing temperature effect on the unhardened seedlings resulted in a significant decrease of oxidative and phosphorylating activities in the isolated mitochondria, while the respiration rate and energy activity in mitochondria of hardened seedlings subjected the cold shock, remained stable. Thus, the functional activity of mitochondria under the cold shock depends on the ROS content and is

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determined by the fatty acid composition, among which the α-linolenic acid plays an important role for the preservation of mitochondrial activity. The entire complex of the parameters (the contents of ROS and LP products, fatty acid composition and oxidative and phosphorylating activity of mitochondria) determines the development of properties of winter wheat cold and frost hardiness.

Keywords: fatty acid composition, mitochondria, reactive oxigen species.

TUE-403
Fatty acid composition of maternal blood and cord blood of women who delivered preterm babies, and full-term small for gestational age infants

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Background & Aims: Fatty acids are required by the developing fetus as a source of energy, to maintain the fluidity, permeability and conformation of membranes and as precursors of many compounds which are necessary in structural and metabolic processes, especially in the central nervous system. The fatty acid composition of maternal blood and cord blood throughout the period of pregnancy is fairly well understood. What is not known, however, is the fatty acid composition of the above-mentioned biological fluids at the interface of physiology and pathology of pregnancy. We therefore decided to analyse and compare the differences in the FA profile of mothers who delivered small for gestational age neonates born at term and infants delivered at 35–37 weeks of gestation, that is "late preterm".

Subject/Methods: The study population was selected randomly. To obtain a homogeneous group of women, the following inclusion criteria were applied: Polish nationality (excluding naturalised Polish citizens); single pregnancy; pregnancy I-III; stable socioeconomic status; secondary or higher level of education; living in a highly industrialised urban region. Women who participated in the research program were classified into two groups according to the following criteria: Group PTB: mothers who gave birth prematurely – between 35–37 weeks (bw 10th–90th percentile). Group SGA: mothers who gave birth to full-term but small for gestational age (SGA) neonates (bw <10th percentile). The fatty acids were analysed by gas chromatography equipped with MS detector.

Results: Maternal peripheral blood contained higher levels of C18:3n6, C20:1, C22:0, C20:5n3 and C24:1 in group B. On analysis of the fatty acid profile of the cord blood, C10:0, C12:0, C14:0, C16:1c, C18:1c, C20:3n6, C20:4n6, C22:6n3 and ∑n6 differed statistically between the two groups. C18:1c, C22:6n3 and ∑n6 were higher in group C. Other fatty acids were more prominent in group B. $P \leq 0.05$.

Conclusion: In our research we have undertaken to determine the dynamics of changes in the fatty acid profile of maternal blood and cord blood of mothers who gave birth to babies at term but small for gestational age, and to infants born between 35 and 37 weeks of gestation. Our studies have shown that the fatty acid profiles of the studied biological materials differed. The FA profiles of both maternal blood and cord blood undergo changes in response to pregnancy duration and the presence of reduced fetal growth. The differences in the FA composition of these groups lead to the conclusion that both "late prematurity" and reduced neonatal weight of children born at term affect the FA composition of maternal and cord blood. Despite a slight degree of deviation groups B and C are not generic against each other.

Keywords: Fatty acids, low birth weight, preterm, SGA.

TUE-404
Features of metabolism of arginine and its derivatives in placenta

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Background: An important problem of perinatal biochemistry is the detection of the functional role of amino acids and their derivatives in the provision of the normal development of placenta. One of the leading places among free amino acids is taken during gestation by arginine, which may be an essential amino acid for a fetus (especially under pathologic conditions) delivered to him from placenta – an intermedium between organisms of a mother and fetus. The role of arginine as a plastic, energy material as well as an independent signaling molecule determines negative consequences of the disturbance of its placental metabolism.

The aim of this research is the study of content of arginine, proline, nitric oxide (NO) and its derivatives as well as activity of arginase and NO-synthase in placenta of women in placental insufficiency (PI).

Methods: Full-term placentas were obtained after delivery from women with physiological pregnancy ($n = 25$) and PI ($n = 30$). Arginine and proline content in extracts of placental tissue was determined using an automatic analyzer. The activity of enzymes, content of NO metabolites, peroxynitrite and other nitro compounds were evaluated using kits.

Results: Arginine level and arginase activity in placenta in PI are rapidly reduced as compared with the control values. At the same time, the production of NO and NO-synthase activity under these conditions are reduced resulting in the change of the balance of vasoactive components and disturbance of the feto-placental blood circulation. In addition to modification of hemodynamic processes, deviations in arginine metabolism may be accompanied with structural changes in vascular connective tissue as a result of reduction of proline concentration (required for the synthesis of collagen). The interrelation between changes in the content of arginine and proline is confirmed by the direct correlation between them. Significant changes are also detected for NO derivatives: peroxynitrite, S-nitrosoglutathione and nitrotyrosine, the placental level of which increases in PI. Oxidative properties of these products increase the disturbance of placental homeostasis (especially in case of NO-protein modification).

Conclusion: The received results indicate an important role of disturbances of metabolism of arginine and its derivatives in the mechanisms of PI formation.

Keywords: arginine and its derivatives, placenta.

TUE-405
Fibroblast growth factor receptor 2 gene, tumor's hormone receptor status and breast cancer risk in Kazakhstan population

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Fibroblast growth factor receptor 2 (*FGFR2*) encodes a transmembrane tyrosine kinase receptor and its expression is increased in 5–10% cases of all breast tumors. Some SNPs in *FGFR2* gene have the clear relevance to breast cancer (BC) as was shown by GWAS. The associations described by GWAS are not universal for all word populations. Therefore three polymorphisms (rs2981582, rs17542768, rs17102287) of *FGFR2* gene were investigated in Kazakhstan population in this study. Multinational pop-

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ulation of Kazakhstan totals 16.9 million with main ethnic groups presented Kazakhs (Asians) – 65.1% and Russians (Europeans) – 21.9% according to statistical data.

The case-control study of 615 BC cases (376 Kazakhs – 49.7 ± 11.2 years, 239 Russians – 51.6 ± 9.0 years) and 530 controls (283 Kazakhs – 49.2 ± 7.5 years, 247 Russians – 49.7 ± 7.5 years) was performed by PCR-RFLP. Estrogen receptor (ER+/ER-), progesterone receptor (PR+/PR-) and HER2 status were estimated in tumors. The informed consent was obtained from BC patients and donors. Pearson χ^2 p value (P) and Fisher's exact test p value (P_f), odds ratios (OR), 95% confidence intervals (CI) tests, dominant and recessive models were applied to data analysis.

The association of rs17542768 with BC was revealed in Russians. Significant difference was detected in allele distribution ($P=P_f=0.04$). The risk G allele has OR value 1.49 (95% CI=1.02–2.16) in Russians. No significant differences were registered in alleles and genotypes distributions at rs2981582, rs17102287 variants of *FGFR2* gene in Russian and Kazakh ethnic groups by dominant and recessive model analysis. All investigated groups were in Hardy-Weinberg equilibrium. Further we examined the association of genotypes and hormone status of BC patient's tumors. This part of analysis have revealed the statistically significant association of *FGFR2* rs17542768 polymorphism with BC in PR+/ER+/HER2- case subjects in the Kazakh ethnic group (P = 0.01 for alleles, P = 0.04 for genotypes, P_f=0.02 for both). The A allele of rs17542768 can be considered as a risk factor in ER+/PR+/HER2- Kazakh BC patients (OR=3.81; 95% CI=1.25–11.64). But these data should be clarified additionally to validate rs17542768 as the hormone related cancer risk factor in Kazakhs because of CI's wide range.

In conclusion, the effect of rs17542768 on risk BC is limited to PR+/ER+/HER2- tumors only in Kazakhs. The G allele of rs17542768 has association with BC without regard for tumors hormone status in Russians.

Keywords: FGFR2, hormone-related breast cancer, Kazakhstan population.

TUE-406
FT-IR spectroscopy in the search for specific metabolic biomarkers for the identification of micromycetes

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Introduction: Identification of micromycetes is time-consuming due to cultivation and microscopic examination of culture morphology and can be relatively expensive due to application of molecular techniques. Moreover, some of these micromycetes are dangerous, because fungal spores have been found to contain specific toxic and non-toxic metabolites which can lead to development of several diseases. Therefore, direct, accurate and robust detection of fungi and their specific metabolic biomarkers is very essential. Fourier-transform infrared (FT-IR) spectroscopy appears very promising as a perfect tool to detect and characterize the main components of such chemically very complex probes as micromycetes.

Purpose: The aim of this study was to find species-specific metabolic biomarkers of micromycetes using FT-IR spectroscopy. For this purpose, the chemical composition of spores from five species of micromycetes – *Pichia pastoris, Ulocladium chartarum, Saccharomyces cerevisiae, Trichoderma viride* and *Penicillium sp.* – was characterized.

Method: The infrared analysis was performed using Nicolet 8700 FT-IR spectrometer (Thermo Scientific). All the spectra were acquired in the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} and analyzed using OriginLab 9 (OriginLab Corporation) and MatLab 7.12.0 (The MathWorks) software. This study was carried out in the Centre for optical and laser materials research of St. Petersburg State University.

Results: The FT-IR analysis enabled us to identify differences and similarities for all five fungal species in the spectral regions known to be characteristic for certain chemical structures and groups. All the examined fungi show the characteristic infrared absorbance of C-H region dominated by fatty acids (3050–2800 cm^{-1}), polypeptide bonds (amide I at 1600–1700 and amide II at 1540 cm^{-1}), and polysaccharide groups (1200–900 cm^{-1}). Another small band appears at 1587 cm^{-1}, possibly due to deacetylated chitin. Bands at 550–900 cm^{-1} region are typical for low-molecular-weight organic compounds. Of particular interest are regions 1200–1500 cm^{-1} and 1700–2700 cm^{-1} which are different for the examined micromycetes and can be considered as fingerprint regions for their identification. FT-IR profile of strong bands at these regions rules out the presence of short aliphatic structures and suggests the occurrence of acid and/or conjugated groups, compatible with fungal metabolites and other aromatic structures.

Conclusion: Our data suggest that FT-IR spectra display a fingerprint of micromycetes under study. As FT-IR spectroscopy is less time-consuming and less expensive method compared to microscopic and molecular techniques, it can be successfully applied for the routine identification of fungal species.

Keywords: FT-IR spectroscopy, Metabolism, Micromycetes.

TUE-407
Generation, release and uptake of the NAD precursor nicotinic acid riboside by human cells

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Nicotinamide adenine dinucleotide (NAD) is essential for cellular metabolism and has also a key role in a variety of central signaling pathways. It is used as substrate by several classes of enzymes including ADP-ribosyltransferases and protein deacetylases (Sirtuins) that control gene expression, progression of the cell cycle, DNA repair, cell death and aging. Given the considerable turnover, NAD must be permanently resynthesized. The vitamin B3 precursors for NAD biosynthesis in humans are nicotinamide (Nam) and nicotinic acid (NA) as well as their corresponding ribosides (NR and NAR). So far, little is known about the relationships between intra- and extracellular pools of NAD and its key metabolites in human cells. Our recent studies have established that all known NAD metabolites can serve as extracellular precursors of intracellular NAD. However, nucleotides are degraded to the ribosides NR or NAR, which then enter cells as NAD precursors.

In this work we observed that human cells convert NA to its riboside, NAR, which is subsequently released into the culture medium. HepG2 cells lack NAPRT activity (converting NA to the mononucleotide NAMN) and are therefore normally unable to utilize NA as NAD precursor. Transient transfection with a vector encoding NAPRT enables these cells to survive in the presence of NA as the single usable source for NAD synthesis. Surprisingly, transient expression of NAPRT rescued not only the transfected

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cells, but essentially all cells in the culture. Since NA could not be metabolized by the NAPRT-deficient cells, an alternative NAD precursor must have been produced and released by the transfected cells. NMR-based analyses of NAD metabolites in the culture medium revealed that the riboside NAR was released by the NAPRT-positive HepG2 cells. These results have important implications: Since NAPRT converts NA to the NAMN, an intracellular 5′-nucleotidase must have cleaved NAMN to generate the riboside, NAR. Moreover, the observed survival of untransfected cells suggests that, in the presence of sufficient amounts of a suitable precursor (such as NA for the transfected cells), human cells can produce and release riboside derivatives. Importantly, these ribosides are taken up by neighboring cells to support their NAD metabolism.

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Disclosure of Interest: None Declared.
Keywords: NAD metabolism.

TUE-409
Ginsenoside Rg3 rejuvenates cellular senescence of human diploid fibroblasts

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Aging is a multifactorial event resulting from the accumulation of cellular damage over time, which leads to physiological deterioration, increased mortality and eventual death. Primary human diploid fibroblasts (HDFs) undergo a limited number of cell divisions in culture and ten reach the replicative senescence. Senescent HDFs have some features such as cell cycle arrest, limited cell division, morphological change, increase of reactive oxygen species (ROS) levels and no response to mitogenic stimuli. Ginsenoside, a class of steroid glycosides and triterpene saponins, found primarily in the roots of *Panax ginseng*, has many remedial effects such as anti-inflammation and anti-tumor.

In this study, we examined the effects of ginsensodies 20(S)-Rg3 on the replicative senescence of HDFs. When the senescent HDFs were incubated with 20(S)-Rg3 for 48 h, the senescence associated-b-galactosidase (SA-b-gal) activity was dramatically decreased only in 20(S)-Rg3-treated HDFs. Moreover, 20(S)-Rg3 reduced ROS level and changed protein expression level of superoxide dismutase (SOD) in senescent HDFs. Western blot analysis demonstrated that 20(S)-Rg3 led to the inactivation of p53 and subsequently down-regulated the expression of $p21^{waf1/cip1}$ in senescent HDFs. In additions, 20(S)-Rg3 inhibited the phosphorylation of Akt/mTOR and AMPK whereas 20(S)-Rg3 improved expression of downstream effectors of mTORC1 such as eukaryotic initiation factor 4E-binding proteins (4E-BPs) and S6 kinase (S6K). On the other hand, 20(S)-Rg3 treated in HDFs influenced Sirtuins and GSK3β expression. Taken together with preliminary data, ginsenoside 20(S)-Rg3 suggests inducing the rejuvenation of senescent HDFs by scavenging ROS and restoring cellular senescence via enhanced cell cycle progression and modulated Akt/mTOR/AMPK signaling.

Keywords: ginsenoside Rg3, human dermal fibroblast, rejuvenation.

TUE-410
Globotriaosylceramide and globotriaosylsphingosine induce epithelial to mesenchymal transition in kidney cell lines

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Fabry disease is a lysosomal storage disorder caused by deficiency of α-galactosidase A (α-gal A), resulting in deposition of globotriaosylceramide (Gb3) in the vascular endothelium. Deacylated Gb3, globotriaosylsphingosine (lyso-Gb3), also accumulated and increased it plasma level in the patients with the Fabry disease. According to renal and cardiac pathological findings, fibrosis due to epithelial-to-mesenchymal transition (EMT) could be a possible pathogenic mechanism in Fabry disease. However, the association of EMT with Fabry disease has not been elucidated yet. Expression level of EMT markers such as E-cadherin, N-cadherin, β-catenin and α-SMA in mouse renal glomerular mesangial cell (SV40MES13) significantly increased when treated with Gb3. Whereas, in human proximal renal tubular epithelial cell (HK-2), expression of EMT markers were significantly altered when treated with lyso-Gb3 rather than treated with Gb3. Additionally, we observed that the activation of TGF-β, phospho-AKT, and PI3K were highly elevated in these cells. Also, inhibitor of the TGF-β signaling and recombinant α-galA have been shown to block EMT-like processes according to Gb3 or lyso-Gb3, and following knockdown of α-galA gene expression by siRNA, high TGF-β expression as well as high Gb3 expression were observed, suggesting that EMT was induced. Our study demonstrates that Gb3 and lyso-Gb3 can potentiate EMT-like processes through AKT/PI3K signaling pathway in kidney mesangial cell and tubular epithelial cell, respectively. Moreover, these findings suggest that not only accumulated Gb3 but also plasma lyso-Gb3 might be a crucial role for nephropathy of Fabry disease by inducing EMT and may contribute to a better understanding of the renal fibrosis.

Keywords: epithelial-to-mesenchymal transition (EMT), Fabry disease, lyso-Gb3.

TUE-411
Glucose concentration is distinctive for *Escherichia coli* hydrogenase 4 (hyf) activity

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Escherichia coli produces molecular hydrogen (H_2) during sugars (glucose), glycerol or other carbon sources fermentation [1]. This production of H_2 occurs via special enzymes namely hydrogenases (Hyd) which reversibly oxidize H_2 to $2H^+$. *E. coli* is able to encode four membrane-bound [Ni-Fe]-hydrogenases [1]. Hyd-1 (*hya*) and Hyd-2 (*hyb*) are reversible enzymes which can operate in different mode depending on carbon source: during glucose or glycerol fermentation they operate in H_2 uptake or producing mode, respectively [2]. Hyd-3 (*hyc)* and Hyd-4 (*hyf*) are H_2 producing enzymes upon glucose fermentation but these enzymes operate in reverse mode during glycerol fermentation [3]. Dependence of Hyd-4 H_2 producing activity (V_{H2}) on glucose concentration at different pHs was studied.

During growth on 0.2% glucose at pH 7.5 in Hyd-4 defective mutants *hyfA-B* and *hyfB-R* V_{H2} was decreased ~6.7 and ~5 fold, respectively, compared to wild type. Only in *hyfB-R* mutant at pH 6.5 and 5.5 V_{H2} was severely decreased ~7.8 and ~3.8 fold, respectively. But surprisingly when cells were grown on 0.8% glucose no any difference between wild type and mutant strains were

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detected at different pHs. Inhibitory effect of glucose on Hyd-4 activity was shown when cells were grown on 0.8% glucose. Taken together these results indicate that *E. coli* Hyd-4 H_2 producing activity is inhibited by high concentration glucose mainly at pH 7.5.

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Keywords
Escherichia coli, Glucose concentration, Hydrogenase 4 activity.

TUE-412
Glutathione biosynthesis in a cold-adapted microorganism

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Glutathione (GSH) is a powerful regulator of the physiological redox environment in eukaryotes and prokaryotes. Its antioxidant action, including defence against oxidative damages, detoxification of foreign compounds and toxic metals, preservation of reduced state of protein sulfhydryls, is involved in several cellular pathways. The mechanism of redox homeostasis is mainly based on the intracellular balance between GSH and its oxidised form, GSSG. Biosynthesis of GSH occurs with a mechanism conserved throughout prokaryotes and eukaryotes and involves two sequential steps, both coupled to ATP hydrolysis. The first step, catalysed by g-glutamyl-cysteine ligase (GshA), leads to the formation of g-glutamylcysteine and the second one, producing GSH, is catalysed by glutathione synthetase (GshB).

GSH has a more crucial role in microorganisms exposed to oxidative stress conditions, such as the psychrophile *Pseudoalteromonas haloplanktis* isolated from the Antarctic sea. To characterize the enzyme system for GSH biosynthesis in the first cold-adapted microorganism, recombinant forms of GshA and GshB from *P. haloplanktis* (r*Ph*GshA II and r*Ph*GshB, respectively) were produced and characterized (Albino et al. *Mol BioSys* 8, 2012, 2405–2414; *Biochimie*, in press). The investigation covered the study of the substrate specificity of both enzymes, setting up the best ionic and pH conditions for triggering their activities, determination of K_m values for all substrates of the catalysed reactions. Both enzymes were already active at 15°C, as required for their cold adaptation. Interestingly and differently from what observed in eukaryotic systems, the reaction rate of r*Ph*GshA II was higher than that reported for r*Ph*GshB, thus suggesting that formation of g-glutamylcysteine was not the rate limiting step of GSH biosynthesis in *P. haloplanktis*. The inhibitory effect of GSH and GSSG on glutathione synthesis was investigated. Indeed, GSH acted as a non-competitive inhibitor of r*Ph*GshA II and GSSG caused the mono-glutathionylation of the enzyme on the target residue Cys 386; vice versa, GSSG acted as an irreversible inhibitor of r*Ph*GshB, forming a disulfide adduct with the enzyme. When compared to r*Ph*GshB, r*Ph*GshA II possessed more typical features of a psychrophilic enzyme, as it was endowed with lower thermodependence and higher heat sensitivity. Curiously and differently from other prokaryotes, *P. haloplanktis* harbors another redundant g-glutamyl-cysteine ligase (*Ph*GshA I), whose characterization is in progress.

Keywords: cold-active enzymes, Glutathione biosynthesis, Pseudoalteromonas haloplanktis.

TUE-413
Heat shock protein 72 protects against lipid-induced insulin resistance via upregulation of AMPK in skeletal muscle

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Heat shock protein 72 (Hsp72) is known to protect against obesity-induced insulin resistance, a condition in which cells fail to respond to insulin. Insulin resistance is associated with many health related complications, including type 2 diabetes and heart disease. It is also known that expression of Hsp72 in human skeletal muscle is positively correlated with insulin sensitivity but the underlying molecular mechanisms are not well understood. Therefore, the aim of this study was to investigate the molecular mechanisms involved in Hsp72-associated regulation of insulin sensitivity in skeletal muscle. Herein we show that overexpression of Hsp72 decreases both palmitic acid- (16:0) and C2 ceramide-induced insulin resistance in C2C12 cells. Additionally, C2C12 cells overexpressing Hsp72 are characterized by decreased lipid content and increased phosphorylation of 5′AMP-activated protein kinase (AMPK), as well as acetyl-coA carboxylase (ACC), its downstream target. Inhibition of AMPK with compound C attenuated the Hsp72-induced improvement in insulin sensitivity in C2C12 myotubes. We also observed that overexpression of Hsp72 with an inactive ATPase domain does not affect insulin sensitivity in C2C12 myotubes. Moreover, the phosphorylation of AMPK was significantly decreased in these cells. Overall, this study showed that overexpression of Hsp72 decreases lipid accumulation and improves insulin sensitivity *via* upregulation of AMPK pathway in C2C12 cells. Furthermore, active ATPase domain in Hsp72 is required to increase insulin sensitivity and AMPK phosphorylation in myotubes.

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Keywords: None.

TUE-414
Hemorphins regulate the activity of nuclear factor-kappa B (NFκB)/p65 in streptozotocin-induced diabetic rats

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In the present work we have studied the effect of hemorphins (hemorphin-7 and LVVYPW) on expression level of NFκB transcription factor in the brain of streptozotocin (STZ)-induced diabetic rats. Recently, we have demonstrated the antidiabetic effect of LVVYPW in pathophysiology of streptozotocin (STZ)-induced diabetes. It has also been revealed that the regulatory influence of hemorphins in pathophysiology takes place on transcriptional level. Determination of NFκB/p65 in brain tissue lysates was performed using rat NFκB/p65 ActivELISA Kit (Imgenex Corpora-

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tion, San Diego, CA, USA) according to manufacturer's recommendations. The intraperitoneal injections of LVVYPW and hemorphin-7 (1 mg/kg) into fasting STZ-induced diabetic rats (male, Wistar line, 180–220 g) significantly decrease the expression level of NFκB/p65, increased in nuclear fraction of brain tissue of STZ-induced diabetic rats. In contrast, the significant increase in expression level of NFκB/p65 was found in the brain tissue cytoplasmic fraction, obtained from hemorphin-treated diabetic rats. It should be noted that we didn't observed such an increase in NFκB/p65 expression level in the brain tissue cytoplasmic fraction of rats, which didn't received hemorphin treatment.

Recently, we have demonstrated the involvement of Ca^{2+}/calmodulin/calcineurin signaling pathway in the molecular mechanisms of antidiabetic effect of LVVYPW. Calcineurin was reported to regulate the activity of NFκB by affecting the phosphorylation of IκBs (nuclear factor κB inhibitors). Earlier it has also been reported the involvement of calcineurin/NFκB pathway in pathophysiology of diabetes. Thus, data obtained indicate that hemorphins, by modulation of Ca^{2+}/calmodulin/calcineurin signaling pathway, are able to regulate the activity of NFκB in pathophysiology of diabetes. It should be emphasized that one of the molecular mechanisms of antidiabetic effect of famous antidiabetic drug metformin is inhibition of nuclear translocation of NFκB as well.

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Keywords: diabetes mellitus, hemorphin, NFkB.

TUE-415

Hepatic mitochondrial content in malondialdehyde may be a marker of sea lamprey contact with atrazine

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The atrazine attracts special attention as pollutant because of its persistence in the aquatic environment. Although this herbicide has been studied in teleost, its toxicity in the sea lamprey, *Petromyzon marinus* is still poorly understood. Oxidative stress may occur if chemical pollutants contribute to block the capacity of mitochondria to generate ATP with continuous production of reactive oxygen species (ROS), disturbing the success of *P. marinus* seawater acclimation. So, the aim of this study was to evaluate how atrazine influences the malondialdehyde (MDA), glutathione (GSH) and glutathione disulfide (GSSG) contents of gills and liver mitochondria of juveniles from Lima river basin, Portugal during salt acclimation. Sampling occurred at the beginning of the *P. marinus* downstream migration. The sampled juveniles were transported alive to the laboratory and maintained in 200 l tanks with LSS 8life support system. Two groups of 40 specimens were hold in tanks with 50 or 100 μg/l atrazine, during 30 days. The salinity was gradually increased from 0 to 35 psu, following a three step procedure during a 30 days period. The control group was maintained in freshwater without atrazine. Mitochondria obtained by centrifugation at 15000 *g*, 30 min, 4°C, of tissues homogenates prepared in 50 mM Tris-HCl pH 7.5 buffer were used in determination of ROS, MDA, GSH and GSSG by fluorescence. The statistical analysis were performed by ANOVA I and Duncan ($p < 0.05$), using SPSS 22 for Windows. The results showed that in *P. marinus* juveniles, no significant changes in the markers of oxidative stress and cell damages were detected in the mitochondrial gills. Nevertheless, in the animals exposed to 50 μg/l atrazine the content in glutathione and GSSG increased. A similar pattern of stress markers was detected in hepatic mitochondria. However, in the presence of atrazine, the MDA level of the mitochondria of liver increased threefold in the animals during salt acclimation. The high level of mitochondrial damages, detected in the hepatic mitochondria of macrophthalmia treated with atrazine, suggests that herbicide exposure caused metabolic failures which can disturb the adaptation of these specimens to the oceanic feeding phase. The hepatic mitochondrial MDA levels of *P. marinus*, may eventually detect sea lamprey contact with chlorine herbicides.

Keywords: Cell damages, Petromyzon marinus, Triazines.

TUE-416

Hepatocyte-specific knockout of Cyp51 from cholesterol synthesis results in sex-specific metabolic adaptations and liver pathologies

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Cholesterol is a key molecule in mammals serving as a major component of cell membranes and a precursor of various signaling molecules. The most striking examples of cholesterol deficiency are the inborn errors of cholesterol biosynthesis that, when compatible with life, manifest with severe whole body phenotypes. Surprisingly, liver as the principal site of cholesterol homeostasis has rarely been investigated in these pathologies. We focus on the hepatocyte-specific deletion of lanosterol 14α-demethylase (CYP51) catalyzing the rate-limiting step in the post-squalene part of cholesterol synthesis. The hepatic loss of *Cyp51*

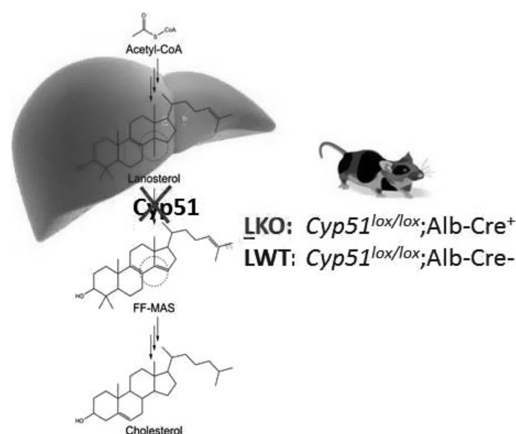

Fig. 1. Hepatocyte-specific ablation of Cyp51 from the cholesterol synthesis pathway leads to accumulation of immediate substrates lanosterol and 24,25 dihydrolanosterol and diminished hepatic cholesterol production.

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in mice causes sex-specific whole body effects with prominent hepatomegaly and hepatic ductular reaction with bridging periportal fibrosis and inflammation, but without steatosis. Accumulation of lanosterol and 24,25-dihydrolanosterol and a depletion of cholesterol esters in the liver could represent the underlying mechanistic events. Transcriptome analysis further suggested hepatocyte senescence and identified a female-specific down-regulation of peroxisome proliferation, fatty- and amino acid metabolism that probably fuel a stronger ductular reaction than in the males. The addition of dietary fats improved liver injury in the females, whereas the addition of fat and cholesterol rescued the phenotype in both sexes. *Conclusion:*

The hepatic loss of *Cyp51* in mice causes hepatic accumulation of CYP51 substrates and lack of cholesterol esters that affected the liver lipid homeostasis and extended to the entire body. The liver-specific *Cyp51* knockout mice represent a new model to study cholesterol-associated liver pathologies in the context of sex and diet. The model shows unequivocally that the disrupted post-lanosterol cholesterol synthesis represents another risk factor for liver pathologies.

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Keywords: cholesterol, Cytochrome P450, Expression regulation.

TUE-417
Hexokinase IV and its regulatory protein in the hypothalamus

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Glucokinase (GK), the hexokinase involved in glucose sensing in pancreatic β cells, is also expressed in hypothalamic tanycytes, which cover the ventricular walls of the basal hypothalamus and are implicated in an indirect control of neuronal activity by glucose. Previously, we demonstrated that GK was preferentially localized in tanycyte nuclei in euglycemic rats, which has been reported in hepatocytes and is suggestive of the presence of the GK regulatory protein, GKRP. In the present study, GK intracellular localization in hypothalamic and hepatic tissues of the same rats under several glycemic conditions was compared using confocal microscopy and Western blot analysis. In the hypothalamus, increased GK nuclear localization was observed in hyperglycemic conditions; however, it was primarily localized in the cytoplasm in hepatic tissue under the same conditions. Both GK and GKRP were next cloned from primary cultures of tanycytes. Expression of GK by *Escherichia coli* revealed a functional cooperative protein with a $S_{0.5}$ of 10 mM. GKRP, expressed in *Saccharomyces cerevisiae*, inhibited GK activity *in vitro* with a K_i 0.2 mM. We also demonstrated increased nuclear reactivity of both GK and GKRP in response to high glucose concentrations in tanycyte cultures. These data were confirmed using Western blot analysis of nuclear extracts. Results indicate that GK undergoes short-term regulation by nuclear compartmentalization. Thus, in tanycytes, GK can act as a molecular switch to arrest cellular responses to increased glucose.

Keywords: enzymatic activities, Glucokinase, regulatory protein.

TUE-418
Hierarchical classification of glycoside hydrolases

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Glycoside hydrolases or glycosidases (EC 3.2.1) are a widespread group of enzymes hydrolyzing various carbohydrates and glycoconjugates. They are represented in almost all living organisms. Their catalytic domains are grouped into 133 sequence-based families in the CAZy database (http://www.cazy.org/): GH1–GH133. 52 of these families compose 14 clans (GH-A–GH-N) at a higher hierarchical level. Enzymes of the same clan have common evolutionary origin of their genes and share the most important functional characteristics: composition of the active center, anomeric configuration of the hydrolyzed glycosidic bond, and molecular mechanism of the catalyzed reaction (either inverting, or retaining). The subfamily level of the classification exists only for GH5, GH13, and GH30 families in the CAZy database. However, extensive data on relationship between glycosidase families belonging to different clans and/or non-included into any clans are available in the literature, as well as information on phylogenetic protein relationship within particular families. Based on this information and on our complementary data we propose a multilevel hierarchical classification of glycosidases and their homologues.

According to the SCOP database, the catalytic domains of almost all families of glycoside hydrolases have one of six basic folds: β/α-barrel, β-propeller, β-jelly roll, α/α-barrel, β-solenoid, and lysozyme-type. Extensive comparative analysis of the primary and tertiary protein structures suggests the common evolutionary origin of essentially all glycosidase domains having the same type of the three-dimensional structure. This fact allows us to classify glycoside hydrolases into six main primary groups based on homology of their catalytic domains. Grouping of glycoside hydrolase families into clans at the Pfam database suggests the subsequent level in the hierarchical classification of glycosidases. Clans from the CAZy database represent a lower level, combining together the closest families. Useful information about homology-based grouping of glycosidases can be obtained from many other on-line protein classifications, including COG, KOG, PANTHER, and Génolevures. Intrafamily phylogenetic analysis of proteins suggests the lowest classification level – the subfamily level. Iterative screening of the protein database by PSI-BLAST program results in finding of interfamily relationships and, in some cases, allows to distinguish additional intermediate level(s) in the classification. Evolutionary connections of glycosidase families with other families, including families of functionally uncharacterized domains (for example, GHL1–GHL50, FURAN1–FURAN39, COG1306, COG1649, COG2342, PF11790), also can be traced by the iterative screening of the database. The obtained results allow to extend the hierarchical classification of glycoside hydrolases on their homologues.

Keywords: CAZy database, protein classification, protein evolution.

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TUE-419

High-pressure and functional studies of glyoxylate-hydroxypyruvate reductases from different Thermococcal species indicate clue to understanding the energy metabolism of deep sea Archaea

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The thermococcales representent a large part of the archaea associated with deep-sea hydrothermal vents. Previously, the archaeal glyoxylate-hydroxypyruvate reductases (GRHPR) were described as glyoxylate reductases [1]. This assignment is not consistent with the metabolic pathway of thermococcales. To specify the functional identity of this important metabolic enzyme we determined its specific activities and enzymatic parameters. For this, we cloned and expressed in E. coli of various purified recombinant GRHPR enzymes arizing from from *P.furiosus*, *P.horikoshii* and *P.yayanosii*. After purification to homogeneity, we determined their respective specific activities and enzymatic parameters, by using different substrate-cofactors combinations. The results showed unambiguously that, at atmospheric pressure, the GRHPR from Pyrococcus species are in fact NADH-dependent hydroxypyruvate reductases. Together with a survey of the presence/absence of the GRHPR enzyme in the archaeal genomes, this study allowed to place HPR enzyme in the metabolism of *Pyrococcus*. Based on this study, a model for the metabolism of thermococcales species will be presented.

The discovery of obligate piezo-hyperthermophilic archaeon such as *Pyrococcus yayanosii* suggests the existence of specific metabolic or structural adaptations with respect to high pressure [2]. We examined whether or not high-pressure conditions could influence GRHPR activity as well as their substrate specificity or NADPH/NADH requirement. Since *P.furiosus*, *P.horikoshii* and *P.yayanosii*, were isolated at different depth and display different pressure optima for their cultivation, we characterized the HPR enzymatic properties at different pressures. This work revealed a modulation of the HPR activities by high pressure. Interestingly, the behavior of HPR enzymes under pressure is modulated by the substrates and the cofactors and we showed that NADPH tends to protect the enzymes from high-pressure inhibition. In order to understand the molecular basis of this pressure the X-ray structures of PfuGRHPR and PyaGRHPR were solved and compared. High pressure X ray crystallography was also employed to study the effect of high pressure on the HPR active sites and quaternary structures. These analyses will be presented and discussed.

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Keywords: extremophiles, Metabolism, Pressure.

TUE-420

Human carbamoyl phosphate synthetase: structure, function and pathology

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Carbamoyl phosphate synthesis from bicarbonate, ammonia and two molecules of ATP, catalyzed by carbamoyl phosphate synthetase 1 (CPS1), is the first step of of the urea cycle, which detoxifies the ammonia produced in protein catabolism. CPS1, a large (1462-residue) multidomain protein having two ATP-binding phosphorylation sites, is very abundant in liver mitochondria (20% of the matrix protein), and is inactive in the absence of the allosteric activator N-acetyl-L-glutamate (NAG). CPS1 deficiency (CPS1D) is an inborn error causing hyperammonemia leading to death or mental retardation. The report of CPS1 regulation by multiple lysine acylation and by deacylation by sirtuin 5 connected the urea cycle with the age-control machinery (Nakagawa et al. Cell 2009; 137:560). The only structure known for a CPS was that of the *Escherichia coli* enzyme, which only has 40% sequence identity with CPS1 and differs from it in key traits such as the use of glutamine instead of ammonia as preferred sub-

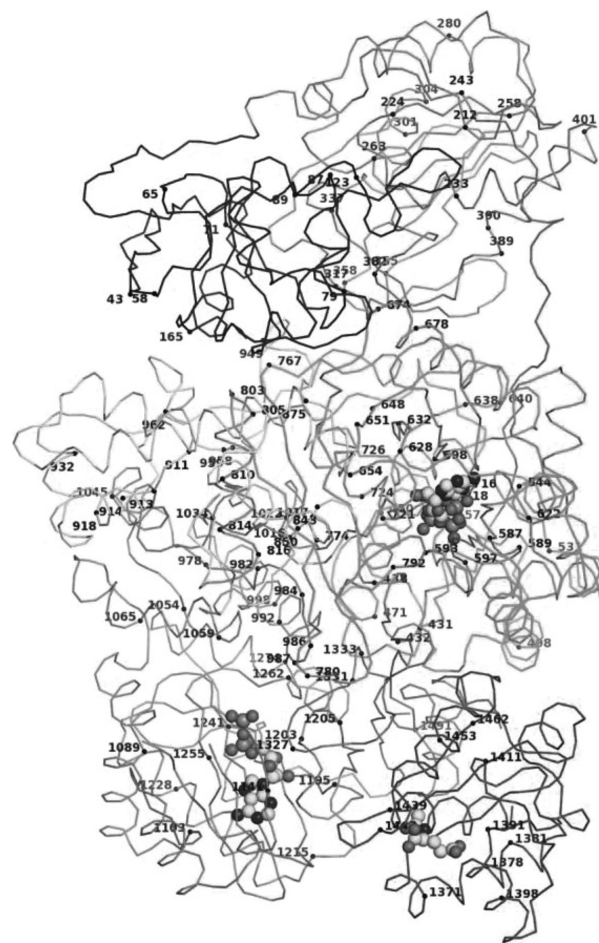

Fig. 1. Structure of active human CPS1 with spheres and residue numbers marking clinical mutations.

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strate (CPS1 cannot use glutamine), its insensitivity to NAG, and for being active in the absence of effectors. Thus, determination of the CPS1 structure appeared essential for understanding CPS1 function and its control by the NAG switch (an extreme case of allosteric activation) and by acylation, and to judge about the pathogenicity of the >130 CPS1 missense mutations reported in CPS1 deficiency. Exploiting our recent baculovirus/insect cell system for recombinant human CPS1 production (Diez-Fernández et al., Hum Mutat. 2013; 34:1149–59), we have crystallized the human enzyme and determined its X-ray structure at up to 2.4 Å-resolution, in apo and ligand-bound (NAG and ADP/Pi) forms. The liganded structure revealed how does NAG bind in a pocket of the C-terminal domain and has identified elements that are stabilized by ADP binding, as well as conformational changes induced by NAG and ADP binding that lead to define the carbamate tunnel, which in the apo form is heavily branched and open to the environment. Our structures decipher the CPS1 inability to use glutamine and reveal a potential channel for ammonia intake. Furthermore, they help rationalize the disease-causing role of most clinical CPS1 mutations. *Supported by Fundación Alicia Koplowitz and Valencian (Prometeo 2009/051) and Spanish (BFU2011-30407; FPU to CD-F) governments.*

Keywords: allosteric activation, protein structure, urea cycle.

TUE-421
Hypothalamic stearoyl-CoA desaturase-2 (SCD2) controls whole body energy expenditure

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Stearoyl-CoA desaturase 2 (SCD2) is the main δ9 desaturase expressed in the central nervous system. Knockout SCD mice unveil a tissue-specific role of this gene on obesity. However, hypothalamic SCD role on obesity has not yet been addressed. Because of its potential involvement in the control of whole body adiposity, we evaluated the expression and function of SCD2 in the hypothalamus of mice. The levels of SCD2 in the hypothalamus are similar to other regions of the central nervous system and are approximately ten-fold higher than in any other region of the body. In the arcuate nucleus, SCD2 is expressed in POMC and AgRP neurons. Upon eight weeks on high fat feeding the levels of hypothalamic SCD2 are increased in mice. Inhibition of hypothalamic SCD2 by two distinct approaches, antisense oligonucleotide and a short hairpin RNA delivered by a lentivirus directly in the arcuate nucleus, resulted in the reduction of body mass gain in control and in diet-induced obese mice mostly due to increased energy expenditure and increased spontaneous activity. Increasing hypothalamic SCD2 by a lentiviral approach resulted in no change in body mass and food intake. Thus, SCD2 is highly expressed in the hypothalamus of rodents and its knockdown reduces body mass due to increased whole body energy expenditure.

Keywords: Hypothalamus, Obesity, SCD2.

TUE-422
Identification of a haloacid inducible regulation of transcription of a glycolate operon in a *Burkholderia caribensis*

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Burkholderia caribensis MBA4 was able to utilize toxic haloacids as the carbon and energy sources. Monochloroacetate (MCA) is one of them. Genes of an inducible dehalogenase (Deh4a) and a haloacid transporter (Deh4p) were found arranged in a transcript. They were involved in dehalogenation and uptake processes, respectively.

Glycolate is a metabolite of MCA which was transformed by Deh4a. Glyoxylate was produced from oxidation of glycolate by glycolate oxidase. Three glycolate oxidases were found in *Burkholderia* species MBA4: ETY79679-81, ETY80271-3 and ETY25123, ETY84258-59. Reads per kilobase transcript per million (RPKM) of the analysis of RNA-seq technology indicated that ETY79679-81 was produced at a level of around 100 regardless of the carbon sources: pyruvate, glycolate or MCA. It seems that the production of ETY79679-81was not affected by any of the substrates. The RPKM values of ETY80271-3 were 7 in pyruvate-, 867 in chloroacetate- and 1260 in glycolate-grown cells. This is quite conventional, as expression of glycolate oxidase of model microorganism, such as *Escherichia coli* K-12, was induced by its substrate: glycolate.

Most interestingly, ETY25123 and ETY84258-59 showed its highest value (1880) in chloroacetate- grown cells which was 10 folds of that in glycolate-grown cells (178), and around 100-fold in pyruvate- grown cells (20). A malate synthase G gene, ETY84261, situated downstream of ETY25123 and ETY84258-59 gave similar response as that of ETY25123 and ETY84258-59. It is envisaged that ETY84261 and ETY25123 and ETY84258-59 were expressed in a single transcript. Putative regulator GlcC genes, ETY80275 and ETY84257 were found, located upstream of ETY80271-3, and ETY25123, ETY84258-59 andETY84261, respectively. ETY84257 was found to express more in chloroacetate-grown cells, respectively. The behaviors of the regulators were possibly different. With the differential expression, ETY84257, it is further characterized to have a complete picture of the regulatory mechanism of this glycolate operon.

Keywords: glycolate operon, regulation, monochloroacetate.

TUE-423
Identification of the main regulatory factors involved in the protective role of thiamine in baker's yeast *Saccharomyces cerevisiae* under stress conditions

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Thiamine (vitamin B_1) is an essential compound for all living organisms, mainly owing to the cofactor role of thiamine diphosphate in the central cellular metabolism. From numerous recent studies a hypothesis has emerged that thiamine, at least partly independently from the cofactor role, functions as a protectant against stress factors, e.g., by affecting the overall cell viability, the expression of antioxidant enzymes and the generation of reactive oxygen/nitrogen species under cellular stress conditions. Depending on the type of stress, the expression changes were more profound in different subcellular compartments, suggesting

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the involvement of various regulatory pathways. Hence, the current study was undertaken to identify the regulatory proteins related to the protective functions of thiamine in baker's yeast exposed to the oxidative and osmotic stress factors, i.e., 1 mM hydrogen peroxide and 1 M sorbitol, respectively.

Two main regulatory pathways associated with stress responses in baker's yeast depend on the Hog1 and Yap1 proteins. In order to test whether they could be involved in the observed protective thiamine action, two mutated strains, *hog1Δ* and *yap1Δ*, were studied. As expected, both regulatory proteins seemed to affect the stress responses, with Hog1 being predominant under osmotic stress and Yap1 under oxidative stress. Both mutated strains showed an elevated accumulation of thiamine (up to 220%) and deregulated expression of several stress markers, as compared to the wild type strain.

Further analyses aimed at the identification of transcription factors, possibly involved in the observed effects. Searching through the promoter regions of selected thiamine-associated genes using the YEASTRACT database revealed several candidates, mainly Msn2, Skn7, Sko1, Hsf1, Gis1 and Stb5. The analysis of mutated strains showed that one of them, *stb5Δ*, was severely impaired when grown without thiamine. Moreover, it was the only mutant that in the presence of thiamine and under oxidative stress was not found to downregulate common stress markers such as superoxide dismutase (*SOD2*) and glycerol dehydrogenase (*GPD1*). Some other connections to the thiamine functions under stress conditions could also be suggested for *msn2Δ* and *skn7Δ* strains.

Taken together, the current study has presented a novel insight into the contribution of thiamine to the stress responses, pointing for the first time at the possible regulatory proteins, involved in these effects in *S. cerevisiae*.

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Keywords: abiotic stress, regulatory proteins, thiamine.

TUE-424
Impacts of toxic metals on glutathione *S*-transferase activities and glutathione and protein levels in selected barley varieties

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Accumulation of heavy metals (HM) in soils has an important impact on the health of animals and humans via food chains. Either directly or indirectly, HM cause oxidative damage in plants through reactive oxygen species (ROS) formation. However, cells can detoxify the harmful effects of ROS with the help of enzymatic or nonenzymatic antioxidant defense mechanisms. Of those, glutathione *S*-transferases (GST) are a diverse group of enzymes catalyzing the conjugation of electrophilic xenobiotic substrates with the tripeptide glutathione (GSH) and found to be present from early embryogenesis to senescence in different plant tissues. On the other hand, GSH is considered as one of the most important metabolite for intracellular defense against ROS induced oxidative damage. It is also important for regulation of sulfate transport, signal transduction and detoxification of xenobiotics through GSTs. In this study, the effects of different concentrations of $CdCl_2$ and $PbCl_2$ treatments on contents of GSH, protein and GST activities in the roots and shoots of *Hordeum vulgare* L. cultivars cv. Bilgi-91 and cv. Kalayci-97 were assessed in hydroponic solutions. The application of HMs to plants caused an increase in protein contents by comparing to their control groups. While the increase in shoots were higher than in the roots of Bilgi-91, protein contents were increased more in roots of Kalayci-97. In GSH concentration measurements, the shoots of Bilgi-91 has shown a dose dependent manner with Cd treatment. However, the dose dependent characteristic was observed with Pb for their roots and 538% increase was noted as the highest increase in GSH levels by comparing to controls. On the other hand, Kalayci-97 shoots and roots have shown the highest GSH concentration with Pb treatment. As a result of GST activity measurements, the highest activities were observed in both plants shoots with 300 μM Cd treatment (123 and 166% of control for Bilgi-91 and Kalayci-97, respectively) and in roots with 150 μM Pb treatment (282 and 291% of control, respectively). Our results indicate that, depending on the plant origin, plant parts, HM used and treatment conditions, HMs had an effect on the parameters measured. The variable results which observed in GST activities and GSH contents are reflecting a difference in the rate of metabolism with regard to HMs between varieties. However, the presence of high GST activities and GSH contents have shown a general adaptability to stress conditions.

Keywords: Barley, Glutathione S-Transferases, Toxic metals.

TUE-425
Impairment of glycogen metabolism by thiocarbamates pesticides through inhibition of brain glycogen phosphorylase

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Glycogen (Gln) is a glucose polymer found primarily in liver, muscles and brain where it represents the main storage-form of glucose. In brain, Gln is mainly found in astrocytes and appears to be critical for certain neurological processes such as learning and long term memory consolidation. Brain glycogen phosphorylase (bGP) is the key enzyme involved in mobilization of Gln. This enzyme is thighly regulated by both phosphorylation and binding of allosteric effectors.

Dithiocarbamates (DTC) are organosulfur compounds widely used as fungicide. These chemicals are known to be neurotoxic and have been linked to Parkinson's disease. Interestingly, recent data have shown that Gln metabolism in brain could be altered by DTC.

In order to better understand the molecular and cellular mechanisms of neurotoxicity linked to DTC, we studied the impact of a panel of known DTC on human recombinant bGP. Thiram (TH) was found to be the most potent inhibitor of bGP. Chemical-labelling, electrophoretic and mass spectrometry analyses showed that inhibition of purified bGP by TH occured through modification of critical cysteines residue and formation of intramolecular disulfide bonds. A sulfoxide-metabolite of TH (DEDTC-sulfoxide) was also found to inhibit bGP through modification of cysteine residues. In agreement with these data, exposure of a human astrocyte cell line to TH led to the inhibition of endogenous bGP with concomitant impairment of glycogen mobilization in treated cells.

Altogether our data indicate that certain DTC inhibit the glycogenolytic activity of bGP through the modification of key cysteine residues with subsequent impairment of Gln catabolism. This may lead to altered energy metabolism or toxic accumulation of Gln in astrocytes. More broadly, owing to the important role of Gln in neurological processes, impairment of bGP functions in brain could contribute to the neurotoxic effects of DTC.

Keywords: Glycogen phosphorylase, Parkinson's disease, Thiram.

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TUE-426
In vivo adenovirus-mediated inhibition of MCT1 and MCT4 in tanycytes affects the brain glucose sensing mechanism

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Hypothalamic glial cells, or tanycytes, are involved in the brain glucose sensing mechanism, a process based on glia-neuron interactions and mediated by lactate. We evaluated the effect of adenoviral-mediated inhibition of tanycytes monocarboxylate transporters (MCT1 and MCT4) expression on the glucosensing mechanism. Here we show that tanycytes are metabolically coupled with neurons of the arcuate nucleous (AN) and that MCT1 inhibition changes feeding behavior. We generated an adenovirus expressing MCT1 shRNA (AdshMCT1-EGFP), MCT4 shRNA (AdshMCT4-tdTomato), and *E. coli* ß-galactosidase shRNA (control). MCT expression and loss of function was analyzed in primary tanycyte cultures using qRT-PCR, Western blot, ^{14}C-lactate uptake and lactate efflux by HPLC. We also characterized the organization of hypothalamic cytoarchitecture by confocal-spectral microscopy. We used qRT-PCR to analyze orexigenic and anorexigenic neuropeptide expression in the AN in response to intracerebroventricular glucose injections, in rats receiving the AdshMCT1-EGFP or AdshMCT4-Tomato. Finally, we evaluate the effects of MCT1 inhibition in feeding behavior in response to fasting-refeeding cycles. Our results indicate that the decreased MCT1 and MCT4 expression and activity was observed in primary tanycyte cultures after transduction with AdshMCT1-EGFP and AdshMCT4-Tomato, respectively. Inhibition of MCT1 and MCT4 expression *in vivo* altered the normal expression of neuropeptides in response to increased glucose concentration. The *in vivo* inhibition of MCT1 altered the normal response to a fasting-refeeding cycle. Inhibition of the expression of glial MCTs alters neuropeptides mRNA levels in the AN, involved in feeding behavior. MCT1 inhibition, produces changes in the normal feeding behavior, supporting the participation of monocarboxylates in the glucosensing mechanism, based on a metabolic interaction between tanycytes and neurons in the hypothalamus.

Disclosure of Interest: None Declared.
Keywords: glucosensing, hypothalamus, tanycytes.

TUE-427
In vivo toxicity studies of dextran coated iron oxide nanoparticles

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The goal of this study was to develop dextran coated iron oxide nanoparticles (DIO-NPs) by an adapted coprecipitation method and evaluate the in vivo toxicity after intratracheal instillation in rats. The adsorption of dextran on surface of iron oxide was evidenced by FTIR spectroscopy analysis. The average size, deduced from the XRD data refinement, has a value of 7.08 nm for DIO-NPs consistent with the mean sizes deduced from TEM observations. To examine the cytotoxicity of the iron oxide nanoparticles, the MTT assay was used. The HeLa cells were treated on/in a medium containing different concentrations (5, 10, 15, 20, 25 and 30 μg/ml) of the suspension of iron oxide nanoparticles. At the tested concentrations, the nanoparticles proved to be not cytotoxic on HeLa cells. On the other hand, we also evaluated the toxicity of dextran coated iron oxide nanoparticles by histological evaluation of the nanoparticles effects on rat tissues after a single intratracheal instillation of DIO-NPs in male Brown Norway rats at concentrations of 10, 20 and 30 mg/kg. All animals survived the administration of dextran coated iron oxide on all tested concentrations, and did not show any sign of discomfort (lethargy, nausea, vomiting or diarrhea) during the whole duration of the experiment. The lung examination at 24 h after the intratracheal instillation with 30 mg/kg of DIO-NPs the lung parenchyma of the rats shows preserved alveolar architecture with rare macrophages in the alveolar septa, discreet anisokaryosis and anisochromia of type II pneumocytes with rare nucleoli. On the other hand, the pathological micrographs of lung in rats after the intratracheal instillation with 10 and 20 mg/kg dose of DIO-NPs show that the lung has preserved the architecture of the control specimen. Pathological sections of spleen after the intratracheal instillation of the rats with a 10 ml/kg and 20 ml/kg dose of DIO-NPs show that the architecture of the spleen was not affected by DIO-NPs compared with the architecture of the control specimen. After the intratracheal instillation of the rats with a 30 ml/kg dose of DIO-NPs, we observed the splenic red pulp with increased number of monocytes, with nuclear contour irregularities.

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Resources Development 2007–2013 of the Ministry of European Funds through the Financial Agreement POSDRU/159/1.5/S/134398.

Keywords: dextran iron oxide, in vivo assays, intratracheal instillation.

TUE-428
Inhibition of doxorubicin metabolizing enzymes by selected cytostatic drugs

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To date, several studies have demonstrated that the elevated activities of carbonyl reducing enzymes followed by increased enzymatic reduction of doxorubicin to its less potent C13-hydroxy metabolite constitute one of the mechanisms causing pharmacokinetic doxorubicin resistance in tumors. However, possible interactions of carbonyl reducing enzymes with cytostatic drugs that are administered concomitantly with doxorubicin in combination chemotherapy regimens, have not been investigated in detail to date and remain to be elucidated. In this study, we investigated the inhibition of recombinant cytosolic carbonyl reducing enzymes by 5-fluorouracil, paclitaxel, docetaxel, tamoxifen, cyclophosphamide and its pre-activated form, 4-hydroperoxycyclophosphamide. These cytostatic drugs are included in first-line breast cancer chemotherapy regimens together with doxorubicin. First, we explored the ability of selected cytosolic carbonyl reducing enzymes to metabolize doxorubicin to doxorubicinol. Regarding their activity, the tested enzymes can be ranked as follows: AKR1C3 > > CBR1 = AKR1A1. Other tested enzymes (CBR3, AKR1B1, AKR1B10, AKR1C1, AKR1C2, AKR1C4) exhibited only negligible activity toward doxorubicin and, therefore, were not further evaluated in subsequent study that investigated possible inhibition of AKR1C3, CBR1 and AKR1A1 by selected anticancer agents. AKR1C3 was shown to be significantly inhibited by paclitaxel, tamoxifen and 4-hydroperoxycyclophosphamide. CBR1 inhibition was induced by the presence of paclitaxel and 4-hydroperoxycyclophosphamide. The last tested enzyme, AKR1A1, was inhibited by paclitaxel and cyclophosphamide. In the follow-up cellular studies, we will focus on the changes in the expression of AKR1C3, CBR1, AKR1A1

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after exposure to tested cytostatic drugs and evaluate the overall effect of these drugs on doxorubicin reduction in MCF7 and HepG2 cell lines. In conclusion, our results describe important molecular events emerging during the combination breast cancer therapy which may modulate the pharmacokinetic doxorubicin resistance. This work is co-financed by the European Social Fund and the state budget of the Czech Republic. Projects no. CZ.1.07/2.3.00/30.0061 and CZ.1.07/2.3.00/20.0235.
Keywords: breast cancer chemotherapy, carbonyl reducing enzymes, doxorubicin resistance.

TUE-429
Inositol phosphates induce DAPI fluorescence shift

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The polymer inorganic polyphosphate (polyP) and inositol phosphates, such as phytic acid (IP6), share many biophysical features. These similarities must be attributed to the phosphate groups present in these molecules. Given the ability of polyP to modify the excitation-emission spectra of DAPI (4′,6- diamidino-2-phenylindole) we decided to investigate if inositol phosphates possess the same property. We discovered that DAPI-IP6 complexes emit at around 550 nm when excited with light of wavelength 410–420 nm. Inositol pentakisphosphate (IP5) is also able to induce a similar shift in DAPI fluorescence. Conversely, inositol trisphosphates (IP3) and inositol tetrakisphosphates (IP4) are unable to shift DAPI fluorescence.

We have employed this newly discovered feature of DAPI to study the enzymatic activity of the inositol polyphosphate multikinase (IPMK) and to monitor phytase phosphatase reactions. Finally, we used DAPI-IP6 fluorescence to determine the amount of IP6 in plant seeds, facilitating the biotechnology industry in their efforts to obtain low phytic acid grain. Using an IP6 standard curve this straight forward analysis revealed that among the samples tested, borlotti beans possess the highest level of IP6 (9.4 mg/g) while the Indian urad bean the lowest (3.2 mg/g). The newly identified fluorescence properties of DAPI-IP5 and DAPI-IP6 complexes allow the levels and enzymatic conversion of these two important messengers to be rapidly and reliably monitored.

We believe that this reliable method can be easily adapted to IP_6 measurement from any biological samples. By degrading polyP, treating the acid extract at high temperature, since IP_6 is not degraded in these conditions and DNA does not induce DAPI shift at 550 nm, the only fluorescent signal will derive from inositol phosphates such as IP_6. However, some caution and adjustment should be taken considering the presence of IP_5 and inositol pyrophosphates in many biological samples. Since inositol pyrophosphates usually represent 4–6% of their IP_5 or IP_6 precursor pools they will influence minimally the fluorescence signal that will be mainly a reflection of both IP_5 and IP_6 presence.
Keywords: inositides, phytic acid, signalling.

TUE-430
Insight on the interaction of an agmatinase-like protein with Mn2+ and evidence for the agmatinase activity of the protein Limch 1

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Agmatine results from decarboxylation of L-arginine by arginine decarboxylase and can be hydrolyzed to putrescine and urea by agmatinase. Putrescine is required for polyamines biosynthesis, being essential for cell replication.Agmatine is involved in modulation of insulin release and inhibition of nitric oxide synthesis. It is also considered a neurotransmitter and has been associated to anticonvulsant, antineurotoxic and antidepressant actions in the brain.We have described a protein, detected in hypothalamic and hippocampal region of rat brain, which hydrolyzes agmatine, although its sequence greatly differs from all known agmatinases and lacks the typical Mn^{2+} ligands of the urea hydrolase family of proteins. This agmatinase-like protein (ALP) exhibits a LIM-like domain, whose removal results in a 10-fold increased k_{cat}, and a 3-fold decreased K_m value for agmatine.We have now examined the interaction of ALP with the catalytically required Mn^{2+} by using mutagenic and kinetic approaches. Purified preparations of wild-type, LIM-domain truncated-ALP, and histidine mutants (H65A, H127A, H206A, H394A and H435A),were active even in the absence of added Mn^{2+}, but were further activated by incubation with $MnCl_2$(2 mM) at 60°C; the activation was not accompanied by changes in K_m for agmatine. We propose that, like arginase and agmatinase,fully activated species of ALP contain 2 Mn^{2+}/active site and that heating at 60°Cprovides the structural flexibility which is required for optimal binding of the Mn^{2+} ions in the active site. With the exception of the H206A variant, K_d values for Mn^{2+} dissociation for all other species were in the order of 10^{-8} M. Considering a 10-fold increased K_d value for the H206A variant, we suggest that His206 is involved in the stabilization of the activating Mn^{2+} ions. Interestingly, a gene data base analysis revealed at least two rat transcripts (designated Limch 1 isoforms I y II), with a 3‘extremes which are identical to ALP. These transcripts contain 3918 and 2871 pb, respectively, whereas ALP contain 1569 pb.We have cloned the Limch 1-isoform II from cDNA of rat brain hypothalamus and the gene was expressed in *S.cerevisiae* strain TRY104Δspe1 (unable to synthesize polyamines, due to the lack of ornithine decarboxylase, which needs exogenous polyamines for growth). The agmatinase activity of the LIMCH 1-isoform II was demonstrated by its ability to support polyamine biosynthesis*in vivo*. Grant Fondecyt 1120663.
Keywords: Agmatinase, Agmatine, Metabolism.

TUE-431
Insights into the catalytic mechanism of PfECT by thermodynamic, kinetic and structural analyses

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Malaria is caused by the infection and destruction of red blood cells by protozoan parasites belonging to the genus *Plasmodium*. Among the five species infecting humans, *Plasmodium falciparum* is by far the deadliest one, accounting for approximately 95% of deaths. During its intra-erythrocytic development, *P. falciparum* requires massive biosynthesis of membranes which are composed of phospholipids and lack cholesterol unlike the host cell membranes. Phosphatidylethanolamine (PE) represents ~ 35% of the total membrane phospholipids and inhibition of its biosynthesis leads to parasite death. PE is synthesized by the parasite's machinery mainly through the *de novo* CDP-ethanolamine (Kennedy) pathway using ethanolamine as precursor. Our studies focus on the rate limiting step of this pathway catalyzed by CTP: phosphoethanolamine cytidylyltransferase (*Pf*ECT, EC 2.7.7.14). *Pf*ECT catalyzes the formation of CDP-ethanolamine (CDP-Etn) from CTP and phosphoethanolamine (P-Etn) and its accurate

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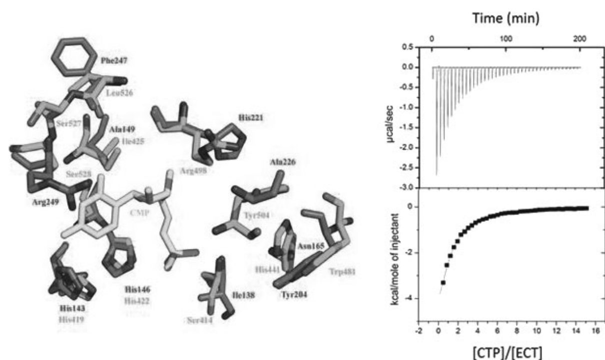

Fig. 1.

mechanism of catalysis has still not been described. Like all ECTs studied so far, *Pf*ECT contains two catalytic cores (CT domains) separated by a long linker. By site-directed mutagenesis of key residues involved in catalysis, we shown that only the N-terminal CT domain of *Pf*ECT is active. This feature raises the question of the role of the C-terminal CT domain. Ligand binding studies by isothermal titration calorimetry showed that *Pf*ECT binds only one molecule of CTP substrate or one molecule of CDP-Etn product with similar affinities while the binding of P-Etn substrate could not be detected. Interestingly, the enzyme is also able to accommodate two molecules of CMP. These results suggest that the C-terminal CT domain lost the ability to bind CTP substrate but retained the binding ability for an analogue like CMP. Analyses of the structural model of *Pf*ECT pointed out critical amino acid differences between the substrate binding sites of both CT domains. By the combination of kinetic, biophysical and structural studies, we now aim at deciphering the precise mechanisms of catalysis and regulation of *Pf*ECT in order to develop inhibitor compounds with anti-malarial activity by rational drug design.

Keywords: Malaria, phospholipid metabolism.

TUE-432
Insights into the sesquiterpenes lactone biosynthetic pathway in *Cynara cardunculus* L.

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Globe artichoke (*Cynara cardunculus* var. *scolymus* L., Asteraceae) is a perennial crop traditionally consumed as a vegetable in the Mediterranean countries and it is rich in secondary metabolites, such as phenolic and terpenoid compounds. Its bitter taste is caused by its high content in cynaropicrin, a sesquiterpene lactone that has attracted attention because of its therapeutic potential as anti-tumor and anti-photoaging agent. One germacrene A synthase (GAS) and two cytochrome P450 genes (CYP71AV9 and CYP71BL5) were isolated in a set of *Cynara cardunculus* unigenes and the encoded enzymes were assessed on their ability to catalyze two consecutive hydroxylation steps leading to costunolide synthesis. When heterologously expressed in *E. coli*, the globe artichoke GAS converted the f*arnesyl* pyrophosphate (FPP) into (+)-germacrene A while the co-expression of CYP71BL5 and CYP71AV9 with GAS led to biosynthesis of the free costunolide in yeast and costunolide conjugates in *N. benthamiana,* demonstrating their involvement in STLs biosynthesis as GAO and COS enzymes. To elucidate the accumulation site of cynaropicrin, we measured its concentration in different tissues at varying developmental stages. The biochemical analyses highlighted that cynaropicrin is mainly concentrated in the leaves, its content decreases progressively during the inflorescence development with almost undetectable levels in roots and stems.

On the basis of the remarkable concentration of cynaropicrin detected in the leaves as compared to the other tissues, we further investigated its localization by isolating the apoplastic fluids and the trichomes. Our results confirmed that cynaropicrin mainly accumulates in the trichomes. Transcript level patterns of the three genes involved in STLs biosynthesis CcGAS, CcGAO and CcCOS were analyzed through q-PCR experiments in leaf and stem tissues respectively characterized by high and undetectable levels of cynaropicrin. The quantitative expression analysis revealed that all three genes involved in cynaropicrin biosynthesis are much more expressed in the leaves, confirming their correlation with the high accumulation of sesquiterpenes in this tissue.

Keywords: globe artichoke, sesquiterpenes, secondary metabolism.

TUE-433
Interaction between the Aryl hydrocarbon receptor pathway and alcohol metabolism enzymes in the liver

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TCDD (2,3,7,8-TetraChlorodiBenzo-*para*-Dioxin) is a persistant xenobiotic pollutant which is classified as a human carcinogen. TCDD binds to and activates the Aryl hydrocarbon Receptor (AhR) which then interacts in the nucleus with ARNT (AhR Nuclear Translocator) to form a transcription factor. This factor regulates the expression of genes involved in the degradation and elimination of xenobiotics. In order to determine whether TCDD can alter hepatic cell function, we performed a transcriptome analysis using a model of differentiated human hepatic cells (HepaRG). Treatment of cells with 25 nM TCDD for 30 h decreased (by 60%) the expression of genes for alcohol metabolism enzymes: cytochrome P450 2E1 (CYP2E1) and the alcohol dehydrogenases ADH1, 4 and 6. In hepatocytes, ADHs and CYP2E1 oxidize ethanol. The overconsumption of alcohol may lead to alcoholic liver diseases (ALD) which are manifested by steatosis, alcoholic cirrhosis and hepatocarcinoma. However, the role of ADHs and CYP2E1 in the toxicity of alcohol is not completely known. Alcohol may exert opposing effects. Its metabolism is toxic due to the production of aldehydes and reactive oxygen species whereas free ethanol itself is toxic. Our objective is to decipher the mechanisms by which alcohol metabolism enzymes are regulated by AhR signaling in HepaRG cells.

We found that ADH and CYP2E1 gene expression decreased as soon as 8 h after treatment with TCDD over a wide range of concentrations (0.1 to 25 nM). The ADHs and CYP2E1 proteins decreased after 72 h of treatment with 25 nM TCDD. The half-lives of the ADH proteins were not modified by treatment with TCDD which suggests a transcriptonal regulation of ADH expression. The AhR antagonist CH-223191 completely inhibits the regulation of expression of ADHs by 25 nM TCDD. The AhR-ARNT complex mediates expression of ADHs as shown by siRNA experiments directed against AhR and ARNT. Finally, the non-canonical pathway of AhR that is mediated by c-Src, is

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not involved in the ADH and CYP2E1 regulation. Similar regulation was observed using other AhR ligands (3-MethylCholanthrene, Benzo(a)pyrene and PolyChloroBiphenyl-126) highlighting the importance of this pathway.

Our results suggest that TCDD regulates expression of ADHs by activation of the AHR/ARNT complex. However, the links between this pathway and the decreases in enzyme expression are still unknown. Study of the promoters of the ADH genes to determine the mechanisms by which transcription factors (AhR or other factors) act in the regulation of ADH expression will help to elucidate how the exposure to pollutants which act through the AhR and which deregulate the expression of ADHs is involved in alcoholic liver disease pathogenesis.

Keywords: ADH, Alcohol, TCDD.

TUE-434

Interactions of tetrapyrrolic antitumor compounds with human serum albumin and low density lipoproteins

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Tetrapyrrole containing compounds (porphyrins, chlorins, chlorophylls) are accumulated predominantly in metabolically active cells, in particular, malignant tumors. These compounds and their derivatives are perspective as photosensitizing agents due to light absorption in the long wavelength spectral region and a deep photodamage of tumor tissues. Boronated porphyrins emerged as promising dual photo- and radiosensitizers for both photodynamic therapy (PDT) and boron neutron capture therapy (BNCT).

However, the question as to how to deliver the bulky molecules to the tumor site remains open. Human serum albumin (HSA) and low density lipoproteins (LDL) are two major carriers of many endogenous and exogenous compounds in the body. In this work the quantitive parameters of interaction of four tetrapyrrole containing compounds (two pheophorbide *a* and two bacteriochlorophyll *a* derivatives; figure 1) with HSA and LDL were determined. Investigated compounds have been synthesized in Nesmeyanov Institute of Organoelement Compounds, RAS (Moscow).

Figure 1. Structures of compounds (**1**) and (**2**), derivatives of pheophorbide *a*; (**3**) and (**4**) derivatives of bacteriochlorophyll *a*.

Adsorption spectra showed that, unlike (**2**) and (**3**), compounds (**1**) and (**4**) were in the aggregated form in aqueous buffer. Increase of HSA or LDL concentrations shifted the equilibrium towards the complex of the monomeric form of these compounds with proteins. Diboronated compound (**2**) did not form complexes with HSA. The estimated values of binding constants K_b to HSA were 5×10^4 M^{-1}, 1×10^4 M^{-1} and 1×10^5 M^{-1} for (**1**), (**3**) and (**4**), respectively. In contrast, (**1**) and (**2**) were similarly affine to LDL with binding constants ~10^8 M^{-1}.

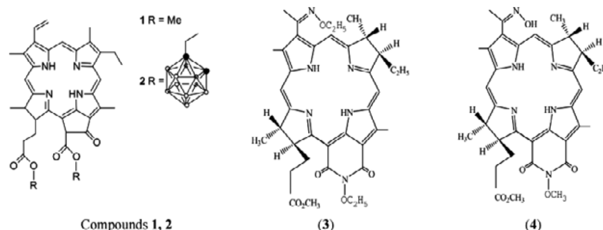

Fig. 1.

Molecular docking showed that (**1**), (**3**) and (**4**) fit tightly into the HSA hemin binding site. In contrast, for the diboronated derivative (**2**) such interactions were sterically hindered by boron polyhedra. Hydrophobic interactions made the main contribution to the binding free energy. Based on their binding free energy, the compounds can be ranked from less to more preferential): (**2**), (**4**), (**3**), (**1**) that is in a good agreement with experimental data.

These results suggest that: A) HSA can be a potential transporter for methylpheophorbide *a* (**1**) and bacteriopurpurinimide (**3**) whereas boron cages might impede the formation of stable drug-protein complexes; B) LDL might be the preferred carrier for polycarborane containing methylpheophorbide *a* derivatives.

Keywords: boron neutron capture therapy, human serum albumin, photodynamic therapy.

TUE-435

Interleukin-10, mean arterial blood pressure and insulin resistance in normal pregnancy

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Problem statement: The purpose of this study was to investigate whether serum of Interleukin-10 (IL-10) Concentration change during normal pregnancy and, if so, to relate these changes corresponding alterations in insulin resistance and blood pressure.

Approach: This cross sectional study was carried out on 86 healthy pregnant women including 26, 23 and 37 individuals in the 1st, 2nd and 3rd trimesters, respectively and in 21 healthy non pregnant women. Serum IL-10 concentration was measured by Enzyme Linked Immunosorbent Assay (ELISA) method. Insulin resistance value was calculated using the homeostasis model assessment, HOMA-IR.

Results: Serum IL-10 concentration was found to be significantly higher in patients in all gestational age as compared non pregnant women. Il-10 level was significantly increased with increase in gestational age. Pregnant women exhibited higher score of HOMA IR compared non pregnant women, but there were no difference in this score between pregnant subjects in different gestational age. There were not significant correlation between IL-10 level with IR and blood pressure.

Conclusion: The results of the study show maternal IL-10 level increase with further increase in gestational age and there is no significant correlation between IL-10 level with Mean Arterial blood Pressure (MAP) and IR.

Keywords: blood pressure, interleukine, pregnancy.

TUE-436

Intracellular localization of Ncb5or, a novel flavoheme reductase likely involved in fatty acid desaturation

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Cytochrome b_5 (b_5) and Cyb_5 reductase (b_5R) are integral membrane proteins in the endoplasmic reticulum (ER). Their concerted action in fatty acid desaturation is well characterized. The recently discovered NADH cytochrome b_5 oxidoreductase (Ncb5or) is a soluble natural fusion protein, which contains both b_5R-like and b_5-like domains. This structural homology along with the marked alterations in lipid metabolism observed in Ncb5or (-/-) mice strongly suggest the involvement of Ncb5or in fatty acid desaturation. The enzyme likely transfers electrons

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from NADPH to the desaturase enzyme in the ER membrane. However, controversial data have been published regarding the cytosolic or ER localization of the protein. In order to clarify whether Ncb5or uses the common cytosolic NADPH or utilizes the separate pyridine nucleotide pool of the ER lumen, we aimed to elucidate the intracellular localization of this soluble protein.

Green fluorescent EGFP-Ncb5or fusion protein was expressed in transiently transfected human HEK293T cells and detected by fluorescent microscopy. The localization of endogenously expressed Ncb5or was assessed in HEK293T cells by two methods. Cells were harvested and homogenized to separate the subcellular fractions by differential centrifugation. Ncb5or and specific marker proteins of various cellular organelles were detected by Western blot. In addition, the endogenous protein was also localized by using in vitro immunocytochemistry.

Endogenous expression of Ncb5or could be demonstrated in HEK293T cell line both at mRNA and protein levels. Purity of the generated nuclear, microsomal, mitochondrial and cytosolic cell fractions were confirmed by immunoblot with characteristic organelle proteins. Ncb5or could only be detected in the cytosolic fraction using an antibody either against the protein, or against the GFP tag. This observation was confirmed using fluorescent microscopy. Ncb5or fusion protein was detected in the cytoplasm of the cells but no co-localization could be detected with fluorescent markers labeling either the nucleus or the endoplasmic reticulum. Similar localization was observed in case of endogenous Ncb5or protein by immunocytochemistry as well.

Or results clearly prove that Ncb5or is localized in the cytoplasm. Therefore, the utilization of ER luminal reducing equivalents by this enzyme can be ruled out. Further research is needed to confirm the putative role of Ncb5or in the fatty acid desaturation, which in turn will help to understand the contribution of this novel protein to the protection of pancreatic β-cells against lipid-induced oxidative and ER-stress.

Keywords: Endoplasmic reticulum, Intracellular localization, Ncb5or.

TUE-437

Investigation of the effect of rosiglitazaonon asymmetric dimethylargnine levels and dimethylarginine dimethylaminohydrolase activity in acute liver failure

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Liver metabolizes ADMA, which is an endogenous NOS inhibitor, by means of DDAH enzyme. Accumulation of ADMA in circulation due to the liver dysfunction has a serious negative impact on the vascular system and perfusion of organ. In our study, we aimed to investigate the effect of acute liver failure on the hepatic handling of ADMA. Beside that, it was invastigated that which direction rosiglitazone (RGZ), a PPAR-g agonist, change the dysfunction developed due to liver damage. Before generating damage with 500 mg/kg body weight,ip, we applied rosiglitazone (5 and 10 mg/kg body weight per day) by way of gavage during 7 days. Acute liver damage has been evaluated by measuring liver DDAH activity, plasma ADMA levels besides plasma ALT and AST activity. Change in the oxidative stress has been determined by tissue levels of malondialdehyde (MDA) and total sulphydryl (t-SH). TAA treatment in this dose, has generated acut liver damage. While increasing the level of MDA in the liver, this treatement has inhibited DDAH enzyme and raised increased the plasma ADMA levels. 5 mg RGZ administration before the liver damage has an antioxidant effect by decreasing tissıe MDA levels and increasing t-SH levels. Moreover, in the DDAH activity it has totally eliminated the inhibition occured due to the damage. However, positive effect of 10 mg RGZ on oxidative stres accuring due to the damage and DDAH inhibition has not been improved. In conclussion, our finding indicates that acute liver damage can seriously affect the hepatic handling of ADMA caused by DDAH inactivation. 5 mg dose of RGZ can improve this function possibly by preventing oxidative stress.

Keywords: acute liver failure, asymmetric dimethylargnine, dimethylarginine dimethylaminohydrolase.

TUE-438

IRBIT, a Inositol 1,4,5-trisphosphate receptor (IP3R) binding protein specifically binds to and inactivates *S*-adenosyl-L-homocysteine hydrolase

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Predominantly, *S*-adenosylhomocysteine hydrolase (AHCY) is a cytoplasmic, homotetrameric enzyme, but some portion of the protein is located to the nucleus. Indeed, it is proposed that the efficiency of transmethylation might profit from a close proximity between methyltransferases and AHCY due to its particular function of rapid removal of S-adenosyl homocysteine (SAH), the byproduct of transmethylation reactions. Rapid removal of SAH is crucial to avoid product inhibition of methyltransferases as it is one of the most potent methyltransferase inhibitors. Very little is known in terms of regulation of enzymatic activity of AHCY, nor its intracellular dynamics. Devogelaere et al (2008) hypothesized that AHCY might interact with a homologous protein, namely S-adenosyl-L-homocystein hydrolase-like protein (AHCYL1, IRBIT). In an effort to evaluate putative interactions between AHCY and AHCYL1 we used molecular, biochemical and cell biological approaches. We performed functional studies of recombinant proteins, Co-IP experiments, FRET in combination with confocal microscopy and live cell imaging, and mass spectrometry. In view of our studies we have confirmed that AHCYL1 specifically binds to and inactivates *S*-adenosylhomocysteine hydrolase. Our results show that besides documented functions of AHCYL1 as inhibitor of inositol 1,4,5-trisphosphate receptor (IP3R), and activator of $Na^+/HCO3^-$ co-transporters (NBC), AHCYL1 has an additional role thus interacting with AHCY and regulating its function. This indicates crosstalk between Ca^{2+} regulation, intracellular pH, methylation potential regulation, and signaling between cytoplasm and the nucleus shedding new light on the AHCY family of proteins.

Keywords: AHCY, IRBIT, S-Adenosyl homocysteine.

TUE-439

Is human dietary methanol an evil that can do good?

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Methanol (MeOH) is considered to be a poison in humans because of the alcohol dehydrogenase (ADH)-mediated conversion of MeOH into toxic formaldehyde (FA). Our recent genome-wide analysis of the mouse brain demonstrated that an

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increase in endogenous MeOH after ADH inhibition led to a significant increase in the plasma MeOH concentration and the modification of mRNA synthesis. These findings suggest endogenous MeOH involvement in homeostasis regulation by controlling mRNA levels.

Here, we demonstrate directly that study volunteers displayed increasing concentrations of MeOH and FA in their blood plasma when consuming citrus pectin, ethanol and red wine. Experiments with volunteers after ethanol intake as an ADH "inhibitor" can assess lower-level MeOH production by endogenous sources. Ignoring the low contribution of renal and pulmonary clearance, we estimated the approximate lower level of endogenous MeOH production is at least = 1.66 mg/kg/h. A microarray analysis of white blood cells (WBC) in volunteers after pectin intake showed various responses for 30 differentially regulated mRNAs. Most of the mRNAs were somehow involved in the pathogenesis of Alzheimer's disease (AD). There was also a decreased synthesis of hemoglobin mRNA, *HBA* and *HBB*, the presence of which in WBC RNA was not a result of red blood cells contamination because erythrocyte-specific marker genes did not show significant change. A qRT-PCR analysis of volunteer WBC after pectin and red wine intake confirmed the complicated dependence between plasma MeOH content and the mRNA accumulation of previously identified genes, namely *GAPDH* and *SNX27*, and *MME*, *SORL1*, *DDIT4*, *HBA* and *HBB* genes revealed in this study. The identification of human genes, the transcriptional activity of which varies with the blood levels of MeOH, would lie between three possible functions of MeOH in humans as follows: (a) MeOH, a poisonous waste product, (b) MeOH, a signaling molecule that regulates the life processes, and (c) MeOH, a Janus-like substance similar to carbon dioxide that is released from the body during respiration, but without which the brain respiration centers cannot be activated. Our microarray analysis allows us to cede to the third hypothesis, which includes not only the inevitable involvement of MeOH-to-FA toxic metabolite formation but also the participation of MeOH in the regulation of gene involved in AD pathogenesis and signaling.

Keywords: diet, human health, regulation of gene expression.

TUE-440
Is there a relation between hemoglobin concenteration and blood lipid profiles?

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Objectives: Both anemia and dyslipidaemia are broadly prevalent public health problems, particularly in the Iranian population. Anemia is defined by the World Health Organization (WHO) as the reduce in haemoglobin concentration under 120 g/l for women and 130 g/l for men. Hyperlipidaemia has been implicated as a risk factor in coronary heart disease (CHD) and atherosclerosis. The aim of this study was to evaluate the plasma lipids and lipoprotein patterns and hemoglobin concenterations in the patients refered in our laboratory.

Design and Methods: In this study participants were selected from those people who refered to Laboratory Dena. Plasma total cholesterol (CHO), HDL-C, and triglyceride (TG) concentrations were measured using enzymatic kits, standardized reagents, and standards (Pars Azmoon Co, Tehran, Iran). The red blood cell (RBC) count and blood concentrations of Hb were assayed.

Results: Out of 102 participates, 50.98% were men and 49.02% were women. The mean serum total CHO level was 4.88 mM, The mean serum TG level was 1.79 mM and the men had Hb and RBC count higher than women. RBC count which takes into account for anemia correlated with HDL-C (r = 0.32, p less than 0.001). There was not a significant correlation between Hb values and the lipid profiles (Total CHO, TG, HDL-C and LDL-C).

Discussion: The present findings indicate that lipid profiles modification generally not be associated with the alteration of RBC count and concentration of hemoglobin (Hb). Conversely, there was a correlation between RBC count and HDL-C.

Keywords: Hemoglobin, Hyperlipidemia, Lipid profile.

TUE-441
Isothiocyanates impair the bioactivation of carcinogenic aromatic amines through irreversible inhibition of arylamine *N*-acetyltransferase

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Aromatic amines (AA) such as 4-aminobiphenyl (4-ABP) are environmental procarcinogens. These chemicals undergo metabolic activation by N-oxidation followed by O-acetylation to form N-acetoxy arylamine that binds to DNA to give carcinogen-DNA adducts. Arylamine N-acetyltransferase (NAT) are xenobiotic metabolizing enzymes that play a key role in both detoxication and bioactivation of AA such as 4-ABP. Isothiocyanates (ITCs) found in cruciferous vegetables including benzyl-ITC (BITC) and phenetyl-ITC (PEITC) with cancer chemopreventive activity. One of the principal mechanisms of action of ITC is to perturb the metabolism of carcinogenic chemicals to limit their metabolic activation. Recent data have shown that ITC inhibit DNA damage induced by 4-ABP in part through Nrf2 activation.

We report here an additional mechanism that may contribute to the chemoprotective effects of ITCs towards AA carcinogens. We found that ITCs such as BITC and PEITC are strongly irreversibles inhibitors of human NAT1 and NAT2 that covalently bind to the active site cysteine of the enzymes. Further mechanistic charaterization was carried out with human NAT1 and we found that BITC was slightly more potent at inhibiting the enzymes than PEITC (k_{inact}=11400 M^{-1} min^{-1} and 3700 M^{-1} min^{-1} for BITC and PEITC, respectively). As expected, both N- and O-acetyltransferase activities of NAT1 were inhibited by BITC. More importantly, we found that BITC inhibited NAT acetylation pathway in MCF-7 cells with concomitant significant decrease in 4-ABP-DNA adducts.

Overall our data suggest direct irreversible inhibition of NAT enzymes by ITCs may alter the NAT-bioactivation pathway of AA thus contributing to the chemopreventive action of ITCs.

Keywords: Carcinogens, Xenobiotics.

TUE-442
Kinetic characterization of enolase enzyme from *Theileria annulata*

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Tropical theileriosis is caused by infection with a tick-borne parasite *Theileria annulata* and this disease has economically considerable importance for livestock industries in many regions of the world. In recent years, parasite resistance has been reported against the most effective antitheilerial drug used for the treatment of tropical theileriosis. This situatuion has given rise to requirement for novel antitheilerial drugs. Enolase (2-phospho-D-

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glycerate hydrolase) is a crucial enzyme that involved in glycolytic pathway for energy production. Therefore, it can be selected as a molecular target for novel antitheilerial therapy methods. In this study, steady state kinetic parameters of the enzyme were determined for the first time. Enzyme kinetic measurements using 2-PGA as substrate gave a specific activity of ~40 U/mg, K_m: 106 μM, k_{cat}: 37 s^{-1} and k_{cat}/K_m: 3.5 × 105 M^{-1} s^{-1}. Determination of kinetic properties of enolase from *Theileria annulata* enables application of further studies on new antitheilerial drug design.

Keywords: drug design, enolase, *Theileria annulata*.

TUE-443
Kinetic mechanism of smooth muscle cell plasma membrane Ca^{2+}, Mg^{2+}-ATPase selective inhibition by calixarene C-90

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Today a great attention of scientists is paid to calixarenes as original molecular platforms which are perspective for designing of biologically-active compounds. Calixarenes are macrocyclic oligophenolic compounds and some of them possess bactericidal, antiviral, antitumoral, antithrombotic activity, they almost have no toxic action on cells.

In our previous investigation carried out on the suspension of myometrium cell plasma membranes we found that calixarene C-90 efficiently inhibited Ca^{2+}, Mg^{2+}-ATPase ($I_{0.5}$ is about 20 μM) and did not influence on activity of other membrane-bound ATPases. Considering further development conception about mechanisms of inhibition of the plasma membrane Ca^{2+}, Mg^{2+}-ATPase of uterus cells by this calixarene we have more thoroughly investigated the influence of this compound on kinetic properties of the Ca^{2+}, Mg^{2+}-ATPase activity of myometrium plasma membrane.

We have shown that calixarene C-90, while inhibiting Ca^{2+}, Mg^{2+}-ATPase, did not influence on kinetic parameters (K_M, n_H) of reaction velocity dependence on substrate concentration. Changes of ATP concentration also did not effect on kinetic of Ca^{2+}, Mg^{2+}-ATPase inhibition by calixarene C-90 and its respective constants (K_i, n_H). The growth of calixarene concentration up to 100 μM caused the slight increase of enzyme activation constants by $MgCl_2$ (K_{Mg}) and by Ca^{2+} (K_{Ca}). The Hill cooperativity coefficients (n_H) of activation by both $MgCl_2$ and Ca^{2+} did almost not varied in the presence of mentioned calixarene. Both $MgCl_2$ and Ca^{2+} also slightly influenced on Ca^{2+}, Mg^{2+}-ATPase cooperativity coefficient n_H and coefficient of inhibition by calixarene C-90 (K_i). The most considerable impact of calixarene C-90 was observed to change the maximal velocity of enzyme reaction. In all cases calixarene C-90 proportionally itself concentration decreased mentioned kinetic parameter. Therefore, we can conclude that inhibitory action of calixarene C-90 on Ca^{2+}, Mg^{2+}-ATPase has mainly uncompetitive character because interaction of calixarene C-90 with enzyme leads to decrease of enzyme turnover number.

We consider that calixarene C-90 is perspective for creation of new pharmaceuticals in order to regulate intracellular Ca^{2+} concentration and therefore muscle tone and contractility.

We are thankful to professor V.I. Kalchenko for helpful discussion and scientific cooperation.

Keywords: Ca^{2+}, Mg^{2+}-ATPase, calixarene, plasma membrane.

TUE-444
Lack of association between genetic variation of -2548G/A of leptin gene polymorphismand hypertension in obese adult Saudi subjects

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Background: Leptin is a polypeptide hormone synthesized mainly by white adipose tissue. Common polymorphism -2548G/A of leptin (LEP) gene has been associated with obesity, but its association with cardiovascular diseases including hypertension has been little studied. Thereforeour study aimedto investigate whether the polymorphism -2548G/A of (LEP) gene is associated or not with hypertension in obesity in a sample of Saudi adult patients.

Patients and Methods: A total of 206 Saudi adult subjects (112 women and 94 men) their aged 40–60 years were recruited and subdivided into three groups: 50 normotensive ND controls (age: 47.9 ± 5.4 y; BMI 22.9 ± 2.1 kg/m^2), 80 normotensive obese ND (age: 47.7 ± 6.0 y; BMI 34.1 ± 4.2 kg/m^2) and 76 hypertensive obese patientswith T2DM (age: 49.4 ± 5.9 y; BMI: 35.1 ± 4.7 kg/m^2). Analyses of -2548G/A polymorphism of LEP gene were made by the polymerase chain reaction restriction-fragment length polymorphism technique (PCR-RFLP). Anthropometric data were collected from all subjects. Serum leptin and insulin concentrations were determined by Luminex as well as fasting blood glucose and serum lipids were determined by a chemical auto analyzer Konelab.

Results: Our study showed that the AA genotype of −2548G/A variant of LEP gene was significantly associated in individuals with higher fasting glucose levels ($p < 0.04$), and HOMA-IR ($p = 0.03$), as well as the A allele of LEP gene variant (−2548G/A) is more prevalent among the subjects with elevated fasting glucose [OR 1.9 (1.2, 3.0), $p = 0.006$], while GA genotype was more common in individuals with hyperleptinemia ($p = 0.04$). On the other hand no association was elicited with either systolic or diastolic blood pressure.

Conclusion: The study showed that the genotypes distribution of −2548G/A variant of LEP gene (GA and AA) are associated with plasma leptin, and glucose levels in set of Saudi individuals. Moreover, these genotypes as well as A allele of this gene might be an important risk factor predisposing healthy subjects to T2DM.

Keywords: gene polymorphism, leptin, obesity.

TUE-445
Lindane disturbs the capacity of *Saccharomyces cerevisiae* to scavenge lipid hydroperoxides via phospholipid hydroperoxide glutathione peroxidase causing cell death

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Reactive oxygen species (ROS) are by-products of aerobic metabolism in cells. Pollutants such as lindane may raise its production, causing cell damages in biomolecules as lipids. Cells possess defence systems to counter oxidative stress including glutathione and glutathione peroxidases (GPx). Phospholipid hydroperoxide glutathione peroxidase (PHGPx) is considered to be the main line of enzymatic defence against membrane damage. The experimental advantages of the yeast model *Saccharomyces cerevisiae* have been exploited extensively for advancing our understanding about

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cell defences against ROS, because the yeast genome sequencing revealed genes that encode GPx which exhibit great homology with other eukaryotes, including man. Lindane has been used as pesticide in agricultural and human health applications. Several factors have contributed in concern over the use of lindane including its persistence, toxicity and bioaccumulation. Thus, the aim of this study was to evaluate the response to lindane mediated by yeast GSH and PHGPx. *Saccharomyces cerevisiae* UE-ME_3, a wild-type yeast deposited in the collection of laboratory of Enology, University of Évora, at mid-exponential phase, were inoculated in YEPD medium, 2% (w/v) glucose, at 28°C, and shaken 150 rpm for 72 h in presence of 5 μM or 50 μM lindane and compared with control. Cell viability was determined by cfu and the biomass by dry weight. Yeasts harvested were suspended in 10 mM phosphate buffer pH 7.0 and disrupted by sonication. The post-12000 *g* pellets were used for determination of malondialdheyde (MDA), glutathione (GSH) and glutathione disulfide (GSSG) by fluorescence and the post-12000 *g* supernatant for PHGPx determination by spectrometry. Statistics were performed by ANOVA I and Duncan ($p < 0.01$) using SPSS for Windows, version 22. The results showed that lindane, at 72 h of exposure, inhibited yeast growth decreasing biomass produced and cell viability. On the other hand, it was observed, for both levels of exposition, an increase in the GSH/GSSG ratio and in the level of proteins, total glutathione, GSH, and MDA of mitochondria as well as a decrease in the PHGPx. The increase in GSH/GSSG ratio of mitochondria probably resulted from the incapacity of the cell to scavenge lipid hydroperoxides, *via* PHGPx, preventing cell damages in mitochondrial membranes. This effect may have determined an increase in the mitochondrial MDA content. This response probably contributed to slowdown the energetic metabolism and over express mitochondrial proteins. So, lindane was toxic to *Saccharomyces cerevisiae* UE-ME_3, probably causing cell death by an active process.

Keywords: Antioxidant enzymes, organochlorine pesticides, yeast.

TUE-446
Lipid profile of the red blood cell membranes in acute pancreatitis: effects of traditional therapy

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The pancreatic acinar cell synthesizes, stores, and secretes digestive enzymes. Observing pancreatic cells is difficult due to time and physiological problems, however, by comparing the red blood cell membranes of people with acute pancreatitis (AP) and those without it is possible to see the damage caused by the activated enzymes to the blood cells and thus conclude that similar damage is taking place in the acinar cells themselves.

In this study the erythrocyte membranes of 110 AP patients were examined before and after traditional therapy. Two study groups comprising 58 patients with acute biliary pancreatitis (ABP) and 52 patients with acute non-biliary pancreatitis (ANBP) were compared. 26 age matched volunteers were used as a control group.

The lipid bilayer is composed of cholesterol and phospholipids in equal proportions by weight. These lipids were separated using the method of thin layer chromatography. Only cholesterol esters (CE), monoacyl glycerol (MAG), and diacyl glycerol (DAG) levels fall into the normal range in patients with ABP on admission. Elevated cholesterol (C) level indicates decreased mobility of fatty acids and deterioration of lateral diffusion; decreased sphyngomyelin (SM) concentration is a sign of impaired lipid phase micro viscosity; reduction in phosphatidyl choline (PC) indicates the decrease in membrane permeability and is a sign of impaired cholesterol metabolism. In contrast, patients with ANBP had normal values of C, TAG, and phospholipids. Decrease in CE, MAG and DAG and rise in FFA indicate the activation of the lipolytic processes.

There is strong correlation between ankyrin and PC levels and clinical symptoms in all the AP patients, whereas in ANBP there is an additional link between the clinical picture and MDA concentration.

In total, 80% of lipids of the RBC membranes were altered in ABP, whereas 50% of lipids were damaged in ANBP. In ABP, traditional therapy improved 25% of lipids, 37.5% fell into the normal range. In ANBP, traditional therapy was less effective and only improved and normalized up to 40% of lipid profile.

The present studies provide evidence that oxidative damage to both lipids and proteins of the RBC membrane occurs in acute pancreatitis. Traditional treatment corrects the structural aberrations in ABP more effectively than in ANBP. This suggests that there is much to be gained by introducing into the traditional therapy drugs with immunomodulating, antioxidant and membrane protective properties.

Keywords: membrane lipids, pancreatitis, red blood cell.

TUE-447
Localization of plasminogen-binding site in fibrin fragment DD

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Fibrinolytic system ensures the destruction of blood clots, but in addition perform other physiological and pathological functions and participates in the process remodulation tissues, reproduction, angiogenesis, inflammation, tumor cell invasion and others.

In order to investigate the mechanism of fibrinolysis and its regulation pathways we studied the potentiating effect of cross-linked fibrin fragments on Glu-plasminogen activation by tissue-type plasminogen activator (t-PA). It was found all plasmic degradation products of fibrin including DDE-polymers, DDE-complexes, fragments E_1E_2 (dissociation products of non-covalent DDE-complexes) and core product DD, apart from core fragment E_3, have potentiating effect on plasminogen activation by t-PA. These results indicate that plasminogen- and t-PA-binding sites on fibrin surface which is exposure under conversion of fibrinogen to fibrin remain on the fibrin fragments during fragmentation by plasmin. Thus, the process is irreversible.

Multifunctional fragment DD molecule is of particular interest because it inhibits platelet aggregation, slows fibrin polymerization, shows an affinity for fibrin clot and stimulates Glu-plasminogen activation.

Using method of izomolar-series, we have established the maximum potentiating effect of fragment DD on activation system plasminogen by t-PA. The effect is observed at a molar ratio of plasminogen to fragment DD 1.0–1.3. Considering this data and structure of fragment DD (identical D-domains of two neighboring covalently cross-linked by γ-chains fibrin molecules) we conclude that the plasminogen-binding site is located in region of γ-γ-cross-linking.

The results of this study can be the basis for the creation of new fibrinolytic drugs and the development of test systems to determine the fibrinolytic system parameters.

Keywords: fibrin fragments, fibrinolytic system, plasminogen activation.

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TUE-448
Low salt intake during pregnancy alters glucose metabolism and DNA methylation in the offspring

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It is known that some maternal nutritional alterations during pregnancy are associated with metabolic disorders in adult offspring, such as insulin resistance, type 2 diabetes mellitus, obesity and arterial hypertension. The period of pregnancy in which these nutritional alterations influence adult offspring remains uncertain. Epigenetic changes are proposed to underlie these metabolic disorders. Twelve-week-old female Wistar rats were fed a low-salt (LS – 0.15% NaCl) or normal-salt (NS – 1.3% NaCl) diet since the first day of gestation until delivery or LS during the first (LS10) or second (LS20) half of gestation. Body weight, food and water intake were weekly evaluated during gestation. Blood glucose, insulin (ITT) and glucose (GTT) tolerance tests, HOMA-IR were performed in adult offspring. Gene expression and DNA methylation were mapped using bisulfite treatment evaluated by pyrosequencing in the male and female neonates and adult offspring. Weight gain was lower in LS and LS20 dams than in NS and LS10 dams in the third week of pregnancy. Birth weights were lower in male and female LS20 and LS rats compared with NS and LS10 neonates. HOMA-IR was higher in 12-week-old LS males compared with NS and in 20-week-old male LS10 rats compared with NS and LS20 rats. In 12-week-old LS10 females, HOMA-IR was higher than in LS. Serum insulin levels were higher in 20 week-old LS10 male compared with NS rats and in 12-week-old LS10 female compared to LS rats. The area under the curve of GTT indicated glucose intolerance in 12- and 20-week-old LS male. Methylation of CpG islands of the *Insr*, *Igf1*, *Igf1r*, *Ins1* and *Ins2* genes in liver in neonates male and female offspring and liver, white adipose tissue and muscle in 20-week-old male offspring were influenced by low-salt intake during pregnancy. None of these alterations was identified in 20-week-old females. In conclusion, low-salt diet consumption in the second half of pregnancy can result in low birth weights in the males and females offspring. Glucose intolerance observed in adult offspring occurred only if low salt intake was given throughout pregnancy. However, insulin resistance in response to low salt intake during pregnancy is related to the time at which this insult occurs and to the age of the offspring. Alterations in the DNA methylation of *Igf1* were observed to be correlated with low birth weight in response to low salt feeding during pregnancy.

Keywords: DNA methylation, fetal programming, Insulin-like growth factor 1.

TUE-449
Low-doses dioxin chronic exposure promotes liver fibrosis development in the C57BL6/J DIO mouse model

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Non-alcoholic fatty liver disease (NAFLD) is strongly associated with obesity, and includes a wide histological spectrum of diseases, ranging from benign steatosis towards pathological non-alcoholic steatohepatitis (NASH) and fibrotic complications, which may progress to end-stage liver diseases. The Aryl hydrocarbon Receptor (AhR) is a ligand-activated transcription factor which binds to a broad variety of environmental pollutants such as the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Beside its detoxification function, a role for AhR has emerged in the regulation of lipid metabolism and hepatic steatosis. Furthermore, previous work from our lab has shown that chronic exposure to high doses of TCDD (25 μg/kg) leads to liver fibrosis. Our aim was to study the effect of low-doses TCDD chronic exposure on NAFLD progression in the C57BL6/J diet-induced obesity (DIO) mouse model. In a preliminary part of the study, a TCDD dose-response (0.1-1-2.5-5-10-25 μg/kg) was performed in C57BL6/J mice to establish that 5 μg/kg TCDD is the threshold dose for liver collagen deposition after chronic administration. Interestingly, a computer modeling of our experimental procedure predicts a final TCDD plasma concentration below 70 ppt, a dose which is relevant to human exposure risk assessment. Then, C57BL6/J male mice were either fed a 10% low fat (LFD) or 45% high fat (HFD) purified diet during 14 weeks and injected once a week with 5 μg/kg TCDD or vehicle in the last 6 weeks of the diet intervention. Histological liver stainings reveal that co-exposure to HFD and TCDD aggravates hepatic lipid accumulation and, more importantly, promotes liver fibrosis development compared to HFD or TCDD alone. In line with these observations, mRNA expression levels of lipid markers, quantified by real-time PCR, were strongly impaired by the co-treatment. Remarkably, TCDD acts in synergy with HFD to increase Cd36 (fatty acid transport), Pparg (lipid storage) and to decrease Srbf1, Acaca (lipogenesis), while it counteracts the HFD-induced increase in Ppara, Cpt1a (fatty acid catabolism) mRNA levels. Moreover, TCDD increases pro-fibrotic (Tgfb1, Col1a1, Col3a1, aSMA) and inflammatory (Il1b, Mcp1, Cd68, Cd11b) gene expression levels although there was no additive effect upon HFD. In conclusion, even if further analyses are needed to better understand the underlying molecular mechanisms, our data strongly support the hypothesis that environmental pollutants could promote liver fibrosis development in obesity-related NAFLD.

Keywords: Aryl hydrocarbon receptor, NAFLD, Obesity.

TUE-450
Luteolin 7-glicoside treatment in normal human keratinocytes: a metabolomic approach

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Flavonoids are a class of plant secondary metabolite. Recent interest in these polyphenolic compounds has been stimulated by the potential health benefits; in particular their antioxidant and anti-inflammatory properties have been shown to protect keratinocytes from environmental stresses.

The epidermis of the skin is a dynamic tissue in which keratinocytes proliferate in the basal layer and undergo a tightly controlled differentiation program upon movement into the suprabasal layers. The final differentiation step of epidermal keratinocytes is the conversion of living cells into corneocytes. The balance between keratinocyte proliferation, differentiation and death it is important in avoiding pathologic changes of the epidermis. Wide-spread skin diseases such as psoriasis, atopic dermatitis and certain forms of ichthyosis are characterized by epidermal defects leading to keratinocyte hyperproliferation or

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hyperkeratinization which are accompanied by inflammatory reactions.

Since it has been reported that topically applied chamomile exert beneficial effects on skin health we selected luteolin-7glucoside (-7G) and apigenin-7glucoside (-7G), the major flavonoids present in this officinal plant, to evaluate the effects on cell death, cell cycle, metabolism, and redox level in normal human keratinocytes (HEKn).

The results of the experiments showed that only treating cells with apigenin-7G leads to the induction of the apoptosis program. Treatment with luteolin-7G do not induce apoptosis in keratinocyte cells, but it leads to a modification of cell cycle, with a consistent accumulation of cells in G1 phase. Luteolin-7G induces also a differentiative stimulus in keratinocytes, indeed demonstrated by the production of differentiation specific molecules such as keratin1, keratin 10 and involucrin. The treatment of human keratinocytes with this flavonoid also induces metabolic alteration (the data sets comprises a total of 279 componds of known identity). The study highlights a decrease in polyamines, a generalised block of energy metabolism (both glycolysis and Krebs cycle) and alteration of fatty acid metabolism. Finally a higher cholesterol level was observed in luteolin treated cells and may be associated with differences in lipid raft generation as well as to the production of the anti-inflammatory glucocorticoid cortisol.

This study demonstrate that flavonoid can deeply modify the normal homeostasis of epidermal tissues, aging on metabolism and differentiation program. The results leads to consider luteolin-7G in the possible treatment of diseases in which the homeostasis of the epidermis is compromised.

Keywords: Flavonoids, Keratinocyte, Metabolomic.

TUE-451
MAO activities and relationships with antioxidant status and lipid peroxidation in human glioma and meningioma type tumours

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Monoamine oxidase (MAO) located in the outer membrane of mitochondria is responsible for oxidative deamination of biogenic and xenobiotic amines, especially in the peripheral and central nervous system. Recent studies revealed that MAO activities were incerased in neurodegenerative diseases as a result of the enhanced metabolism of dopamine leading to lipid peroxidation and membrane damage via H_2O_2 and ROS formations.

In our study, MAO activities, antioxidant status and lipid peroxidation products in brain tumours (glial and meningiomal) were determined. Otherwise the relationships were intevtigated.

Our study was performed with 16 patients hospitalized due to brain tumour surgery in Sisli Etfal Hospital Neurosurgical Department. Working group were generated from 8 gliomas and 8 meningiomas. Peritumoral tissues (8) were used as control group.

Superoxide dismutase (SOD), MAO, glutathione (GSH) and lipid peroxidation (TBAR-S) were studied spectrophotometrically .ELISA method was used for the determination of the glutathione reductase (GR).

MAO and GR activities and TBAR-S were found statistically significant ($p < 0.05$) in gliomas. On the other hand SOD activity was found highly significant, whereas no statistically significant result were obtained in GSH level in gliomas.

In meningiomas MAO and SOD activities were found statistically significant ($p < 0.05$), TBAR-S levels high significant ($p < 0.001$), and no significant values in GSH levels ($p > 0.05$).

Significant İncreased in MAO and antioxidant enzyme activities and lpid peroxidation level were obtained in both type of tumours compared to peritumoral tissues. TBAR-S levels reflecting the lipid peroxidation suggests that MAO activity might increase lipid peroxidation via the H_2O_2 in human brain tumour.

Keywords: Antioxidant status, Brain tumours, Monoamine Oxidase.

TUE-453
Metabolic changes in soybean roots inoculated by *Bradyrhizobium japonicum* strains

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Unique way to enrich the soil with biological nitrogen as well as to increase yield capacity and quality of agricultural products is the creation of effective symbiotic systems of legumes with nodule bacteria. The development of symbiotic interrelations between legumes and nodule bacteria is one of the most important periods in the life of these plants. At the same time the metabolite profile is poorly studied.

The aim of our investigations was to estimate the metabolic profile of soybean roots, inoculated with active and inactive strains of *Bradyrhizobium japonicum* during symbiosis formation under different nitrogen doses. The analysis of metabolites was performed using GC-MS.

Experimental data showed that the soybean roots inoculated with active nodule bacteria had a lot of polyhydric alcohol, amino acids and sugars as compared to roots treated with inactive strain. Inoculation with active strain caused the changes in the quantitative ratio of succinic acid and malonic acid. Application of active nodule bacteria led also to increasing all the metabolites, especially organic acids, in the soybean roots while the enhancing nitrogen fixation was observed. There were some differences in the composition of root metabolites of plants, which were supplied with different doses of nitrogen. The formation of free fatty acids may be due increasing activity of cell biosynthesis of membrane lipids. It is known that the level of membrane fatty acids influences essentially on plant resistance to drought. The increase of contents of osmoprotector proline under water stress promotes the water retention in plant cells and prevents protein dehydration as well as increases the irrigation of membranes and stabilizes the structure of latter.

The studies suggest that the effective inoculation of soybean seeds induces the synthesis of physiological active products in plants affected by stress and thereby creates conditions for increasing plant resistance to moisture deficiency.

Thus, our findings contribute to the understanding of some aspects of the interaction between legumes and nodule bacteria. Besides, these data can be used to develop the strategy for the creation of plants with high ecological plasticity.

Keywords: *Bradyrhizobium japonicum*, metabolic profile, soybean.

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TUE-454
Metabolomic analysis of fission yeast at the onset of nitrogen starvation

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Microorganisms naturally respond to changes in nutritional conditions by adjusting their morphology and physiology. The cellular response of the fission yeast *S. pombe* to nitrogen starvation has been extensively examined by genetic, transcriptomic and proteomic studies. However, since nitrogen starvation is ultimately a metabolic condition, complete understanding of its effect cannot be achieved without studying the intracellular metabolome. In addition, the most immediate response to nitrogen starvation might not be mediated on the transcriptional level, as the transcription/translation machinery responds with an inevitable delay. In this study, we conducted time course metabolomic analysis immediately after nitrogen starvation, prior to any visible changes in cell morphology. We semi-quantitatively measured 75 distinct metabolites, 60% of which changed their level over 2-fold. The most significant changes occurred during the first 15 min after nitrogen source removal, are the rapid increase of trehalose, 2-oxoglutarate, and succinate, and the sharp decline of purine biosynthesis intermediates. At 30–60 min, free amino acids decreased, although several modified amino acids, including trimethylated amino acids, increased. Most energy metabolites such as ATP, S-adenosylmethionine or NAD^+ remained stable during the first 1 h. The fast shut-off of purine biosynthesis and the sharp rise of 2-oxoglutarate and succinate may be caused by the depletion of NH_4Cl from the culture medium. Glutamate dehydrogenase catalyzes the reaction of 2-oxoglutarate and NH_4Cl to synthesize glutamate and subsequently succinate. The gene *gdh1* + encodeing glutamate dehydrogenase is essential for *S. pombe* enter the G0 phase under nitrogen starvation. The level change of key metabolites such as 2-oxoglutarate, succinate and glutamate, may represent an important mechanistic step to trigger subsequent cellular regulations under nitrogen starvation. We extened our metabolome experiments for longer time after nitrogen starvation (6, 12 and 24 h), and will present results that suggest the importance of 2-oxoglutarte udner long-term nitrogen starvation.

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Sajiki, K., Pluskal, T., Shimanuki, M., Yanagida, M. (2013): Metabolomic Analysis of Fission Yeast at the Onset of Nitrogen Starvation. Metabolites 3: 1118–1129.

Keywords: 2-oxoglutarate, Nitrogen starvation, Yeast metabolism.

TUE-455
Metformin reduces palmitate induced endoplasmic reticulum stress and apoptosis in rat insulinoma cell line

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Lipotoxicity refers to cellular dysfunctions caused by elevated free fatty acid levels playing a central role in the development and progression of obesity related diseases. Saturated fatty acids cause insulin resistance and reduce insulin production in the pancreatic islets, thereby generating a vicious cycle, which potentially culminates in type 2 diabetes. The underlying endoplasmic reticulum (ER) stress response can lead to even β-cell death (lipoapoptosis). Since improvement of β-cell viability is a promising antidiabetic strategy, the protective effect of metformin, a known insulin sensitizer was studied in rat insulinoma cells. We measured palmitate induced cell death using Tryphan Blue exclusion method. Assessment of palmitate-induced lipoapoptosis by detection of caspase-3 showed a significant decrease in metformin treated cells. Attenuation of β-cell lipotoxicity was also revealed by lower induction/activation of various ER stress markers, e.g. phosphorylation of eukaryotic initiation factor 2α (eIF2α), c-Jun N-terminal kinase (JNK), splicing of X box binding protein 1 mRNA and induction of CCAAT/enhancer binding protein homologous protein (CHOP). Our results indicate that the β-cell protective activity of metformin in lipotoxicity can be at least partly attributed to suppression of ER stress.

Keywords: apoptosis, Endoplasmic reticulum stress.

TUE-456
Microbial removal of remazol blue by different bacteria

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Textile, pulp, paper, tanning, and dyeing wastewaters include toxic many substances such as highly colored chemicals. These wastewaters affect the environment negatively and need to be treated. In the current study, 3 different bacteria (isolate A, isolate B, and isolate C) were isolated from dye containing textile wastewaters. These bacteria were tested with their possible usage in treating wastewaters including dyestuff. For this purpose, molasses media was prepared and Remazol Blue (RB) was used as the pollutant. Experiments were carried out in a rotary shaker (100 rpm) with an incubation period of 7 days at 30°C. Isolate A, isolate B, and isolate C were investigated with regards to different pH levels (6, 7, 8, and, 9) and dye concentrations (approximately 25, 50, 75, and 100 mg/l). All the tested bacteria were cultivated in molasses media with approximately 25 mg/l dye at different pH levels. The residual dye concentration and optical densities of the bacteria were determined spectrophotometrically. According to the data from the experiments, all the three bacteria removed this dye with the maximum levels at pH 7. In the experiments investigating the effect of initial dye concentration onto dye removal, with an increase in pollutant concentration removal of dye decreased. Within the three bacteria, isolate C, removed dye with higher efficiencies than other bacteria tested.

Keywords: Bioremoval, Remazol Blue, Wastewater.

TUE-457
Modulation of global gene expression profile with resveratrol in rat liver tissues

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Analyzing gene expression profile by using microarray technology is one of the fastest-growing new technologies in genetic researches. Resveratrol, a strong antioxidant in plants, is a natural phytoalexin and polyphenolic compound and regulates the expression of various genes. In this study, microarray analysis was performed to indicate effects of resveratrol treatment on global gene expression profiles of rat liver tissues. RMA algorithm (Robust Multiarray Analysis) was used for microarray raw data normalization. Significantly expressed probe sets with p-values lower than 0.05 were determined by One way ANOVA. Among

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significantly expressed probe sets, fold change of at least two was considered as differentially expressed probe sets. Principal components analysis (PCA) was used to simplify the analysis and visualization of multidimensional data sets. PCA revealed that three biological replicates within a one group clustered together and separated from control group. The Venn diagram was constructed to indicate the number of separated and overlapping probe sets between resveratrol and control groups. After the resveratrol treatment, 186 and 494 transcripts were up and down regulated, respectively. Gene Ontology Enrichment Analysis Software Toolkit (GOEAST) was used for determination of annotation and biological processes for significantly different probe sets. According to the GO database, after resveratrol treatment, upregulated differentially expressed probes were functionally categorized into 10 groups. These included not only functionally well-defined categories, such as cell part, cytoplasm, positive regulation of biological process but also response to stress, organic substance, immune system, and biotic stimulus. Resveratrol treatment led to an increase in the expression of genes including Lcn2 and Usp2 which are responsible for regulation of apoptosis. Additionally, expression level of cellular mobility element genes (Akta1-Mylpf), and acute inflammation of the calcium binding protein gene (S100a9) increased. So, it can be concluded that resveratrol reduced apoptosis and increased cell motility, and immune response. Expression levels of genes involved in the synthesis of the proteins found in the nucleus and nucleolus reduced after resveratrol treatment. Expression level of Pdcd4 gene acting as an inhibitor of apoptosis and Tp53 bp2 gene encoding tumor protein p53 binding protein decreased by 8.8 and 2.8 folds, respectively. It can be suggested that resveratrol has ability to reduce cell death. Finally, microarray results were validated with qRT-PCR using selected genes such as Cat, Usp2, Igfbp2, Cyp1a1, and Cyp8b1.

Keywords: Microarray, Rat Liver Tissue, Resveratrol.

TUE-458
Modulation of metallothionein and apoptotic activities in zinc nanooxide exposed mussel by heat stress and nifedipine

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Nanooxide ZnO (n-ZnO) is one of the most common types of used nanoparticles. N-ZnO can be a source of Zn for the aquatic organisms. Zn is an essential co-factor of many metalloezymes and transcription factors and can also modulate function of ionic channels; however, it is toxic at high concentrations. This study investigated the effects of n-ZnO and Zn^{2+} on metal binding and cellular stress response, and possible modulation of these effects by heat stress (25°C) or Ca-channel blocker nifedipine (NFD) in a model organism, a mussel *Unio pictorum*. Male *U. pictorum* were exposed for 14 days to Zn^{2+} (3.1 μM), NFD (10 μM), n-ZnO (3.1 μM), combination of n-ZnO and NFD at 18°C (n-ZnO + NFD), and n-ZnO at 25°C (n-ZnO + T). Metal binding capacity was determined by measuring levels of metallothioneins (MT) in the digestive gland tissue. Cellular stress response was assessed in the digestive gland by measuring levels of antioxidants (reduced and oxidized glutathione, GSH & GSSG, respectively) and activity of superoxide dismutase (SOD), levels of oxiradicals and oxidative lesions of proteins (carbonyls, PC) and lipids (LPO), DNA fragmentation, and activity of the main effector enzymes in the apoptotic cascades, caspase-3 and cathepsin D (total and free). Exposure to Zn, n-ZnO and n-ZnO + NFD induced significant upregulation of MT levels by ~ 30%, while NFD alone depleted the MT level. Notably, the upregulation of MT in response to n-ZnO exposure was abolished at the elevated temperature (25°C). All exposures except n-ZnO + T led to upregulation of the activity of an antioxidant enzyme, SOD, accompanied by a 2–3-fold decrease in the levels of PC, while the concentrations of LPO products did not change. In contrast, the combined exposure to n-ZnO + T abolished upregulation of SOD activity and induced oxidative stress as indicated by elevated levels of protein carbonyls (by ~40%), LPO products (by over 100%) and a ~2-fold increase in the levels of oxidized glutathione (GSSG). Exposures to the Zn, n-ZnO + T and NFD led to a significant increase in DNA fragmentation. Cathepsin D-related apoptotic activity was induced by all exposures except n-ZnO + T and NFD, while the caspase-3 mediated cascade was induced prominently by n-ZnO + T and decreased by n-ZnO, NFD and n-ZnO + NFD. NFD exposure alone caused elevation of oxyradical formation and release of cathepsin D. Overall, our data show that in the complex exposures, the heat stress drastically impacts MT-dependent metal binding and oxidative stress responses on the exposure to n-ZnO, whereas NFD leads to a change in the apoptotic activity and doesn't affect MT functions.

Keywords: apoptosis, heat stress, metallothionein.

TUE-459
Molecular characterization of nicotinate phosphoribosyltransferase from *Mycobacterium tuberculosis* H37Rv, and inhibition of its activity by pyrazinoic acid

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Nicotinate phosphoribosyltransferase (NAPRTase) catalyzes the reaction of nicotinic acid (NA) with 5-phosphoribosyl-1-pyrophosphate (PRPP) to produce nicotinic acid mononucleotide and diphosphate. It plays an important role in nicotinamide adenine dinucleotide biosynthesis. Recently, two enzymes in *Mycobacterium tuberculosis* H37Rv—PncB1 (Rv1330c) and PncB2 (Rv0573c)—were reported to exhibit NAPRTase activity. In this study, we investigated the properties and kinetics of PncB1. PncB1 requires bivalent metal ions for its catalytic activity, and its optimal temperature is 40°C. In addition, PncB1 exhibits ATP hydrolysis activity. PncB1 can catalyze the NAPRTase reaction in the presence and absence of ATP. However, by comparing the PncB1 kinetic values in the presence and absence of ATP, it was found that ATP affects the reaction by altering the apparent K_m values, whereas, the K_{cat} values were only slightly affected. The K_m values for NA and PRPP in the presence of ATP were 0.16 mM and 0.22 mM, respectively; and 3.22 mM and 2.71 mM, respectively, in the absence of ATP. On the other hand, the K_{cat} values for NA and PRPP in the presence of ATP were 0.14 and 0.15 s^{-1}, respectively; and 0.13 and 0.09 s^{-1}, respectively, in the absence of ATP. The K_{cat}/K_m values for both NA and PRPP were approximately 25-fold greater in the presence of ATP than in the absence of ATP. Hence, it is suggested that PncB1 utilizes the energy obtained through its facultative ATPase activity for effective catalysis. Pyrazinamide (PZA) is an anti-tuberculosis drug, which is converted to its active form, pyrazinoic acid (POA), by pyrazinamidase/nicotinamidase. PZA and POA are nicotinamide and nicotinic acid analogs, respectively. Although PZA and POA cannot be used as substrates for PncB1 instead of NA, the enzymatic activity of PncB1 is strongly inhibited by POA at pH 5.4. The results of molecular modeling and molecular simulation of PncB1 revealed that the binding site of POA is the same as that of NA. POA requires an acidic environ-

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ment for its anti-tuberculosis activity and PncB1 may be one of its drug targets.
Keywords: mycobacteria, NAD.

TUE-460
Molecular diagnosis of mental retardation in children: our experience in Algeria

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Mental retardation (MR) is a major handicap, it may be syndromic involving neurological abnormalities, morphological, biochemical or visceral. Or more frequently non syndromic, defined by a single RM, without other clinical abnormalities.

The number of genes responsible for RM is estimated at about 1000, only 90 of them have been identified.

Our laboratory performs the last few years the molecular diagnosis of some syndromes such as Fragile X syndrome (FMR1 gene), the Prader Willi syndrome (SNRPN), Angelman syndrome (UBE3A) and Rett syndrome (MECP2).

Although the evocation of these syndromes is clinical, the identification of the molecular defect responsible for the RM is required to confirm the diagnosis.

Our study was conducted on 857 childrens from the different departments of pediatrics and neurology of the country, over a period that spans six years (2009–2014).

The analysis of our activity report shows that these affections are not rare in our population. A reliable etiologic diagnosis of RM is essential, ensuring better care for patients, and provide a suitable genetic counseling to affected families.
Keywords: genes, Mental retardation, molecular abnormality.

TUE-461
Molecular targets and pharmacokinetics of cucumarioside A2-2

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At this moment the significant growth of acute and chronic infectious diseases of bacterial, fungal, protozoal and viral nature is observed. Application of highly effective modern antibiotics does not always give a good clinical effect, but may cause a further suppress of immunity. Therefore, the creation of new effective immunostimulants is an important scientific aim. One of the promising immunomodulatory candidates are holothurian triterpene glycosides. Despite the large number of works related to the physiological activity of triterpene glycosides the available data does not give a clear picture of the molecular mechanisms underlying the immunomodulatory effect.

This study reports the investigation of triterpene glycoside cucumarioside A_2-2, the main glycoside isolated from the holothurian *Cucumaria japonica*. It describes a study of pharmacokinetic behavior of cucumarioside A_2-2, its spatial distribution in mouse spleen tissue and some peptide/protein molecular targets of its immunomodulatory action. The methods of radiospectroscopy, MALDI-MS and MALDI-IMS was applied for this purpose.

Cucumarioside A_2-2 is reliably detected by MALDI-MS in the mouse spleen tissue after single intraperitoneal (*i.p.*) injection at a dosage of 5 mg/kg. The glycoside is stable in the spleen and does not undergo metabolic transformation in either tissue homogenates or in the intact organ within 24 h after *i.p.* injection. The cucumarioside A_2-2 was absorbed and eliminated fairly rapidly. It was established by MALDI-IMS that glycoside was mainly located in the tunica serosa part of the spleen and only a small amount was detected within the red and white pulp of the organ. MALDI-MS images obtained 15–30 min post dosage clearly reflect high drug concentrations in the regions surrounding the organ followed by its decline in the surface part and a very slight redistribution to the internal part of the spleen. It has been shown that stimulation of animals with LPS (positive control) or cucmarioside A_2-2 leads to marked changes in the mass-spectrometric peptide/proteine profile of the spleen. These changes were accompanied by notable morphological alteration in spleen reflecting the immune response. The characteristic m/z peaks the intensity of which significantly varied after exposure to immunostimulants were revealed. It is possible that established peptides/proteins are the specific targets (biomarkers) for studied compounds.

This work was supported by RFBR Grant No 14-04-31435 mol_a.
Keywords: molecular targets, pharmacokinetics, triterpene glycosides.

TUE-462
Nephrin, a transmembrane protein, is pivotal for pancreatic beta-cell survival signaling

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Nephrin, a cell surface signaling receptor, contributes to regulation of podocyte function in health and disease. Pancreatic β-cells express nephrin, however its function remains largely unknown. We used a mouse pancreatic β-cell line (βTC-6 cells), to study the role of nephrin in β-cell survival signaling. Beta TC-6 cells express nephrin which is associated and partly co-localized with PI3-kinase. Incubation of βTC-6 cells with functional anti-nephrin antibodies induced nephrin clustering at the plasma membrane and recruitment of a considerable portion of PI3K to clustered nephrin, as indicated by a significant increase of nephrin-PI3K co-localization. This process led to activation of PI3K which associated with increased phosphorylation of Akt; this effect was inhibited by wortmannin and LY294002, indicating that Akt activation was PI3K-dependent. Akt activation resulted in increased phosphorylation/inhibition of pro-apoptotic Bad and FoxO transcription factors; apparently then, nephrin–mediated PI3K-Akt activation triggered anti-apoptotic signaling. Pre-treatment of cells with PP1, a selective inhibitor of Src kinases, inhibited the interaction of nephrin with PI3K and Akt activation, demonstrating that Src kinases mediate nephrin downstream signaling. Silencing of nephrin expression by nephrin-siRNA abolished nephrin-mediated Akt activation and increased susceptibility of cells to apoptosis, as indicated by enhanced caspase-3 activity. High glucose concentration impaired nephrin-mediated Akt activation without affecting nephrin expression; this effect was accompanied by increased nephrin endocytosis and up-regulation of PKCα expression. Interestingly, a marked decrease in nephrin expression was found in pancreatic islets of *db/db lepr-/-* diabetic mice. Our findings revealed that nephrin plays a major role in pancreatic β-cell survival and suggest that glucose-induced changes in nephrin signaling and/or expression may contribute to gradual pancreatic β-cell loss which occurs

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in type 2 diabetes. Further understanding of the mechanism(s) of islet beta-cell degeneration at the molecular level will identify novel drug/therapeutic targets for the treatment of type 2 diabetes.

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Keywords: PI3K-Akt survival signaling, Nephrin signaling, Pancreatic beta-cells.

TUE-463
New insights into the function of the uncharacterized gene aim4 using NMR based metabolomics

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The functionally uncharacterized gene *AIM4* of the eukaryote model organism *Saccharomyces cerevisiae* seems to play a key role in mitochondrial activity and stress response[1]. In this study, Nuclear Magnetic Resonance (NMR) based metabonomics is used to get a closer insight in the metabolic routes in which this gene is involved. To this end, an optimized method to extract and analyze *S. cerevisiae* metabolites has been developed, allowing the identification of a wide range of yeast metabolites.[2] In addition, analysis of intact *S. cerevisiae* cells by HRMAS has been optimized as a complementary method. The metabolic profiles of wildtype versus *aim4*Δ mutant cells have been studied after induction of diverse stress situations (oxidative stress, osmotic stress and glucose deprivation). Interestingly, *aim4*Δ cells show a characteristic delayed stress response enabling metabolomics to be efficiently used to test stress adaptation in yeast. Moreover, metabolic differences between wildtype and mutant cells suggest a crucial role for *AIM4* in fermentative and lipid metabolism as well as in membrane organization.

Keywords: intact cell analysis, lipid metabolism, stress regulation.

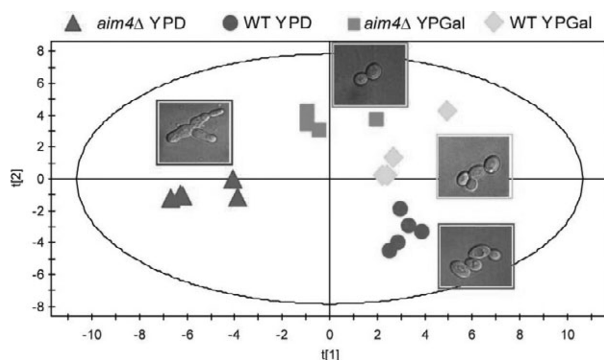

Fig. 1.

TUE-464
New tools for the analysis and biocatalytic synthesis of central metabolites

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Metabolites and metabolic enzymes have been of fundamental importance in the history of biochemistry and the life sciences as components of metabolism in healthy and diseased biological cells. Although a large number of milestone discoveries in classical biochemistry have revealed the molecular details of many metabolic pathways, many central metabolites of well-established pathways have not been available in pure and stable form. It is however essential to synthesize pure metabolites in sufficient amounts, not only for robust functional bioassays and for investigating stability issues, but also to discover novel enzyme functions, other biological functions, metabolic pathways and systems. The widening gap between the human metabolites annotated in databases and the number of compounds with synthesis references underlines the need to focus attention also on the small molecule space of molecular biology. Therefore the Metabolite Synthesis Initiative has been started to address this gap and to synthesize the required metabolite standards of central biochemical pathways. The expansion of enantiomerically pure metabolites enables better stereochemical characterizations of pathway steps in healthy biological systems as well as in inborn errors of metabolism and acquired diseases. Thereby new chiral separation techniques, quantitative NMR, LC-MS of enzymatic reactions and the use of metabolic enzymes have enabled successful biocatalytic asymmetric syntheses of central and chiral metabolites with high step economy. The most recent discoveries on the stability, synthesis and analysis of metabolites in central biochemical pathways will be presented.

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Keywords: metabolic engineering, metabolism, metabolomics.

TUE-465
Nitrogen acquisition in *Agave tequilana* from endophytic bacteria

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Like all living things, plants require nitrogen (N) throughout their development. The N incorporated as NO_3^- and NH_4^+ represents about 2% of total plant dry matter, and is a component

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of proteins, nucleic acids, cofactors, signalling molecules, storage and numerous plant secondary products. The availability of N to plant roots is often an important limiting factor for plant growth. Plants obtain N from nitrogen fixing bacteria and decomposition of dead tissues of both plants and animals by microorganisms. It is unclear, however, how plants obtain nitrogen from these endophytic bacteria within tissues of leaves, stems, and roots. Recent observations suggest that plants may degrade bacteria associated with their tissues in order to extract nutrients. Although, uncertainty still exists as to whether microbial degradation in plant tissues functions in nutrient acquisition or instead is a defensive response to microbes. Here we present experimental data that supports the hypothesis that plant degradation of endophytic diazotrophic bacteria functions as a means to acquire organic nitrogen. We further show that the degradation process is associated with secretion of reactive oxygen, H_2O_2. In these experiments we used *Agave tequilana* and its diazotrophic endophyte *Bacillus tequilensis* to elucidate nitrogen transfer from ^{15}[N]-labeled bacteria to plants. *Bacillus tequilensis* cells grown in a medium with $^{15}NH_4Cl$ as nitrogen source were inoculated into plants growing in sand. We traced incorporation of ^{15}N into tryptophan and pheophytin derived from chlorophyll *a* using liquid chromatography coupled to mass spectrometry. Direct microscopic observations of bacteria in plant tissues visualize the bacterial oxidation process using probes for H_2O_2. These findings challenge the current paradigm for autotrophs, suggesting that plants may degrade endophytic microbes as a nutrient source to maintain growth under circumstances where adequate nitrogen cannot be extracted from soils. Supported by FAPESP (2012/12663-1, 2009/51850-9), CAPES, INCT Redoxoma (FAPESP/CNPq/CAPES; 573530/2008-4), NAP Redoxoma (PRPUSP; 2011.1.9352.1.8), CEPID Redoxoma (FAPESP; 2013/07937-8).

Keywords: 15[N]-labeled bacteria, Endophytic Bacteria, Nitrogen Acquisition.

TUE-466
Nitrosative stress effect on the development of alimentary osteoporosis in rats

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The alimentary osteoporosis remains a serious clinical problem, and the underlying mechanism has not been completely understood yet. The aim of the research was the investigation of nitrosative stress influence on the bone metabolism under alimentary osteoporosis development.

White female W*istar* rats were used in the research. The experimental model of alimentary osteoporosis was performed by using of a synthetic diet without vitamin D_3 combined with balanced content of calcium (1.2%) and phosphorus (0.7%). Rats were divided into 3 groups. 1st – control rats were kept on vitamin D_3 balanced diet, 2nd and 3rd groups were held on vitamin D_3-deficiency diet for 45 days, additionally animals of 3rd group were intraperitoneal injected with 0.05 mg of Bacillus Calmette-Guérin (BCG) vaccine for nitrosative stress development at 30 day of experiment. After decapitation at 45 day in blood samples were determined the level of NO with DAF-2DA by flow cytometry, total calcium, inorganic phosphate and alkaline phosphatase. Also osteometric and X-ray analyses were performed.

Results: In rats with alimentary osteoporosis level of NO in leukocytes was elevated by 17% as compared to control. Level of alkaline phosphatase was also increased more than 2-fold (291 ± 50 U/l) vs. control (134 ± 22 U/l). Total calcium and inorganic phosphate level was decreased by 23 % ((2.3 ± 0.2) mmol/l, control (3.0 ± 0.3) mmol/l) and 79% ((0.4 ± 0.1) mmol/l, control (1.9 ± 0.2) mmol/l) respectively.

In animals of 3rd group with nitrosative stress the level of NO in leukocytes was increased by 17% vs. 2nd group. It was also observed normalization of the main mineral parameters. Thus, the level of total calcium and inorganic phosphate was increased by 22% and 25% relatively, alkaline phosphatase level was decreased by 41% compared to 2nd group – osteoporosis.

Results of osteometric investigation indicated about significant development of osteoporosis. The femur decreased in weight and shortened in length by 47% and 32%, the vertical femoral head diameter decreased by 14% compared to control. The X-ray investigation showed some significant changes in the structure of experimental animal's skeleton. So, in control rats it was normal X-ray picture unlike in animals of 2nd group. The rats with alimentary osteoporosis have unexpressed cortical layer of the tubular bones, deformed thorax, "square" vertebral bodies and rough spine line. In rats with nitrosative stress were normalized the form of thorax, became visible the iliac bone wings and transverse outgrowth of the caudal vertebrae.

Thus, vitamin D_3 deficiency diet caused osteoporosis development. Induced nitrosative stress led to positive effect in mineral metabolism and in structure of rat's skeleton with alimentary osteoporosis.

Keywords: nitrosative stress, osteoporosis.

TUE-467
Oncolytic adenovirus loaded with L-carnosine as a drug delivery system for cancer therapy

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L-carnosine (β-Ala-His) is a naturally occurring histidine dipeptide, normally found in brain, kidney and in large amounts in muscle. L-carnosine has biological functions, including antioxidant activity, ability to chelate metal ions, as well as anti-inflammatory and anti-senescence properties. Recently, we have found that 50–100 mM of L-carnosine decreases cell proliferation in a colon cancer cell line HCT116, bearing a mutation in codon 13 of the RAS proto-oncogene. In addition, pre-treatment with L-carnosine decreases the intracellular concentration of Adenosine Triphosphate (ATP) and Reactive Oxygen Species (ROS) and inhibits the cell cycle progression in the G1 phase. The proto-oncogene KRAS is mutated in a wide array of human cancers and is important both in tumour progression and resistance to anticancer drugs. From this point of view, L-carnosine could represent a good adjuvant candidate for the treatment of colon cancer. To overcome treatment limitations due to the high intracellular concentration required we have hypothesized that L-carnosine cellular uptake could be enhanced by conjugation with the capsid of oncolytic viruses. Indeed, by using L-carnosine-coated oncolytic viruses we believe we can exert a positive anticancer synergistic effect. In fact, it is known that peptides can be used for anti-cancer vaccine, though they require an adjuvant to work, while oncolytic adenoviruses are very efficient in killing cancer cells and have also a strong adjuvant effect. First, we developed a strategy to conjugate peptides on the viral capsid based on electrostatic interaction. Then, using A549 cell line we have demonstrated that oncolytic virus coated with L-carnosine with a tail of positively charged polylysine was able to reduce cell viability compared to the virus alone. In conclusion, we have developed a model to use oncolytic adenovirus as a scaffold to deliver active

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drugs. Once validated the proposed model could be used as a novel drug delivery system for cancer therapy.

Keywords: L-carnosine, Cancer therapy, Oncolytic adenoviruses.

TUE-468
Organotypic cultures of HepG2 spheroids for toxicological studies *in vitro*

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Predictive *in vitro* models alternative to *in vivo* experiments will have a significant impact in future toxicological studies. However, conventional 2D models do not reflect the complexity of a 3D organ resulting in discrepancies between experimental *in vitro* and *in vivo* data. Hence, 3D models are becoming more popular.

In our laboratory, we tried to compare the toxic profile of palmatine and the effect of palmatine on the expression of cytochromes P450 1A1 and 1A2 (CYPs) in primary cultures of human hepatocytes and in HepG2 cells growing as a monolayer or spheroids (Vrba et al.: *Toxicology In Vitro 28*: 693–699, 2014). HepG2 spheroids were produced using the hanging drop method.

Our results suggest that the effect of palmatine on HepG2 spheroids in terms of toxicity and the expression of CYPs is comparable with the results obtained in experiments with primary cultures of human hepatocytes, i.e. in a cell system, which is closer to the *in vivo* situation. Moreover, HepG2 spheroids are more suitable for long-term cultivation than cells growing as a monolayer. Suitability of HepG2 spheroids for longer cultivation was verified by live/dead staining and Alamar blue assay.

Based on our experiments with palmatine we can confirm that organotypic cultures of HepG2 cells are a more complex system useful in toxicology, which better reflects *in vivo* experiments than a conventional cell monolayer.

We gratefully acknowledge financial support of grants LO1304 and NT 13591.
Keywords: cytochromes P450, HepG2, palmatine.

TUE-469
Oxidative stress gene glutathione peroxidase 1 (GPX1) Pro198Leu polymorphism is associated with the gender-specific risk for panic disorder

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Oxidative stress (OS) plays a crucial role in the pathophysiology of psychiatric disorders. Recently, antioxidant system have been shown to affect some brain pathologies. Panic disorder (PD) is an anxiety disorder characterized by sudden attacks of intense fear. Biochemical and genetic studies suggest that oxidative stress index is significantly higher in PD patients, and also the role of OS genes in anxiety-like behavioural phenotypes have been reported. The aim of the present study is to investigate the role of the polymorphisms in OS gene, glutathione peroxidase-1 (GPX1), and DNA repair enzyme gene, 8-oxoguanine glycosylase-1 (OGG1), in PD patients. In this study 127 patients with PD and 151 disease-free controls were included to analyse GPX1 Pro198Leu (rs1050450) and OGG1 Ser326Cys (rs1052133) polymorphisms. The severity of PD symptoms was assessed by Panic and Agoraphobia Scale (PAS). GPX1 Pro198Leu and OGG1 Ser326Cys polymorphisms were analysed with real-time polymerase chain reaction (RT-PCR). No significant relationship was observed in genotype distributions of OGG1 Ser326Cys and GPX1 Pro198Leu polymorphisms between PD and control groups ($p > 0.05$). There was also no significant relationship between OGG1 and GPX1 polymorphisms and age onset, agoraphobia, or PAS scores in PD group ($p > 0.05$). However, in GPX1 Pro198Leu polymorphism, C allele was found to be more frequent in female subgroup of PD patients compared to males ($p = 0.027$). In conclusion, GPX1 Pro198Leu and OGG1 Ser326Cys polymorphisms were not associated with PD risk in Turkish patients. However, a gender specific effect was found in the C allele frequency of GPX1 Pro198Leu polymorphism for the PD risk.

Keywords: GPX gene, Oxidative stress, Panic disorder.

TUE-470
Oxygen-independent regulation of HIF-1 and its role in metabolic adaptation of cancer cells to hypoxia

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Hypoxia-inducible factors (HIFs) are transcriptional activators essential for adaptation to low oxygen conditions. They mediate changes in O_2 delivery and consumption by regulating erythropoiesis, angiogenesis and reprogramming of cellular metabolism under both physiological and pathological conditions. Cancer cells take advantage of HIFs in order to proliferate in the hypoxic microenvironment of solid tumors. HIFs are, therefore, considered promising targets of anticancer therapy.

The activation of HIFs, and especially HIF-1, in cancer cells is often mediated by oncogenic signaling pathways in an oxygen-independent manner. We have previously identified two kinases that phosphorylate and regulate the activity of HIF-1α. ERK1/2 modifies the C-terminal domain of HIF-1α and stimulates its transcriptional activity by blocking its CRM1-dependent nuclear export (1), inhibiting its interaction with non-nuclear proteins or promoting its binding to chromatin. On the other hand, CK1δ modifies the PAS-B domain of HIF-1α and impairs its association with ARNT, thereby inhibiting the formation of an active nuclear HIF-1 heterodimer (2). Our recent microscopical and live-cell imaging studies confirm the operation of these mechanisms in vivo and their importance for hypoxia target gene expression and cancer cell adaptation to low oxygen conditions.

Major characteristic of this adaptation is the shift from oxidative (aerobic) to glycolytic (anaerobic) metabolism, which cancer cells can employ even when grown under normal O_2 levels (Warburg effect). Although the role of HIF-1 in carbohydrate metabolism is well established, much less is known about the part that HIFs play in the metabolism of lipids. We, therefore, examined their involvement in regulation of lipid synthesis under hypoxia. We could show that hypoxia stimulates triglyceride formation and identified lipin 1, a phosphatidate phosphatase isoform that catalyzes the penultimate step in triglyceride synthesis, as a direct transcriptional target of HIF-1 (3). This HIF-1-dependent up-regulation of lipid formation may serve to safely store toxic free fatty acids that accumulate due to deficient oxidative degradation. Inhibitors of ERK-dependent HIF-1α phosphorylation impair this process and also reduce cancer cell viability under hypoxia.

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Keywords: Cancer, Hypoxia, Metabolism.

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TUE-471
Phosphoglucomutase1 is necessary for sustained cell growth under repetitive glucose depletion

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Phosphoglucomutase (PGM) 1 catalyzes the reversible conversion reaction between glucose-1-phosphate (G-1-P) and glucose-6-phosphate (G-6-P). Although both G-1-P and G-6-P are important intermediates for glucose and glycogen metabolism, the biological roles and regulatory mechanisms of PGM1 are largely unknown. In this study we found that T553 is obligatory for PGM1 stability and the last C-terminal residue, T562, is critical for its activity. Interestingly, depletion of PGM1 was associated with declined cellular glycogen content and decreased rates of glycogenolysis and glycogenesis. Furthermore, cells lacking PGM1 showed suppressed proliferation under long-term repetitive glucose depletion. Our results suggest that PGM1 is required for sustained cell growth during frequent nutritional changes, probably through regulating the balance of G-1-P and G-6-P in order to satisfy the cellular demands during nutritional stress.
Keywords: cell growth, glucose depletion, phosphoglucomutase.

TUE-472
Photoperiodic control of carbon distribution during the floral transition in *Arabidopsis thaliana*

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Flowering is a crucial process that demands substantial resources. Carbon metabolism must be coordinated with development through a fine-tuning control that optimises fitness for any physiological need and growth stage of the plant. The circadian-regulated gene *CONSTANS* (*CO*) plays a central role in the photoperiodic control of the floral transition. Recently, a *CO* homologue gene present in the genome of the unicellular green alga *Chlamydomonas reinhardtii* (*CrCO*) was identified. *CrCO* has a conserved role in the coordination of processes regulated by photoperiod and the circadian clock [1]. To study the rate of conservation of the photoperiodic signaling, the analysis of transitory starch accumulation and glycan composition during the floral transition in *Arabidopsis* were analyzed. *As GBSS* (Granule Bound Starch Synthase) expression is controlled by *CrCO* in *Chlamydomonas reinhardtii* [2], in order to study the same effect in *Arabidopsis thaliana*, we followed the expression of the *GBSS* gene in several mutants in short and long day conditions [3]. Moreover, *GBSS* was fused to GFP to study its tisular localization during a 24 h period. *CO*-overexpressing and *CO* mutant plants were crossed to *GBSS* mutant and their floral phenotype observed. The effect of *CO* on *GBSS* could be part of a new regulatory mechanism connecting photoperiodic signaling with carbon metabolism in plants [4]. Photoperiod modification of starch homeostasis by CO may be crucial to increase the sugar mobilization demanded by the floral transition, contributing to our understanding of the flowering process [5].

Photoperiodic control of carbon distribution during the floral transition in *Arabidopsis thaliana*

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Flowering is a crucial process that demands substantial resources. Carbon metabolism must be coordinated with development through a fine-tuning control that optimises fitness for any physiological need and growth stage of the plant. The circadian-regulated gene *CONSTANS* (*CO*) plays a central role in the photoperiodic control of the floral transition. Recently, a *CO* homologue gene present in the genome of the unicellular green alga *Chlamydomonas reinhardtii* (*CrCO*) was identified. *CrCO* has a conserved role in the coordination of processes regulated by photoperiod and the circadian clock [1]. To study the rate of conservation of the photoperiodic signaling, the analysis of transitory starch accumulation and glycan composition during the floral transition in *Arabidopsis* were analyzed. *As GBSS* (Granule Bound Starch Synthase) expression is controlled by *CrCO* in *Chlamydomonas reinhardtii* [2], in order to study the same effect in *Arabidopsis thaliana*, we followed the expression of the *GBSS* gene in several mutants in short and long day conditions [3]. Moreover, *GBSS* was fused to GFP to study its tisular localization during a 24 hour period. *CO*-overexpressing and *CO* mutant plants were crossed to *GBSS* mutant and their floral phenotype observed. The effect of *CO* on *GBSS* could be part of a new regulatory mechanism connecting photoperiodic signaling with carbon metabolism in plants [4]. Photoperiod modification of starch homeostasis by CO may be crucial to increase the sugar mobilization demanded by the floral transition, contributing to our understanding of the flowering process [5].

[1] Serrano, G. *et al.*, (2009). Current Biology 19: 359-368.
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Fig. 1.

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Keywords: CONSTANS, Flowering, Starch.

TUE-473
Physiological-biochemical adaptation of herbs

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The research of state of ecological systems both Lake Baikal and area, testing climate-generate influence of this lake, was actually. The plant adaptation to unfavorable factors of environment, including a low temperature, touch on protein, lipids, carbohydrate metabolism. Some signal molecule is necessary to switching on adaptive process. One of them are oxygen active forms. So long as active forms of oxygen play the important role in beginnings of adaptation, the peroxidase is attract attention of researcher. We was research leafs of plants of fives species, growing in Irkutsk and on the southeastern shore of Lake Baikal. These species are *Achillea asiatica* Serg., *Taraxacum officinale* Wigg., *Plantago major* L., *Veronica chamaedrys* L., *Alchemilla subcrenata* Buser. In leafs of it we was identify the activity of peroxidase, sugars, stress-induced proteins. The variation of activity of weak-associated with plant cell wall and soluble peroxidases of five species of medicinal herbs has been identified for the first time subject to thermal regime and subject to place of growth. In October the coming of negative temperature led to slump of peroxidase activity of plants, growing in Irkutsk. Inversely, plants, sprouting on shores of Lake Baikal, were intensifying activity of this ferment. All described above can be evidence of initiation of unlike adaptation mechanisms in Irkutsk and at Baikal shores subject to environmental conditions. Also the activity of peroxidase was variable during vegetation period. Dehydrine expression

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was followed by spike of peroxidase activity. Dehydrine into leafs in August and middle of October was shown. The reinforcement of dehydrine biosynthesis to medium of October was observed. Also synthesis of dehydrine in summer was happened and this may take place under dehydration of both tissues end cells under sunlight end high temperature. Increase of content of sugar, doing cryoprotector function, was shown to end of vegetation period. The accumulation of sugars on shores of Lake Baikal was going on greater than it in Irkutsk. In August plants of shores of Lake Baikal was being adapt for soft autumn climate better than plants, growing in Irkutsk, and the former are maintaining the prolonged adaptation to frost. Differences of plants adaptation mechanisms at the time and the power were detect. These differences apparently are related to distinctions in both power and combination of stressors in spots of sampling. Particularly, at Lake Baikal the peroxidase activity inside leafs of majority of studied plants was more than it in Irkutsk. In autumn, the accumulation of sugars, stressful proteins (stress-induced proteins) in leafs of plants have been registered in Lake Baikal larger then in Irkutsk.

Keywords: activity of peroxidase, content of sugars, dehydrine biosynthesis.

TUE-474

PKD links golgi to nutrient starvation response in pancreatic β cells

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Pancreatic β cells lower insulin release in response to nutrient depletion. Whether nutrient-deprived β cells induce macroautophagy, a predominant cellular mechanism maintaining energy homeostasis upon starvation, remains poorly explored. We demonstrate herein that, in contrast to other mammalian cells, macroautophagy in β cells is suppressed upon nutrient deprivation. Instead of macroautophagy, starved β cells induce lysosomal degradation of nascent secretory insulin granules in the vicinity of the Golgi. Starvation-Induced Nascent Granule Degradation (SINGD) is controlled by Protein Kinase 1 (PKD1), a key player in Secretory Granule (SG) biogenesis. SINGD in turn triggers lysosomal recruitment and activation of mechanistic Target Of Rapamycin (mTOR) that suppresses macroautophagy. Switching from macroautophagy to SINGD is important to keep insulin secretion low under nutrient-poor conditions. Hence, β cells employ a PKD1-dependent mechanism to adapt to nutrient availability coupling autophagy flux to secretory function.

Keywords: autophagy, mTOR, secretory granules.

TUE-475

Polyglucosan molecules induce testis degeneration and apoptosis in germ cells without affecting the integrity and functionality of sertoli cells

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Glycogen is the main storage form of glucose for most tissues; however, the accumulation of aberrant polyglucosan molecules can lead to degeneration and death in some cell types. Previously, we reported that the accumulation of glycogen in testis of transgenic animals overexpressing a constitutively active form of glycogen synthase (KIN-GS) enhances the apoptosis of pre-meiotic cells in seminiferous tubules. Similarly, the activation of endogenous glycogen synthase (GS) in a germ cell line (GC-1) stimulates the deposition of glycogen and triggers the activation of caspase 3. Here we sought to further identify the effects of glycogen storage in GC-1 and Sertoli cells (42GPA9 cell line) and the mechanism behind the pro-apoptotic activity induced. By spectrophotometric analysis, we found that glycogen synthesized in both cell lines—by expression of a superactive form of GS or by activation of endogenous GS by PTG (*Protein Targeting to Glycogen*) expression—is poorly branched. In addition, the immunodetection of cleaved caspase 3/9 suggests that cellular death induced by polyglucosan molecules affects GC-1 but not 42GPA9 cells. The former cells showed changes in intracellular ATP and cytochrome C content after polyglucosan accumulation, thereby suggesting mitochondrial impairment and activation of an intrinsic apoptotic pathway. Furthermore, we analyzed the effects of glycogen deposition during the establishment of an *in vitro* blood-testis barrier. The results using a non-permeable fluorescent molecule (Evans blue) showed that, in conditions of over-synthesis of glycogen, 42GPA9 cells do not lose their capacity to generate an impermeable barrier. In the same cell line, immunodetection showed that the levels of connexin43 (Cnx43), occludin (Occl), and ZO1 proteins were not affected by glycogen accumulation. Similarly, in the KIN-GS mice, the distribution and intensity of signals for Cnx43, Occl, and ZO1 point to a Sertoli cell-only syndrome in this model, affecting the viability of male germ cells but not the viability or stability of Sertoli cells, as shown by confocal microscopy analysis. These results confirm that the accumulation of polyglucosan molecules has a selective effect—triggered by the intrinsic activation of the apoptotic pathway—in germ cells. [Supported by grants: FONDECYT 3130449 (FVE), 1110508 (ICC) and 1141033 (JCS)]. FRANZVILLARROEL@UACH.CL

Keywords: apoptosis, Glycogen metabolism, testicle.

TUE-476

Polyreactivity of human milk catalytic antibodies as a result of HL-fragments exchange

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The key feature of antibodies is monoreactivity of the antigen binding sites within a single molecule. Monospecific antibodies play a key role in the removal of antigens from the organism. Catalytically active antibodies were found in the blood of patients with autoimmune, viral and bacterial infections, as well as in the human milk. We propose that the human milk bispecific antibodies exhibit other function than the excretion of the antigens.

We have demonstrated that human milk contain substantial amounts of the catalytic bispecific IgG and polyspecific sIgA. We have shown that IgG and sIgA of the human milk eluted from the DNA-cellulose, ATP-Sepharose, casein-Sepharose posess hydrolysis of DNA, ATP, oligosaccharides and phosphorylation of proteins, lipids and oligosaccharides.

In the preparations of human milk IgG and sIgA we have found chimeric antibodies which contain kappa and lambda light chains simultaneously, the chimeric κλ-IgGs were presented by all subclasses (IgG1-IgG4). Adding of the reduced glutathione and the components of milk plasma to the immunoglobulin fractions with different affinities to the DNA-cellulose leads to transi-

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tion from the one fraction to the other. This phenomenon can be explained by the HL-fragments exchange between different immunoglobulin molecules, but not the exchange of light chains.

We plan a detailed study of the human milk factor, providing such HL-fragments exchange.

The reported study was partially supported by RFBR, research projects 13-04-00205, 13-04-00208.
Keywords: antibody polyreactivity, catalytic antibodies.

TUE-477
Possible implications of copper chelation on iron homeostasis in Wilson disease patients. Penicillamine effect on ceruloplasmin ferroxidase activity

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Introduction: Ceruloplasmin (Cp) belongs to multi-copper ferroxidase family of proteins. It is involved in Fe loading into serum transferrin. Defective copper loading into apo-ceruloplasmin (apo-Cp) may result from mutations in the ATP7B gene characterizing Wilson disease (WD). Patients with WD are treated with copper chelators such as penicillamine (PA)

Goals & Methods: We postulate that Cu- chelators will reduce copper incorporation into apo-Cp which will affect ferroxidase activity; consequently disturb iron homeostasis. We hereby investigate the *in vitro* effect of Cu, & Cu-PA on HepG2 viability (MTT assay), ferroxidase activity of Cp (pPD oxidase assay) and expression of Cp, ferritin and transferrin (Western blotting). In addition we examine the activity of serum Cp ferroxidase activity in 5 WD patients who are homozygous for a mutation in WND gene & are treated by PA.

Results & conclusions:

In vitro: Cu decreased (50%) viability of HepG2 cells while co-treatment with Cu-PA altered cell morphology significantly, decreased further cell viability (70%) and induced cell cycle arrest at G2/M phase.

Cp expression in HepG2 cells increased with Cu, decreased with PA, & did not vary with Cu-PA.

Ferritin expression in HepG2 cells decreased with Cu, PA and Cu-PA treated cells

Transferrin expression show qualitative decrease in PA and Cu-PA treated cells

In Vivo: All patients had very low serum Cp ferroxidase activity (0.007–0.01 units) compared to a control (0.022–0.027 units). Mild elevation was noted in 2 patients while transferrin level was in normal range.

Cp Ferroxidase activity in both HepG2 cells and Serum of WD patients was significantly decreased. The recommended life treatment with Cu chelators would influence Fe incorporation into transferrin that may consequently lead to Fe deposition in liver.

The implication of our findings necessitate the re-evaluation of PA treated WD patients for iron complications.

Keywords: ferroxidase, penicillamine, Wilson disease.

TUE-478
Potential role of Focal Adhesion Kinase in lipid metabolism

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Non alcoholic fatty liver disease (NAFLD) is a common metabolic disorder in Western countries, characterized by pathological hepatic lipid accumulation in the absence of alcohol abuse. Simple steatosis can progress to non alcoholic steatohepatitis (NASH), which includes hepatocyte ballooning, inflammation and hepatic fibrosis.

The NAFLD multifactoriality has been profoundly investigated, highlighting a complex network of interactions between genetic, epigenetic and environmental factors. However, the full mechanism underlying NAFLD onset and progression remains still unclear.

Interestingly, the Focal Adhesion Kinase (FAK) family interacting FIP200 protein, active as FAK inhibitor and autophagy inductor, has been recently associated to liver steatosis development. Although the role of FAK as mediator of integrin signalling is well known in liver cancer, its involvement in hepatic lipid metabolism during NAFLD has not been investigated so far.

To study the relationship between FAK and hepatic lipid accumulation, we performed an *in vitro* silencing of FAK in HepG2 cells. Oil-red-O staining, Real-Time PCR and Western Blotting were performed to assess the effect of FAK silencing on lipid accumulation and expression of lipid metabolism-related genes.

Interestingly, FAK-silenced HepG2 cells accumulated more lipids than control cells, but they showed a decreased expression of genes involved in lipid synthesis such as *SREBF1, FASN* and *LIPIN1*. These results suggest that the excessive lipid storage in FAK-silenced cells could be dependent on defects in the β-oxidation pathway.

In a recent study, we showed that inhibition of the histone-lysine N-methyltransferase EZH2 caused increased lipid accumulation and up-regulation of inflammatory genes encoding for TNF-α and TGF-β and mir-200b in *in vitro* NAFLD. So, we hypothesize a possible link between FAK and EZH2 in fatty liver development. Interestingly, we found a down-regulation of EZH2 at the transcript level in FAK silenced HepG cells. Since we found no changes in the protein levels of EZH2, a deregulation of its activity is expected. In fact, we found an increased expression of the EZH2-targeted mir-200b in FAK-depleted cells. Furthermore, FAK silencing, similarly to EZH2 inhibition, induced an up-regulation of TNF-α and TGF-β expressing genes.

In conclusion our results suggest a direct/indirect link between steatogenic effects of FAK silencing and EZH2 inhibition. However, further experiments are needed to better understand the molecular nexus between FAK and EZH2 Lys-27 Histone3 trimethylation activity and the down-stream targets involved in the control of lipid homeostasis.

Keywords: Focal Adhesion Kinase, lipid metabolism, NAFLD.

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TUE-480
Prognostic significance of serum angiopoietin-2 levels and protein redox regulation in chronic lymphocytic leukemia patients

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Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder with the highest incidence in adult population and it has a wide variety of clinical manifestations among the suffers. The aim of current study is to reveal the prognostic importance of 20S proteasome and angiopoietin-2 (Ang-2) parameters and the relationship of those parameters with other prognostic markers of CLL. The systemic levels of oxidative protein damage markers such as advanced oxidative protein products (AOPP) and protein carbonyl (PCO), and proteasome concentrations were also evaluated to clarify protein redox homeostasis in CLL patients. The patient group was formed by the 60 patients who were admitted to Istanbul University Cerrahpasa Medical Faculty, Hematology Clinic. 20 individuals were considered as healthy volunteers to form control group. In patient group, Ang-2, AOPP, PCO and proteasome concentrations were significantly higher than corresponding healthy controls (for all parameters $p < 0.001$). According to modified Rai grading system high risk grade was accepted as state variable, thus diagnostic performance of Ang-2, AOPP, PCO parameters were evaluated by using ROC analysis and Ang-2 levels showed highest specificity (% 82.9) and sensitivity (% 84.6) ($p < 0.001$). There was no significant variation among grades for AOPP. In conclusion, because of high levels of PCO, AOPP and proteasome; redox homeostasis may be impaired in patients with CLL. Systemic Ang-2 and 20S proteasome concentrations may be used for better evaluation of clinical grade and as well as independent of grade, for the purpose of rutine clinical laboratory analysis. Further clinical studies are needed to support our current findings and conclusions.

Keywords: angiopoietin-2, protein redox regulation, chronic lymphocytic leukemia.

TUE-481
Properties and conformational changes in mutants from *Spodoptera frugiperda* midgut trehalase

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Trehalase specifically hydrolyses trehalose to the two constituent glucose units. The enzyme is important for metabolism, since trehalose is the insect main circulating sugar. Previous studies amassed evidence that *S. frugiperda* midgut trehalase has substantial conformational changes on binding different substances. These changes may be responsible for our failure in obtaining the enzyme crystal. Our goal is to produce mutants with less molecular mobility and compare its properties with the wild type enzyme. Based in molecular modeling of *S. frugiperda* trehalase with *Escherichia coli* enzyme we found an N-terminal and a C-terminal region with no defined structure. Two deletion mutants were produced, one lacking 102 residues from the N-terminus (NT) and other lacking this portion plus 31 residues from the C-terminus (NCT). The wild type (WT, 64.7 kDa), NT (52.6 kDa) and NCT (49.6 kDa) were actively expressed. Both mutants have similar Km values (NT 0.85 mM; NCT 0.68 mM) lower than the WT (1.1 mM). The interaction with inhibitors are different among the enzymes. As a rule, the inhibition Ki of amygdalin (WT= 0.21, NT= 0.40 and NCT= 0.48 mM) and mandelonitrile (WT= 13, NT= 27 and NCT= 12 mM) is higher in the mutants. The overall results show that the removal of 102 or 133 amino acids does not greatly change the interaction with ligands, but leads to a considerable decrease in the kcat value, resulting in the following kcat/Km values: WT 74 500 M^{-1} s^{-1}; NT 647 M^{-1} s^{-1} and NCT 1044 M^{-1} s^{-1}. Changes in the molecular folding on ligand binding affects the fluorescence emission of Sypro Orange dye, which binds to hydrophobic regions increasing its fluorescence. The enzymes were incubated with or without substrate, glucose and several inhibitors (gentiobiose, methyl-alpha-mannoside, methyl-alpha-glucoside, amygdalin, prunasin, mandelonitrile and mandelonitrile plus gentiobiose) in the presence of the dye. Trehalose did not cause any change in fluorescence of the enzymes. Two substances increase the fluorescence when WT is present, whereas 5 and 6 compounds affected the fluorescence when respectively NT and NCT are present in the medium. This means that WT was the less affected enzyme and that the shorter enzyme (NCT) is the more mobile one. Four compounds decrease, whereas 5 compounds increase the fluorescence of the dye when respectively NT and NCT are present, pointing out that on binding, NCT exposes hydrophobic residues, whereas the contrary is true for NT. The data suggest that conformational changes occur only in the vicinity of the active site. The wild type has external loops that hinder the mobile region, which is more exposed in the truncated mutants.

Supported by FAPESP, CNPq, INCT-EM.
Keywords: conformational changes, trehalase, truncated mutants.

TUE-482
Properties of arginase-NO-synthase system in peripheral blood lymphocytes of ovarian cancer patients

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Peripheral blood lymphocytes, which provide compensatory-adaptive reactions in the body are important objects for research, related to study of the toxic effect of the products from the tumor cells. In particular, the role of NO-dependent mechanisms in the development of cancer, is relatively novel topic. For the synthesis of NO by involving the enzyme NO-synthase, L-arginine is used as a substrate, as well as a substrate for arginase. Arginase, as a competing substrate for the enzyme is able to significantly influence the activity of NO-synthase reaction and represents an important link in the development of several pathological conditions. Thus, arginase-NO-synthase system may also be involved in the mechanisms of anticancer drugs effects. The aim of this work was to study the properties of arginase and the kinetic characteristics of this enzyme in the peripheral blood lymphocytes of female subjects suffering from ovarian cancer before and after treatment with cyclophosphamide ((RS)-2-[bis(2-chloroethyl) amino] tetrahydro -2H-1,3,2-oksazafosforyn 2-oxide). Cyclophosphamide is a cytostatic drug. Its mechanism of action involves the formation of the transverse cross-links between the chains of DNA and inhibition of protein synthesis. This drug also has a strong immunosuppressive effect on predominant inhibition of B- and T-lymphocytes. Lymphocytes were extracted from freshly received, heparin treated blood, from patients with ovarian cancer and a control group (clinically healthy female patients) using fikol-urohrafin concentration gradient. To reveal of the latent enzymatic activities, they were trea-

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ted with 0.2% saponin. It was shown that arginase activity of lymphocytes in female patient with ovarian cancer increased by 3.8 times, and eNOS activity was reduced by 4.1 times compared to the control values. We found a significant increase in activity of iNOS in ovarian cancer patients with inhibited eNOS in blood lymphocytes. After surgical and chemotherapeutic treatment with cyclophosphamide, arginase activity remained higher compared to control values by 1.7 times. While the activity of eNOS was increasing, iNOS activity was decreasing in these patients. The use of cyclophosphamide chemotherapy in ovarian cancer patients in this study led to the normalization of the arginase and NOS activities.

Key words: lymphocytes, ovarian cancer, NO-synthase
Keywords: None.

TUE-483
Protective effects of exogenously administered testosteron on oxidative damage in rat testicular tissue

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Spermatogenesis is a lifelong process which requires approximately 64 days. Reduction in the number of Leydig and Sertoli cells, diminished capacity of testosterone synthesis, thickening of the basal membrane of the seminiferous tubules and accumulation of lipofuscin granules by oxidative stress are commonly seen in testicular aging. Not only spermatogenesis but also androgen synthesis in testicular tissue adversely affected by impaired redox status. Thus, the optimal regulation of various antioxidant enzymes and the efficiency of the free radical scavenger systems play a critically important role in aging males.

Experimental animals divided into three groups: naturally aged rats (NA:% 0.9 NaCl), testosterone administrated naturally aged rats (TANA: single dose, 25 mg/kg testosterone subcutaneously administered) and their corresponding young controls (YC:% 0.9 NaCl subcutaneously). We analyzed protein oxidation markers: protein carbonyl (PCO) and thiol groups (P-SH); lipid peroxidation markers: lipid hydroperoxides (LHP) and malondialdehyde (MDA); glycoxidation markers: advanced glycation end products (AGEs); antioxidant status parameters: total thiol (T-SH), low-molecular weight nonprotein thiol (NP-SH) groups and Cu,Zn-superoxide dismutase (Cu-Zn,SOD) activity in rat testicular tissue.

As a protein oxidation biomarker, only the levels of PCO groups of TANA rats were found to be significantly lower compared to those in the NA ($p < 0.001$). Although there was a trend toward higher P-SH levels in TANA rats compared to those in NA rats, P-SH levels were not found to be significant. Both TANA and YC groups showed significantly lower LHP and MDA levels as compared to NA rats ($p < 0.01$ NA vs TANA for LHP and MDA; $p < 0.01$, $p < 0.001$ NA vs YC for LHP and MDA respectively). Testicular protein-bound AGEs levels of TANA rats were found to be significantly lower compared to those in NA rats ($p < 0.001$). As antioxidant status parameters only NP-SH concentrations in TANA rats were significantly higher than NA rats ($p < 0.001$). Although there was a trend toward higher T-SH and Cu,Zn-SOD activities, they were not found significant among the groups.

Our results showed that exogenous testosteron administration to the aged rats restored redox homeostasis of proteins, lipids, and low-molecular weight non-protein thiol groups in rat testicular tissue without any alteration Cu,Zn- SOD activity. Additionally, decreased protein bound AGEs levels in TANA rats may play a protective role in keeping the optimal status of protein redox regulation.

Keywords: aging, testicle, testosterone.

TUE-484
Protein structure and structural ordering versus concentration dependence

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Here, we study the mechanism of iron metabolism in ferritin using complimentary techniques for structural studies of multi-subunit protein complexes in solution, such as the small-angle scattering (SAS) method and computational docking. The interest in studying ferritin (apoferritin) arises because it is very likely to be a candidate for cancer and biological age marker [1]. Some apoferritin constructions could also be used as a vaccine against influenza diseases [2].

The function of ferritins in living organisms is iron storage. The fundamental problem is associated with the question of solving ferritin/apoferritin structure, which is essential for understanding molecular mechanisms of iron metabolism activity of these proteins. Despite the intensive research, the large-scale arrangement of the protein complexes is still unknown.

We studied the heavy-water solutions of apoferritin with the help of SAS X-Rays and neutrons. Three instruments have been used for the experiments: BM29, ESRF, Grenoble, France; installation Rigaku, Laboratory of Advanced Studies of membrane proteins, MIPT, Russia; and YuMO spectrometer, IBR-2, Dubna, Russia [3]. We obtained radii of gyration, volume, and the intensity extrapolated in zero value of modulus scattering vector. We demonstrated that in water solutions the proteins interact

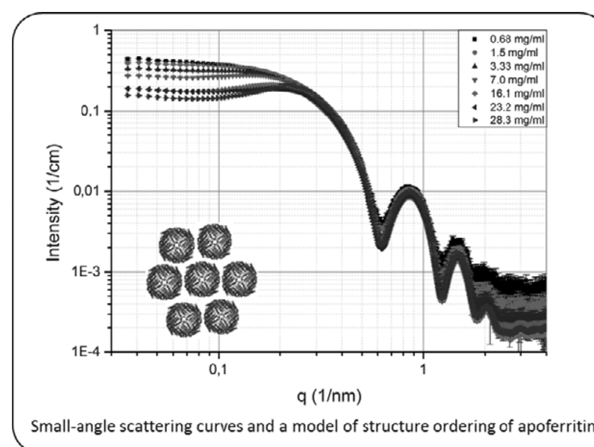

Fig. 1. Small-angle scattering curves and a model of structure ordering of apoferritin.

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with each other even at low concentrations. Also, we recovered the form-factor and the structural factor from the SAS curves. It follows that the distance between individual protein assemblies is about 150 Å for some of the highest concentrations. This allowed us to suggest a model of structure ordering for protein molecules in solution. Finally, we studied the sensitivity of SAS curves to fluctuations in protein structure by computational methods of molecular docking.

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Keywords: iron metabolism, proteins, proteins interaction in solution, apoferritin, small angle scattering.

TUE-485
Proteomics and biochemical strategies for the development of breast cancer biomarker and treatment selection

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Breast cancer is one of the most common women oncological heterogeneous disease subdivided into different subtypes. Very often its therapy depends on early diagnostics. Proteomics analysis is known to be an effective approach for identification of disease biomarkers. 2D gel-electrophoresis represents a powerful and widely used proteomic method for the analysis of complex protein mixtures extracted from cells, tissues, or other biological samples. The diagnostic profile of protein expression in blood plasma of breast cancer patients has been defined previously. But all of differentially expressed proteins were nonspecific markers and corresponded to acute phase response.

In this work we tried to compare the secretome of breast tumor cells with blood plasma proteome of breast cancer patients to find unique biomarkers for each breast cancer subtypes. All patients were divided into groups according to breast cancer subtypes (Luminal A, Luminal B, Triple negative/basal-like and HER2 type). Patients with fibroadenoma (a benign breast tumor) were considered as a control group. The method of primary culturing of breast tumor cells from surgery and bioptic samples of patient have been developed. It was found that cell secretome and proteome composition profiles strongly depend on type of cell culture. The qualitative compositions of blood plasma proteins of patients were also different for each group of patients. The correlation analysis of 2D maps for obtained cell culture and blood plasma revealed potential markers some of which were identified by mass spectrometry.

It was also shown that acetylcholinesterase activity of breast cancer cells correlates with their proliferative activity. The biochemical parameters characterizing the influence of drugs on metabolic processes in primary culture of breast cancer cells have been determined. Thus, acetylcholinesterase activity can be used as criterion for assessment of tumor cells sensitivity to anticancer drugs during the treatment selection. We suppose that obtained results can be used in breast cancer diagnosis and choice of the treatment

Keywords: breast cancer, markers, proteomics.

TUE-486
Proton translocating ATPase activity of *Escherichia coli* membrane vesicles under mixed carbon fermentation at alkaline and acidic pHs

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Escherichia coli is able to ferment glycerol and to produce molecular hydrogen (H_2) by using different hydrogenases (Hyd). Hyd-3 and Hyd-4, together with formate dehydrogenase H (Fdh-H), forms the formate hydrogenlyase (FHL) complexes, which are responsible for H_2 evolution by intact cells. Hyd-4 activity has been shown to be inhibited by *N,N'*-dicyclohexylcarbodiimide (DCCD) [1]. The requirement of F_0F_1-ATPase for Hyd activity has been shown for glycerol fermentation conditions depending on pH, and some relationship of F_0F_1 with Hyd enzymes is suggested [2].

In this study was investigated overall and DCCD-sensitive ATPase activity of mixed carbon fermented (glucose and glycerol) *E. coli* wild type BW25113, *E. coli* FM460 (with deficiency of Fdh-H, Fdh-O, Fdh-N), and *E. coli* KT2110 (with Fdh-H, Fdh-O, Fdh-N, HyaB, HybC deficiency) mutant strains membrane vesicles at different pHs.

ATPase activity of wild type was ~10-fold higher ($p \leq 0.05$) at pH 7.5 compared with that at pH 5.5. DCCD inhibited ATPase activity of wild type ~2.5-fold ($p \leq 0.02$) at pH 7.5, but at pH 5.5 – ~1.2-fold ($p \leq 0.025$). In mixed carbon grown cells, compared with wild type cells, ATPase activity at pH 7.5 was lowered ~1.3-fold ($p \leq 0.02$) in FM460 and ~5-fold ($p \leq 0.025$) in KT2110 mutants. At pH 5.5 ATPase activity of FM460 mutant membrane vesicles was shown to be ~3-fold lower ($p \leq 0.05$) compared with those at pH 7.5. Moreover, ATPase activity of KT2110 at pH 5.5 was ~1.7-fold higher compared with those at pH 7.5. Note that ATPase activity of FM460 and KT2110 mutants at pH 5.5 was similar. DCCD inhibited ATPase activity of FM460 and KT2110 mutants ~3-fold and ~1.2-fold ($p \leq 0.05$) at pH 7.5, respectively.

The suppressed ATPase activity in KT2110 mutant membrane vesicles suggests the cooperation of different components of F_oF_1-FHL supercomplex upon mixed carbon fermentation at alkaline pH. The results also show that alkaline pH is more optimal for the *E. coli* F_oF_1-ATPase activity upon mixed carbon (glucose and glycerol) fermentation.

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Keywords: ATPase activity, *Escherichia coli*, Mixed carbon fermentation.

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TUE-487
Purification of Euphorbia characias endochitinase III

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Chitinases, enzymes hydrolyzing β-1,4 glycosidic bonds of chitin, have been found animals, plants, insects, fungi, bacteria, and viruses. *Euphorbia characias* is a shrub growing abundantly in vast Mediterranean areas. *E. characias* is characterized by the presence of laticifers highly specialized cells. Laticifers contain a sticky milky sap called latex. A Class III endochitinase from *E. characias* latex (ELC) was purified. The purification method includes an effective step using magnetic chitin particles. *E. characias* latex was collected into test tubes and centrifuged at 19000 rpm for 30 min. The supernatant was incubated with magnetic chitin equilibrated in 10 mM KPi buffer, pH 7.0. The incubation was carried out for 1 h, at 4°C, under continuous stirring. Afterwards, the complex enzyme-magnetic chitin was separated from latex using a strong magnetic separator; this complex was washed with 10 mM KPi buffer pH 7.0 to remove the ballast proteins. Then, the chitinase was eluted from magnetic chitin with 100 mM acetic acid, pH 2.8. The solution was immediately adjusted to pH 7.0 with 1 M NaOH and dialyzed against 10 mM KPi buffer pH 7.0. The dialyzed chitinase was loaded on DEAE cellulose column equilibrated with the same buffer. In these conditions the enzyme was not bound to the column and was eluted and collected until the A_{280} became ≤0.01. SDS-PAGE profile reveals a unique protein band of 36.5 ± 2 kDa. The optimal chitinase activity is observed at pH 5.0 in 100 mM NaOAc buffer and at 40°C. *E. characias* latex chitinase hydrolyzes colloidal chitin, and N-acetyl D-glucosamine, chitobiose and chitotriose are observed as reaction products. ELC has both endo and exo chitinase activities. Calcium ions enhance chitinase activity 5–8 times and magnesium ions 2–3 times.The partial ELC cDNA contained an open reading frame of 762 bp, encoding a protein of 254 amino acids (GenBank JX564541). ELC amino acid sequence showed a very high degree of identity (92–99%) to chitinases isolated from several other higher plants. It also contained the conserved sequence of the catalytic domain of family 18 glycoside hydrolases, LDGIDFDIE, from position 145–154 and the presence of six cysteine residues at conserved positions in all class III chitinases.

Keywords: Euphorbia characias; magnetic particles; chitinase.

TUE-488
Recombinant microbial creatinine deiminase as a bioanalytical tool

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Creatinine is one of the most important diagnostic biomarkers for detection of kidney and muscle dysfunctions. To determine creatinine content in biological fluids, creatinine deiminase can be used as a bioanalytical tool. This enzyme is also very important for the construction of creatinine-sensitive biosensor to be used in hemodialysis control. Up to now, the unsolved problem is insufficient stability of commercial available creatinine deiminase, so overproduction of the stable form of this enzyme is very important.

The recombinant strain of *Escherichia coli* that is capable of overproducing microbial creatinine deiminase has been constructed. The $(His)_6$-tagged enzyme was purified in one step from the cell-free extract of the recombinant *E. coli* strain by metal-affinity chromatography. The specific activity of the purified enzyme is 10 μmoles per min and mg of protein. The yield of the recombinant protein is approx. 50 mg from 1 l of culture.

The isolated recombinant creatinine deiminase can be applied for the determination of creatinine in clinical samples using enzymatic method, as well as an automated biosensor-based system.

Keywords: recombinant creatinine deiminase, over-production, purification.

TUE-489
Redox regulation mechanisms in cardiac tissue of testosterone administrated rats

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We analyzed various oxidative stress biomarkers including protein carbonyl groups (PCOs), lipid hydroperoxides (LOOHs), malondialdehyde (MDA), advanced glycation end products (AGEs) and antioxidant status parameters such as total thiol (T-SH), non-protein thiol (NP-SH), protein thiol groups and Cu,Zn superoxide dismutase (Cu,Zn-SOD) activity in cardiac tissue of rats. Experimental animals divided into three groups: naturally aged rats (NA) (IP: %0.9 saline), testosterone administrated naturally aged rats (TANA: 25 mg/kg testosteron) and their corresponding young controls (YC:% 0.9 saline).

PCO, LOOHs, MDA, AGE concentrations in cardiac tissue of NA rats were significantly higher than TANA and YC rats (Both NA vs YC and NA vs TANA: $p < 0.05$; $p < 0.001$; $p < 0.01$ and $p < 0.05$ respectively for each parameters). T-SH and P-SH concentrations in NA rats were significantly lower than YC groups ($p < 0.05$ for both parameters). On the other hand, T-SH and P-SH concentrations significantly lower and LHP in testosterone administered group significantly higher than YC ($p < 0.05$; $p < 0.001$ respectively). NP-SH concentrations were not different among groups. Cu,Zn-SOD activities in NA group were significantly lower than YC group ($p < 0.05$). Additionally Cu-Zn SOD activities in TANA group tended to increase but they did not reach the statistical significance levels.

Our current results related to TANA rats show that the testosterone administration has a positive effects on redox status of the aged cardiac tissue.

Keywords: cardiac tissue, oxidative stress, testosterone.

TUE-490
Regulation and dynamics of human serine racemase

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Human serine racemase (hSR), predominantly localized in neurons and astrocytes, is a dimeric pyridoxal 5'-phosphate (PLP)

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enzyme that catalyzes the reversible racemization of L-serine to D-serine, the physiologically relevant co-agonist of N-methyl-D-aspartate receptors. Because both high and low D-serine levels are associated with neuropathologies, hSR is a promising drug target. We have shown that that:

- ATP, an allosteric effector of hSR, binds in a strongly cooperative fashion, with a Hill coefficient close to 2, and causes a 30-fold increase in enzyme efficiency by reducing K_m and increasing k_{cat}.

- Ligands of the active site, such as glycine or malonate, allosterically increase ATP affinity by 100 fold, and, conversely, ATP binding increases malonate and glycine affinity by 9 and 15 fold, respectively.

- Binding of ATP to the external aldimine intermediate, formed by hSR with glycine, stabilizes a closed conformation of the active site, as probed by the coenzyme fluorescence quenching by iodide.

- Chloride and fluoride anions increase 1.5- and 3-fold, respectively, the beta-elimination activity of hSR, whereas iodide abolishes it. Unlike with other PLP-dependent enzymes, monovalent cations do not have significant effects on catalysis.

- Metal chelators (such as EDTA), phosphate, and, to a lesser extent, imidazole, strongly stabilize the enzyme.

These findings were interpreted on the basis of a model in which different ligands bring about the selective stabilization of alternative enzyme conformations, characterized by high and low affinity and reactivity.

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Keywords: enzyme activity, neurodegenerative diseases, NMDA.

TUE-491
Regulation of GLUT transporters in prostate cancer cells

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Cancer cells show different metabolic requirements than normal cells and this metabolic switch was already described by Otto Warburg in the 20's. One of the most important aspects of tumor metabolism is the high rate of glucose uptake by cancer cells. An increase in glucose uptake has been associated mainly with GLUT1 overexpression but may also involve other glucose transporters, including GLUT4. Despite this, the regulation of GLUT transporters in cell culture has been scarcely studied. In prostate cancer, particularly, glycolytic metabolism differs in androgen-responsive and nonresponsive cells. GLUT1 expression was previously described in prostate cancer cells but functional GLUT4 was recently found in our laboratory. Thus, the aim of this work was to study the regulation of GLUT transporters in prostate cancer cells and its relation with the glycolytic pathway. GLUT1/4 protein levels were analyzed by immunoblot with different glucose concentration in cell media at different times and with constant medium renewal. Actinomycin D (AD) and cicloheximide (CX) were used to evaluate the half-lives of these proteins. Synchronization of cell culture in G1/S and in G2/M was realized using respectively thymidine (DTB) and DTB plus nocodazole (Noc), and analysis of cell cycle phases was evaluated by flow cytometry. Glucose uptake was measured using nonradiolabeled 2-deoxyglucose. The evaluation of subcellular distribution of GLUT1/4 was analyzed by immunocytochemistry. Finally, nucleotides concentration was estimated by HPLC. Results show that both GLUT1 and GLUT4 are glucose and time-dependent, changing their levels along with time after medium renewal. Differences were found among cell lines and GLUT proteins. GLUT levels increased with time of culture and with glucose concentration in LNCaP cells while they decreased in PC-3 cells. Constant medium renewal caused a decrease in GLUT levels at 24 h in LNCaP cells but an increment in PC-3 cells, being similar to levels at 6 h. Half-life of GLUT1 was found to be 18 h while GLUT4 has a posttranscriptional regulation. Finally, GLUT1/4 levels changed differently in G1/S and G2/M cells synchronized cells. In conclusion, GLUT1/4 protein levels and glycolytic metabolism of prostate cancer cells are carefully regulate and they change in relation to the availability of glucose in culture media and more importantly depending of proliferation status of culture cells.

Keywords: GLUT transporters, Metabolism, prostate cancer.

TUE-492
Regulation of retinoid metabolism by FoxO transcription factors in retinal pigment epithelium

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The inactivation of phosphatase and tensin homolog (PTEN) and consequent hyperactivation of Akt not only elevated a chance for tumorigenesis, but also caused age-related macular degeneration (AMD)-like retinal degeneration by triggering degeneration of retinal pigment epithelium (RPE) in mice. The activated Akt in PTEN-inactivated RPE phosphorylates various cellular proteins, including the forkhead box O (FoxO) transcription factors, to induce a series of changes in the cell. In this study, we investigated roles of FoxO in RPE homeostasis. The mice lacking *FoxO1,3,4* genes specifically in RPE develop retinal degeneration in a delayed time course of that observed in *Pten-cko* mice. We discovered retinol dehydrogenases are one of the targets of FoxO and were significantly reduced in *Pten-* and *FoxO*-deficient RPE. The importance of retinoid metabolism regulated by PTEN-Akt-FOXO axis in RPE was proven by the inhibition of RPE degeneration in *Pten-* and *FoxO-cko* mice after dietary alteration of retinoid. Furthermore, *daf-16* mutant *C. elegans* also showed hypersensitivity to retinoid toxicity, implicating a potential inhibitory role of PTEN-FOXO-RDHs in aging. Together, our study not only provides a mechanistic understanding to RPE degeneration, but it further disclosed a link between retinoid metabolism and aging.

Keywords: age-related macular degeneration (AMD), forkhead box O (FoxO), retinal pigment epithelium (RPE).

TUE-493
REPTOR and REPTOR-BP regulate organismal metabolism and transcription downstream of mTORC1

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mTORC1 is a central integrator of nutrient, energy and stress information in a cell. It regulates cellular growth and metabolism

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on a short timescale by post-translationally modifying key signaling components, and on a longer timescale by influencing transcriptional programs. We identify here the two so far uncharacterized fly genes REPTOR and REPTOR-BP and their mammalian orthologs as transcription factors downstream of mTORC1. REPTOR and REPTOR-BP are required for almost all of the transcriptional induction that occurs upon mTORC1 inhibition in *Drosophila*. We find that when mTORC1 is active, it phosphorylates REPTOR on two serine residues, leading to its cytoplasmic retention. Upon mTORC1 inhibition, REPTOR becomes dephosphorylated in a PP2A dependent manner, shuttles into the nucleus, joins its partner REPTOR-BP to bind target genes, and activates their transcription. *In vivo* functional analysis using knockout flies reveals that REPTOR and REPTOR-BP play critical roles in maintaining energy homeostasis and promoting animal survival upon nutrient restriction.

Keywords: Drosophila melanogaster, Metabolism, mTORC1.

TUE-494

Resveratrol ameliorates fatty liver by reducing the size of lipid droplet without changing liver fat species

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Resveratrol has been suggested to have various beneficial health effects. We focussed on its effects in protecting the liver against lipid accumulation induced by a high-fat (HF) diet because fatty liver is closely associated with lifestyle-related diseases, such as type 2 diabetes and coronary heart disease. Among lipids, triacylglycerols (TGs) are a major component of food and animal fats. TGs are a diverse class of molecules, and structural isomers also exist. In the present study, we characterised the components and structures of TGs in lipid droplets by matrix-assisted laser desorption/ionisation spiral orbit-type time-of-flight mass spectrometry (MALDI-SpiralTOF) to investigate the effect of resveratrol on TGs in liver. Mass spectrometry (MS) analysis was used to characterise lipid components and structures, and imaging MS (IMS) then provided distribution maps of selected *m/z* values.

The most intense peak identified in liver and food extracts from both HF (50% fat) and resveratrol-supplemented diet (HF + Res; 50% fat, 0.2% resveratrol) was located at *m/z* 881.7 (52:2). Notably, tandem MS (MS/MS) analysis revealed structural differences between the food and liver TGs, despite apparently having the same precursor at *m/z* 881.7. Palmitic acid (16:0) was located at either of the exterior sites on the glycerol backbone in liver TGs, but at the central site in dietary TGs. These results suggest that dietary TGs affect liver TGs, but that structural differences exist between them. Dietary TGs are digested and hydrolysed after oral intake, and then the hydrolysed TG is re-synthesised before it accumulates in the liver. In this process, resveratrol showed no effect on the components and strucutres of

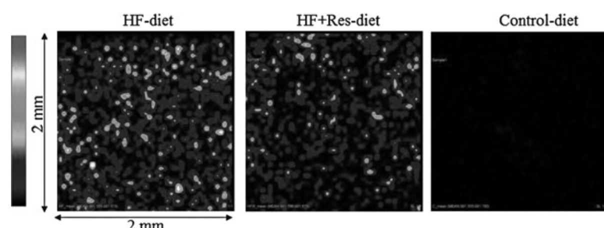

Fig. 1.

TGs in the liver. In contrast to the MS/MS analysis, resveratrol did show differences in IMG and histology analyses. The TG peak at *m/z* 881.7 was found throughout the liver specimens on the HF diet. However, the HF + Res diet diminished the peak intensity. Peak intensity also reflected the amounts of TGs in lipid droplets. These results were consistent with the liver histology, as assessed by haematoxylin and eosin (H&E) staining. IMG also provided better semi-quantitative visualisation of the TG peak. Resveratrol effectively reduced the size of the lipid droplets. In conclusion, we investigated the effects of resveratrol on TGs in fatty liver. Resveratrol did not change the composition or structure of the TG species accumulated in the liver, but did ameliorate fatty liver by reducing the size of lipid droplets.

Keywords: resveratrol, fatty liver, lipid droplets.

TUE-496

Role of cardiac lipid metabolism in the development of hypertension-induced heart steatosis

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Spontaneously hypertensive rat (SHR), an animal model of hypertension and cardiovascular disease, is characterized by increased cardiac triglyceride (TG) level, however the mechanism responsible for the development of heart steatosis in this model is unknown. Therefore, we investigated the expression of lipogenic and oxidative factors in the hearts of SHR and nonrmotensive Wistar-Kyoto rats (WKY). Plasma and cardiac TG contents were significantly higher in SHR than in WKY rats at 18 weeks of age. The protein levels of sterol regulatory element-binding protein 1 and fatty acid synthase were not different, whereas levels of stearoyl-CoA desaturase 1 and acetyl-co carboxylase were decreased in the heart of SHR when compared with WKY rats. Obtained results showed that lipogenesis is not upregulated in the myocardium of SHR rats. AMP-activated protein kinase (AMPK) and peroxisome proliferator activated receptor α (PPARα) are important regulators of mitochondrial fatty acid oxidation. AMPK phosphorylation level was lower and PPARα protein level was decreased in the myocardium of SHR rats when compared with WKY control indicating decreased fatty acid oxidation in the heart of SHR rats. Together, obtained results suggest that decreased rate of fatty acid oxidation but not increased lipogenesis contributes to the TG accumulation in the myocardium of SHR rats.

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Keywords: fatty acid oxidation, hypertension, lipogenesis.

TUE-498

Screening of anti-oxidative effects of boron treatment in *Camellia sinensis* L

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Camellia sinensis L. (tea) is one of the most widely consumed drinks in the World. It is suggested that *C. sinensis* have varial medicinal properties one of which is its anti-oxidative attribute. Treatments with Boron, an essential element for plants, has been reported to increase this anti-oxidative property. The present study aims to evaluate the alteration of anti-oxidative effects of boron treated *C. sinensis* L. leaves extracts.

This experiment was carried out in Rize, Turkey. The experiment area was divided into four groups. Each group was grown

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in five randomly organized sample areas (2 m-line) and the tea samples were collected from those five areas. The first group is the control. Boron in concentration range of 0, 100, 300 or 500 g B da^{-1} in sodium tetraborate buffer were applied as a single dose in March 2013. *C. sinensis* leaves were collected in two different periods (May and July 2013). The levels of malondialdehyde (MDA) and reduced glutathione (GSH), the activities of superoxide dismutase (SOD) and catalase (CAT) were measured in *C. sinensis* leaf samples.

MDA level in tea leave extracts showed a significant decrease in all groups at first period. At second period, it was seen that MDA level increased at 100 g B da^{-1} concentration of boron although MDA level was lower in 300 g B da^{-1} concentration of boron. There was a significant decrease in GSH levels of all groups at first period. The reduction of GSH levels in second period was less than in the first period. A difference was not determined in SOD levels among 100, 300, 500 g B da^{-1} concentration of boron at both periods. However, CAT levels elevated at 500 g B da^{-1} concentration of boron at first period.

In conclusion, it is suggested that boron treatment may cause the elevation of antioxidant status of *Camellia sinensis* at the second collection period.

Keywords: *Camellia sinensis*, boron, antioxidant.

TUE-499

Selective inhibitors of glycogen synthase, a key enzyme in glycogen metabolism

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Owing to the vast number of functions performed by oligo- and polysaccharides and glycoconjugates, the ability to selectively inhibit the enzymes that catalyse the synthesis of these glycoderivatives, namely glycosyltransfereases (GTs), is of great interest in biology and medicine. Specifically, disorders related to glycogen metabolism, such as Lafora disease or type 2 diabetes mellitus, might be treated using specific inhibitors of glycogen synthase (GS) or glycogen phosphorylase (GP), which are responsible for the synthesis and degradation of glycogen, respectively. GS catalyses the successive addition of glucose moieties to the non-reducing end of glycogen, while GP catalyses the phosphorolytic cleavage of the α-1,4-glycosidic bonds of the polymer.

With the aim to find novel inhibitors of these two GTs, we have analysed the effect of several glycocompounds, which mimic the donor or the acceptor substrates, on the catalytic activity of glycogen synthase from *E. coli* (*Ec*GS) and glycogen phosphorylase from rabbit muscle (RMGP). Among the compounds studied, ascorbic acid, whose structure resembles that of the glucose unit of the donor substrate, showed the greatest inhibitory potential of *Ec*GS with an IC_{50} of 4.5 nM, a value that was further reduced by 100-fold in the presence of 0.15 mM ADP. Kinetic studies indicate that ascorbic acid mimics the glucosil donor. Interestingly, ascorbic acid did not show any inhibitory effect on RMGP activity.

We also report on the synthesis and biological evaluation of a novel photo-active and selective inhibitor of GS. The designed molecule consists of a glucose molecule with an appended azobenzene moiety, which not only contributes to mimic glycogen, but also enables the photocontrol of the enzyme activity, through a conformational change of this part of the molecule upon irradiation with UV-light. The more stable form of the azoglucoside (*E*) had a slight inhibitory effect of both RMGP (IC_{50} = 4.9 mM) and *Ec*GS (IC_{50} = 1.6 mM). After irradiation and conversion of the azoderivative to the *Z* form, its inhibitory potency of RMGP activity did not significantly change (IC_{50} = 2.4 mM), but its effect on *Ec*GS increased by 50-fold (IC_{50} = 32 μM).

The ability to photo-regulate the activity of key enzymes of glycogen metabolism, such as GP and GS, may provide a way to modulate cellular glycogen content and a new promising tool for biomedical purposes.

Keywords: glycogen metabolism, glycogen synthase, inhibitors.

TUE-500

Serine-arginine protein kinase 1 (SRPK1) is resilient to nuclear translocation under different metabolic stimuli

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Serine-arginine protein kinases (SRPKs) are a subfamily of serine-threonine kinases that specifically phosphorylate serine residues located in regions rich in Ser-Arg/Arg-Ser dipeptide motifs, known as RS domains. SRPK1 was the first SR protein kinase to be purified and characterized. The first known and basic role of SRPK1 is the regulation of mRNA splicing. More recently, however, it is well established that SRPK1 is involved in other cellular activities such as chromatin reorganization, cell cycle regulation and metabolic signaling. Additionally, SRPKs emerge as interesting pharmaceutical targets since they show increased expression in several types of cancers.

Although the most prominent functions of SR protein kinases are nuclear, their sub-cellular localization is primarily cytoplasmic in all tissues and cell lines examined. Additionally, SRPKs are considered to be constitutively active kinases and the way of their regulation is not completely clarified. A factor reported as determining their regulation is their sub-cellular partitioning.

The aim of this work was to study the intracellular localization of SRPK1 under metabolic stimuli such as glucose and amino acid deprivation, oxidative and osmotic stress, hypoxia and others. To this end, HeLa and MCF-7 cells were cultured under the above mentioned conditions or their combinations and their effect on SRPK1 protein levels and intracellular localization was determined by western blotting, immunofluorescence and biochemical fractionation.

Interestingly, under only osmotic stress and glucose deprivation, a low range reorganization of SRPK1 molecules was detected, whereas no changes were detectable either in the localization or the levels of the protein under any of the other stimuli implemented.

Our results show that SRPK1 remains mainly cytoplasmic under most of the conditions tested, implying a fine tuning cellular mechanism that precisely controls the number of SRPK molecules temporarily residing in the nucleus.

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Keywords: metabolic stimuli, nuclear translocation, SRPK1.

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TUE-501

Short treatment with a grape-seed procyanidin extract, in two different rat models of metabolic syndrome, reduces weight gain and leads to metabolic lipid oxidation

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Procyanidins are phenolic compounds with several benefits against risk factors of the metabolic syndrome (MS), but their effects in weight loss haven't been sufficiently studied. In this work we evaluated the effects of two intragastric doses of a grape seed procyanidin extract (GSPE) on weight gain and energy metabolism in two different rat models of MS.

In the first study, 32-weeks-old male Wistar rats were daily treated (1 h before the dark onset) with 0.5 g or 1 g GSPE/kg during 8 consecutive days. Afterwards, rats were subjected to 30 days of a recovery phase without any treatment. Thereafter, each rat underwent an equal treatment period in a staggered design and metabolic energy expenditure and substrate utilization were assessed by indirect calorimetry at the last day of treatment.

In the second study, 12-weeks-old female Wistar rats were treated (1 h before the dark onset) with 0.5 g or 1 g GSPE/kg during 10 consecutive days, with concomitant access to a high-sucrose tasty solution (33 kcal/day). After 18 days of a recovery phase on standard diet, rats were fed a hypercaloric cafeteria diet ($\approx$75 kcal/day). Indirect calorimetry was performed between days 25 and 30 of the cafeteria period to assess energy expenditure and substrate utilization. In both studies, body weight was measured during all the stages.

In male Wistar rats, GSPE administration reduced body weight gain since the 4th to the last day of treatment. The 1 g/kg dose reduced body weight gain more markedly than the 0.5 g/kg dose, but also produced a rebound effect during the recovery phase. After the second treatment, indirect calorimetry revealed a lower respiratory quotient in both treated groups, but only significant in the 0.5 g/kg group. This lower dose also showed a higher energy expenditure.

In female Wistar rats, GSPE administration didn't produce changes in body weight gain during the treatment period or its recovery phase. However, after introducing the cafeteria diet, the 0.5 g/kg group presented a lower body weight gain from the 10th day of the cafeteria. There was no effect in body weight gain in the 1 g/kg group, thus pointing out to a dose-specific preventive effect of GSPE against hypercaloric diet challenges. Indirect calorimetry of the 0.5 g/kg group revealed a lower respiratory quotient than the control group.

GSPE effects on energetic metabolism were highly dependent on the metabolic situation without a dose-response effect. The 0.5 g/kg dose showed very interesting effects for its preventive action after large resting intervals and should be further analyzed to better understand the molecular mechanisms of GSPE activity.

(AGL2011-23879 from the Spanish Government).

Keywords: body weight loss, procyanidins, respiratory quotient.

TUE-502

Some aspects of respiratory metabolism of winter wheat seedlings treated by atmospheric surface dielectric barrier discharge plasma

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The studies concern actions of the exogenous reactive oxygen species (for example, ozone) on biological objects, including seeds of agricultural plants, have established a big presence. One way of the ozone synthesis is the surface dielectric barrier discharge (SDBD). In this work the impact of plasma products of SDBD on the samples of winter wheat (variety Irkutskaya) is examined. The electrode system consists of three strip electrodes radially located via 120 degrees of 1 mm wide and 10 microns thick, the length of the discharge zone on one electrode is 85 mm. Electrodes were located on the surface of the alumina ceramics of 1 mm thick (dimensions of the alumina ceramics plate is 14*95 mm). The sinusoidal voltage was applied to the stripe electrodes, amplitude is 4.2 kV, frequency is 14 kHz. Ceramic barriers placed on a grounded disk, which also served as a radiator for this system. The distance between processed samples, located on a grounded plate, and high voltage electrodes was 8 mm. Exposure time of the sample in ambient air with humidity of 60% was 15 min. Gas flow through the treatment zone was absent. The treatment of wheat seeds was carried out through one day after the beginning of germination. It was shown that the studied type of exposure caused inhibition of growth of winter wheat seedlings: after three days of germination the inhibition of growth was 25%. At the early stages of germination in the absence of light required for the formation of the photosynthetic apparatus and processes of photosynthesis, the main source of ATP and reduced equivalents for metabolic processes in etiolated seedlings is the respiration process. The products of SDBD plasmas action on the winter wheat seedlings resulted in an almost 2-fold increase of the alternative cyanide-resistant pathway contribution in the process of respiration, while the rate of phosphorylating respiration did not change. At the same time, a decrease of non-phosphorylating respiratory rate and an increase of the respiratory control coefficient. Probably, such changes of the respiratory metabolism like increased activity of alternative oxidase, were due to the intensification of the reactive oxygen species generation process in the cells of seedlings, caused by the studied type of the treatment, since its activation occurs under conditions of inhibition of the electron transport by the main cytochrome pathway.

Keywords: respiratory metabolism, surface dielectric barrier discharge, winter wheat.

TUE-504

Structural and functional characteristics of the first 13-specific divinyl ether synthases: LuDES (CYP74B16) and RaDES (CYP74Q1)

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Keywords: divinyl ether synthase, lipoxygenase pathway, site-directed mutagenesis.

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TUE-505
Structural and functional studies of a high-activity [NiFeSe] hydrogenase

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Hydrogen (H2) is an energy carrier with the potential to become an environment-friendly fuel in the future. However, its production at present still relies on the thermocatalytic transformation of fossil fuels. The biological production of H2 offers the opportunity of generating H2 as a clean technology from renewable sources [1,2]. The [NiFeSe] hydrogenases belong to a subgroup of the [NiFe] proteins in which a selenocysteine is a ligand of the Ni atom. These enzymes are attractive candidates for the biological production of hydrogen, since they display faster reactivation after O2 exposure than the standard [NiFe] Hases and also exhibit much higher H2 production activities [4,5]. For the first time, we developed a homologous system for recombinant expression of a [NiFeSe] Hase in *Desulfovibrio vulgaris* Hildenborough. In this work we fully characterize the recombinant [NiFeSe] Hase, presenting also high-resolution 3D structures obtained by X-ray crystallography that provide further insights into the process of O2 inactivation of this group of enzymes. This expression system will enable the production of mutant variants of the [NiFeSe] Hase that will permit more detailed functional studies. Moreover, this study will contribute to the design and improvement of biocatalysts for H2 production.

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Keywords: hydrogenases, recombinant expression, structure.

TUE-506
Structural characterization of multidrug resistance protein 6 (MRP 6) and its involvment in the ectopic calcification

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In humans, ATP-binding cassette (ABC) transporter superfamily includes 48 proteins divided into 7 subfamilies (A-G) that hydrolyze ATP and transport a wide variety of substrates across membranes. Multidrug Resistance Protein 6 (MRP6) is codified from ABCC6 gene and it is expressed primarly in liver and kidneys at the basolateral membrane [1].

MRP6 transports glutathione S-conjugates, but its natural substrates remain undefined; it confers low levels of resistance to several anticancer drugs leading to multidrug resistance [2]. Mutations of ABCC6 gene cause Pseudoxanthoma Elasticum (PXE), a recessive genetic disorder characterized by ectopic calcification of connective fibers [3].

MRP6 topology presents three transmembrane domains (TMDs) and two nucleotide-binding domains (NBDs), that function cooperatively to bind and hydrolyze ATP for the transport of substrates across biological membranes. Studies performed on isolated NBD domains demonstrated that NBD1 has a higher tendency to form an active homodimer while NBD2 binds ATP and presents ATPase activity although significantly lower compared with isolated NBD1. The mixture of NBD2 and NBD1 exhibited an activity similar to NBD2 alone [4–6]. Moreover, since the role of the additional NH_2-terminal domain (TMD0) is not characterized, we decided to investigate about its function.

As the pathophysiological mechanism whereby MRP6 deficiency results in the onset of PXE is unknown [7], we evaluate in ABCC6-knockdown HepG2 the expression of genes involved in the pathogenesis of ectopic mineralization.

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Keywords: ABCC6, NBDs, PXE

TUE-507
Structure elucidation of novel cold-adapted esterase with unusual thermostability from psychrophilic bacterium *Psychrobacter cryohalolentis* K5T

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Cold environments cover more than 75% of the Earth's surface and include Arctic and Antarctic ice, soils and sediments, ocean water and others. Cold-adapted microorganisms are known to produce cold-active enzymes with highest activity at low and moderate temperatures and easy inactivation upon heating. Elucidation of structural aspects responsible for such enzymes characteristics has fundamental interest and from the other hand could be useful for development of taylor-made industrial biocatalysts.

Psychrophilic bacterium *Psychrobacter cryohalolentis* $K5^T$, was isolated from 110 000-year old cryopeg in Kolyma lowland and was capable of growth in the wide temperature interval from −10°C to +30°C and increased salinity (up to 13% NaCl). Examination of *P. cryohalolentis* $K5^T$ genomic sequence revealed the presence of several genes coding for potential lipases/esterases. A potential esterase EstPc, belonging to lipase Family V was identified in bacterial genome and subsequently cloned and expressed in *E. coli* cells. Recombinant esterase displayed high

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activity at 0–35°C as well as relative thermostability. It was half-inactivated only after 45 min heating at 90°C.

To reveal molecular basis of EstPc unusual characteristics we have solved its 3D structure by X-ray crystallography. Structure was solved at 2.15 Å resolution and refined to R_f=18.7% and R_{free}=25.4%. Despite the protein has typical α/β hydrolase fold and its structure is similar to known esterases, comprehensive structure analysis revealed some special features possibly responsible for unusual enzyme characteristics.

This work is supported by scholarship of the President of Russian Federation (СП-1390.2012.4) and RFBR grant 14-04-31573 mol_a.
Keywords: cold-active enzymes, esterase, Protein structure.

TUE-508
Structure features of TBN1, a P1/S1-like nuclease

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Tomato bifunctional nuclease 1 is a multifunctional zinc-dependent, acidic, plant nuclease with P1/S1-like fold. Exact role of TBN1 is not known but it plays an important role in specific apoptotic functions, cell senescence vascular system development, stress response and tissue differentiation in plants. It shows anti-cancerogenic properties which were proven using mice bearing human tumours [1]. Variants of TBN1 used in our studies were produced recombinantly in *Nicotiana benthamiana* leafs. Presence of zinc in the protein was confirmed by X-ray fluorescence and absorption edge scan. The phase problem was solved using a combination of multi-wavelength anomalous dispersion and real space molecular replacement [2]. TBN1 has a P1/S1-like nuclease fold with a zinc cluster placed in the active site in the centre of the wide groove. Three oligosaccharides bonded on the surface serve primarily as a shielding of the hydrophobic regions and therefore contribute to solubility and stability of the enzyme. TBN1 acts as phosphodiesterase (and phosphomonoesterase) cleaving the bond between phosphorus and 3' hydroxyl group in both single stranded and double stranded forms of DNA and RNA and it also shows 3'-nucleotidase activity. Newly, a phospholipase C-like activity was also discovered. Comparison of TBN1 with single-strand specific P1/S1-like nucleases and other zinc dependent enzymes led to our better understanding of their substrate promiscuity and natural properties [3].

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Keywords: P1/S1-like nuclease, zinc dependence.

TUE-509
Study of anti-Warburg effect of AMPK activators and antimetabolite drugs on MCF7 cell model

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AMPK is an energy sensor that regulates cellular metabolism. When activated by a deficit in nutrient status, AMPK stimulates mitochondrial energy production, while turning off energy-consuming processes to restore energy balance. We analyzed AMPK activation in MCF7 ductal breast cancer cells.

AMPK can work as an anti-Warburg agent via enhancing mitochondrial biogenesis.

In MCF7 cells we studied the combined action of the AMPK activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), and the antimetabolite folate analog, Methotrexate. We observed increased mitochondrial activity, while glycolytic flux decreased. The combination of drugs also disrupted the phases of the cell cycle, and the cells were accumulated in the G1 and G2 phase. These data suggest that the combined application of AICAR and MTX brings about synthetic lethality through an anti-Warburg effect.

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Keywords: AMPK, anti-Warburg, mitochondria.

TUE-510
Study of enzyme activity in mutant lines of soft wheat obtained under the surfactants action

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Over the past decade, a large number of mutants were obtained experimentally for agricultural crops, which differ from the original in several features. One of the most reliable indicators of changes in the genetic apparatus is enzyme activity alteration. Those are the enzymes, influencing the intensity of the most important parts of metabolism, leading to an accurate estimation of the viability of obtained mutant genotypes.

It has been shown that exposure to aqueous solutions of various surfactants (1%) prior to sowing induced changes in traits, which are inherited through generations M_1-M_4.

The aim of this work is study on activity of key enzymes of nitrogen and energy metabolism in the obtained mutant lines (glutamate dehydrogenase – GDH, enzyme complex of malate dehydrogenase and glutamate aminotransferase – EC MDH-GOAT, malate dehydrogenase – MDH, alcohol dehydrogenase – ADH). Seeds of third generation mutant lines of common wheat and the original varieties Zhenis, Kazakhstanskaya 3, Shagala were used as research material. The reaction mixtures for estimation of enzyme activity contained: for GDG – NADH, 2-oksoglyutarat, ammonium sulfate for EC MDH-GOAT – NAD, malate, glutamate, for MDH – NAD and malate, for ADH – NAD and 96% ethanol. Estimation of enzyme activity (μM coenzyme/mg protein per min) was determined at the length of 340 nm, the total protein content (mg/ml) – at 330 nm.

It has been noted that mutant lines differ in content of soluble protein. For instance, in Shagala variety lines 3,4,5 its content is

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increased up to 9% (0.198 ± 0.02 mg/ml, 0.205 ± 0.01 mg/ml, 0.200 ± 0.02 mg/ml, respectively, in control – 0.188 ± 0.01 mg/ml). Study of four enzyme systems activity shows the lines variation in their spectrum of activities. Lines selection on decreased GDH activity (10 lines in the range of 53.22 ± 0.01 to 64.51 ± 0.01 mM/mg; the initial variety Shagala-72.58 ± 0.01 mM/mg) and increased activity of EC MDH-GOAT (5 lines in the range of 403.17 ± 0.01 to 443.27 ± 0.01 mM/mg; the initial variety Shagala-344.44 ± 0.01 mM/mg) was conducted. For genetic and breeding work mutants with reduced GDH activity are interesting, since this enzyme releases toxic ammonia destroying biomembrane. MDH-GOAT plays an important role in the detoxification of products of protein degradation under abiotic and biotic stresses such as salinity, drought, etc. Plants with reduced GDH and high MDH-GOAT activities survive better in case of infection and maintain high productivity. Also the number of lines displayed an increase in ADH and MDH enzyme activity in comparison to the initial variety, the latter might indicate the activation of the respiratory processes. All of this suggests a large term use of surfactants for creation of valuable genotypes of major crops.

Keywords: crop breeding, enzyme activity.

TUE-511
Study of the expression pattern of L-Dopa decarboxylase (DDC) in human β-pancreatic cells: indications of involvement in the insulin-biosynthesis pathway

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The link between the dopaminergic system and glucose metabolism is largely supported by in-vitro and in-vivo studies in animals, and clinical data from psychiatric individuals. However, little is known about the exact molecular pathways underlying this relationship. Herein, we sought to investigate the expression pattern of 3,4-Dihydroxy-L-phenylalanine (*L*-Dopa) decarboxylase (DDC), the enzyme catalyzing the biosynthesis of the neurotransmitters dopamine and probably serotonin, under insulin-pathway stimulating conditions in human β-pancreatic cells.

Methods: The 1.2B4 immortalized human β-cell line was subjected to a titration of human insulin concentrations corresponding to normal insulin blood levels (0–100 μU/ml). In another set of experiments, insulin was added in higher concentration (14.5 mU/ml) with and without the presence of the LY294002 PI3K inhibitor (50 μM). The DDC mRNA and its predicted regulator miR-145 levels were estimated by qRT-PCR, while DDC protein levels by Western blot. The intra- and extra-cellular insulin contents were measured with a commercial ELISA assay. Each condition was tested in duplicates and in 3 independent experiments.

Results: Our data revealed negative correlation between the *DDC* mRNA levels and the amount of insulin added to the culture ($r = -0.88$, $p = 0.01$), as well as the intra- ($r = -0.78$, $p = 0.03$) and extra-cellular insulin content ($r = -0.99$, $p < 0.0001$). Yet, when insulin was added in higher concentration the DDC mRNA levels were increased (~1.5-fold) compared to untreated cells, and that was reversed by the addition of the LY294002 insulin pathway inhibitor. MiR-145 levels were by ~2-fold up-regulated by insulin addition, and by ~4-fold following LY294002 treatment. Likely changes in DDC protein levels were proved not possible to be detected by the method used.

Conclusion: Our preliminary data indicate significant association of the mRNA levels of *DDC*, the key-molecule in neurotransmitters' biosynthesis, with the insulin pathway in human β-cells supporting the widely accepted notion of communication between the dopaminergic system and glucose metabolism, that is, herein, for the first time studied in-vitro on cells of human origin. Further investigation is needed to elucidate the subtending molecular mechanisms that may serve as potential targets for the treatment of type 2 diabetes.

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Keywords: human β-pancreatic cells, insulin biosynthesis, L-Dopa decarboxylase.

TUE-512
Study on responses of ceramide metabolism pathway enzymes in mice liver tissue culture treated with withaferin A and withanolide A

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Ceramide play important roles in intracellular signaling involve in differentiation, proliferation and apoptosis. Withaferin A and Withanolide A are main bioactive compound that traditionally use to cure ulcer, rheumatism and leucoderama. In this investigation, we evaluated ceramide synthase, serine-palmitoyl transferase and dihydroceramide desaturase as anabolic pathway enzymes and ceramidase activity as anabolic enzyme in liver tissue culture treated with 0 to 120 μg/kg. Results showed markedly significant inhibition on ceramidase activity (61%) at 80 to 120 μg/kg of withaferin A and slightly increase (18%) on ceramide synthase and dihydroceramide desaturase (15%) activities with respect to control. There was no considerable effect on activity of serine-palmitoyl transferase in liver for both of these compounds. In addition, sphingosine level did not vary considerably in response to each compound. However, ceramide level increased in a dose dependent manner of withaferin A treatment and reached the highest level at 80 μg/kg exposure. On the other hand Withanolide A treatment did not elevated ceramide considerably as compared with control.

Our findings clarified the role of ceramide elevation in response to withaferin A in comparison to Withanolide A treatment that may involve in reported biological activities of this medicinal plant compound.

Keywords: Withaferin A, ceramide synthesis, sphingosine, liver tissue.

TUE-513
Substrate specific gene expression profiles on different kidney cell types associated with Fabry disease

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Fabry disease is an X-linked inborn error of glycosphingolipid metabolism associated with deficient alpha-galactosidase A activity. The major clinical feature of fabry disease, such as fibrosis in

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cardiomyopathy and renal failure has been observed with abnormal accumulation of globotriaosylceramide (Gb3) in biological fluids, vascular endothelium, heart and kidney. In addition to Gb3, increased concentration of deacylated Gb3 (lyso-Gb3) in the plasma of symptomatic patients also has been suggested as a causative molecular event. However, the correlation between fibrogenesis and elevated levels of these sphingolipids is poorly understood.

To identify the genetic mechanisms involved in renal fibrosis in fabry disease, we analyzed the changes of global gene expression before and after Gb3 or lyso-Gb3 treatment on two types of kidney cell lines, human proximal renal tubular epithelial cells (HK-2) and mouse renal glomerular mesangial cells (SV40MES13), using microarray. The results showed that Gb3 and lyso-Gb3 regulate the expression of 199 and 328 genes in each cell type, with at least 2.0-fold change considerations. To identify key biological functions, we classified all the genes into 4 groups based on the expression patterns (1) Gb3 up, (2) Gb3 down, (3) lyso-Gb3 up, (4) lyso-Gb3 down regulated genes and performed functional annotation analysis using the DAVID tool. Most biological functions were related to fibrogenesis or epithelial-mesenchymal transition (EMT). Interestingly, the gene expression patterns were significantly different between treated with Gb3 and treated with lyso-Gb3. The expressions of EMT related genes (F8, FOXP2, HOXA11, DLL1 and WT1) were confirmed by realtime-PCR or western blot. These findings suggested that Gb3 and lyso-Gb3 lead to renal fibrosis in Fabry disease with different biochemical modulations.

Keywords: EMT, Fabry disease, Gb3.

TUE-514
Surprise effects of Beta-blocker timolol on diabetes-induced pancreas tissue

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Almost 40 years ago, Timolol was a novel drug proposed as an antihypertensive, antiarrhythmic, antiangina, and antiglaucoma agent. And during these years, it was proposed that no significant side effects were observed. It is also used in the treatment of migraine and tremor. β-blockers have beneficial effects on heart dysfunction by neutralizing reactive oxygen species.

In this current study, we have investigated the Timolol treatment of streptozotocin-induced diabetic rats (timolol for 12-week with 5 mg/kg daily following diabetes-induction) has efficiency to prevent hyperglycemia-induced pancreas damage by enhancing the depressed antioxidant defense in pancreas.

Intracellular $NADP^+$/NADPH ratio is of critical importance for maintaining not only cellular homeostasis but also in regulation of many metabolic pathways and Pentose Phosphate Pathway (PPP) accounts for almost %60 production of reducing molecule, NAPDH. One of the main role of NADPH is regeneration of reduced Glutathione and thereby preventing oxidative stress. It is for that reason, enzymes associated with antioxidant defense system, namely Glucose-6-phosphate dehydrogenase (G6PD) and 6-Phosphogluconate Dehydrogenase (6PGD), were analyzed in pancreas tissues. Both of these enzymes have key roles in the synthesis of ribose 5-phosphate, which is required for cell proliferation, and in NADPH production, which participates in biosynthetic and detoxification pathways.

Polypharmacology is a rising, innovative concept that describes the activity of compounds at numerous targets. By this study, we have investigated the Timolol-treatment also significantly normalized these antioxidant enzyme levels in diabetic rat pancreas tissues. By this way we have concluded that Timolol has a beneficial effect on diabetic rat tissues by up-regulating the activities of antioxidant enzymes G6PD and 6PGD. Therefore, increased concentration of NADPH enables pancreas cells to reverse the oxidative stress by the action of the glutathione system.

Keywords: Antioxidants, Beta-blockers, Diabetes.

TUE-515
The activity of band 3 protein of erythrocytes in patients with chronic kidney disease

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Development of chronic kidney disease (CKD) is accompanied by a change in physical and chemical properties of erythrocytes membranes.

The aim of the study was to assess the activity band 3 protein of erythrocytes in patients with CKD 1 and 2 stages depending on the initial etiological nosology.

Two groups were formed. The 1st – patients with chronic glomerulonephritis (CG), CKD 1, 2. The 2nd – patients with chronic pyelonephritis (CP), CKD 1, 2. The activity of the Cl^-/HCO_3^- exchanger of band 3 protein was judged by the dynamics of changes in the erythrocytes volume during the incubation of ammonium medium by the method of Mindukshev (2010). Ammonium load run for 15 min with the measurement of MCV in every odd minute of incubation. Was determined the difference between the initial value of MCV (without ammonium load) and maximum MCV, obtained during the incubation. The distribution of ΔMCV values was described by median and quartiles. Statistically significant differences of data in the comparison groups were assessed using the Mann-Whitney test.

In patients with CG value of ΔMCV Md = 9.3 fl (8.2, 11.2) was higher than this indicator in patients with CP Md = 8.0 fl (5.4, 8.1), these changes were not significant, $p = 0.09$.

In patients with CG in 42.8% of cases the swelling of erythrocytes occurred during the initial minutes of incubation, with maximum MCV in the 3rd minute. In 28.6% of cases maximum MVC was determined in the 5th minute and 28.6% in the 7th minute of incubation.

In patients with CP in 44.4% of cases maximum MCV reached in the 3rd minute, and in 55.6% of cases – in the 5th minute.

The incubation of erythrocytes in ammonium medium leads to activation of band 3 protein. The degree of increase in MCV doesn't depend on nosological forms of CKD. However, in the 1st group there are the patients with resistant erythrocytes, which can survive during 7 min of incubation in ammonium medium. Further research is needed for studying this phenomenon.

Keywords: band 3 protein, chronic kidney disease, erythrocytes.

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TUE-516
The binding of phycocyanobilin to human hemoglobin and serum albumin: comparison with bilirubin

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Phycocyanobilin (PCB) is covalently attached linear tetrapyrrole chromophore of phycocyanin, a major protein of filamentous cyanobacteria Spirulina (genus *Arthrospira*). Phycocyanin shows a wide range of antioxidant, anti-inflammatory and immunomodulatory properties, and promising anti-cancer and hypocholesterolemic activity. Most of the mentioned effects are ascribed to phycocyanobilin. Blue phycocyanobilin is very similar in chemical structure to bile pigment bilirubin, the end product of heme metabolism. It is well documented bilirubin binding to human serum albumin (HSA).

The present study was undertaken to determine whether the PCB molecule binds to HSA and hemoglobin (Hb), and to partially characterize its binding to these proteins. High purity PCB was isolated from commercial *Spirulina pacifica* powder. OxyHb and HSA were purified from the fresh blood of healthy donors.

Influx of both bilirubin and PCB was detected into erythrocytes' cytosol under simulated physiological conditions. Spectrophotometry data indicated binding of PCB (a red spectral Soret band shift) and bilirubin (a blue shift and decrease in Soret band intensity) to Hb, and also PCB for HSA (a red spectral shift of pigment at 360 nm). Analysis of the data obtained by spectrofluorimetry and equilibration dialysis revealed the similarity in binding, stoichiometry (approx. 1:1) and chromophores binding constants (10^6 for HSA and 10^5 for Hb). The binding of PCB does not cause dramatic changes in the structure of proteins (CD spectra) or hemoglobin oxidation (MetHb formation).

Our results could contribute to the biochemistry of bilins in the systemic circulation, as well as to physiological and pharmacological effects of protein components of Spirulina, as a highly valuable functional foods.

Disclosure of Interest: None Declared.
Keywords: hemoglobin, human serum albumin, phycocyanobilin.

TUE-517
The CBLc proteome: *in vivo* elucidation of altered cellular pathway in humans

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Cobalamin deficiency type C with methylmalonic aciduria and homocystinuria (Cbl-C; MMACHC; MIM#s 277400 and 609831;) is the most frequent genetic disorder of vitamin B12 metabolism (1). Even if the exact function of the MMACHC protein is still unclear, recent studies reported that it may act both as an intracellular cobalamin trafficking chaperone and as a decyanase, catalysing the reductive decyanation of cyanocobalamin thus generating the substrate for assimilation into the active cofactor forms of methylcobalamin (meCbl) and adenosylcobalamin (AdoCbl) (2). Moreover, the MMACHC protein may catalyse the dealkylation of newly internalised methylcobalamin and 5'-deoxyadenosylcobalamin, the naturally occurring alkylcobalamin present in the diet (3). To improve metabolic abnormalities, Cbl-C patients were treated with a combined therapy, which included hydroxocobalamin (OHcbl), betaine, and folic acid. Nevertheless, despite this treatment, the long-term follow-up is unsatisfactory (4). In the present study, the in-vivo proteome of MMACHC patients was quantitatively examined by two dimensional difference in-gel electrophoresis (2D-DIGE) and mass spectrometry. Six biological replicates of protein lysates from lymphocytes from CblC patients were used to identify deregulated proteins employing as a control lymphocytes from healthy donors. The experimental procedure allowed us to identify 63 proteins differentially expressed in pathological tissues. Western blot analyses were used to validate deregulated proteins. Consistent with in-vivo studies showing relevant disturbance of glutathione metabolism (5), we found a deregulation in protein involved in cellular detoxification, especially in glutathione metabolism. Our study demonstrate an in-vivo relevant changes in the proteome profile of treated cblC patients, and confirms previous results observed in-vitro (6). These observations may be helpful for better understanding the pathophysiology of the disease and in addressing future research and novel therapeutical strategies.

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Keywords: None.

TUE-518
The degree of genetic variability on zinc in mutation germpasm of spring wheat (M5 generation) obtained through gamma irradiation

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Zinc (Zn) deficiency associated with low dietary intake is a well-documented public health problem, resulting in serious health and socioeconomic problems. Wheat is a crop of major importance and supply the bulk of nutrients in the diets. It is known that genotypic variation for Zn content in grain among wheat cultivars relatively low. Mutation breeding is one of an important tool in crop improvement with increased agronomic values. We developed high-yielding M_5 mutant lines on yield components such as grain weight per plant and thousands grain weight through gamma radiation by 100 Gy doses of and 200.

We found in screening results on grain zinc content that extensive variation exists for grains Zn concentrations in germplasm developed by 100 and 200 Gy having its spectrum from 14.6 to 60.93 mg kg^{-1} with mean = 30.78 mg kg^{-1}, SD = 3.17 mg kg^{-1}; n = 15) and from 17.9 to 91.40 mg kg^{-1} with mean = 53.05 mg kg^{-1}, SD = 23.70 mg kg^{-1}; n = 15). respectively. The parental cultivar zhenis has 13.11 mg kg^{-1} ± 1.85. Thus, the variations in Fe content in germplasm induced by treatment of 200 Gy with number of significant positive lines was higher than in germplasm developed by 100 Gy. In 100 Gy germplasm seven lines (■5(4), ■6(4), ■6(13), ■18(5), (■24(2), ■25 (2) and ■26(1) had significantly enhanced grain zinc content compared to cv. Zhenis. In these lines the Zn content was by 4.7, 4.6,

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3.3, 3.4, 3.0, 2.1, and 2.3 times higher than in cv. Zhenis, respectively. Thirteen M5 lines from 200 Gy germplasm were by from 2.1 to.6.9 times higher them that in cv. Zhenis. These results clearly indicate that enough genetic variation exists in mutation germplasm to increase Fe concentrations substantially in wheat grain. Six in each M5 mutant germplasm developed by 100 and 200 Gy showed positive correlation between this trait and such yield component as thousand grain weight (r = 0.53–0.99).

Keywords: zinc enriched mutation lines of spring wheat.

TUE-519
The effect of proteasome on c-jun mediated signaling mechanisms in hypercholesterolemia induced oxidative stress

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Atherosclerosis and its complications are major causes of the death all over the world. One of the major risks for atherosclerosis is hypercholesterolemia. Increased reactive oxygen species (ROS) production and the resulting oxidative cell stress that occurs in many disease states has been shown to induce signaling mechanisms. During atherosclerosis, oxLDL regulates CD36-mediated activation of JNK1 and modulates MMP induction which stimulates inflammation with an invasion of monocytes. Additionally, inhibition of proteasome leads to an accumulation of c-jun and phosphorylated c-jun and activation of AP-1 related increase of MMP expression.

We have previously reported a significant increase in CD36 mRNA levels in hypercholesterolemic rabbits and shown that vitamin E treatment prevented the cholesterol induced increase in CD36 mRNA expression. In the present study, our aim was to identify the signaling molecules/transcription factors involved in the progression of atherosclerosis following CD36 activation in an *in vivo* model of hypercholesterolemic (induced by 2% cholesterol containing diet) rabbits. In this direction, proteasomal activities by fluorometry and c-jun, phospo c-jun, JNK1, MMP-9 expressions by quantitative RT-PCR and immunoblotting were tested in aortic tissues.

The effects of vitamin E on these changes were also investigated in this model. As a result, c-jun was phosphorylated following decreased proteasomal degradation in hypercholesterolemic group. MMP-9 expression was also increased in cholesterol group rabbits contributing to the development of atherosclerosis. In addition, vitamin E showed its effect by decreasing MMP-9 levels and phosphorylation of c-jun.

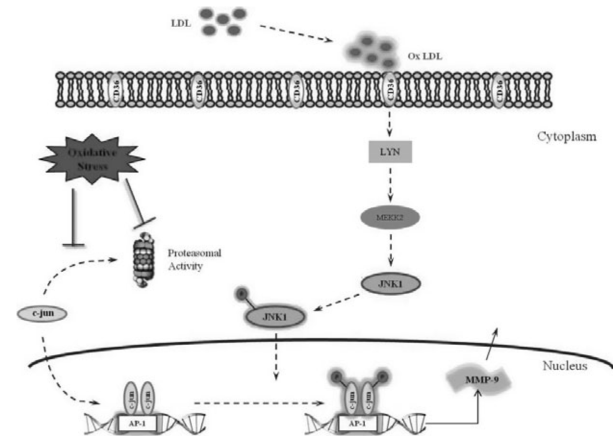

Fig. 1.

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Keywords: Atherosclerosis, Hypercholesterolemia, Proteasome.

TUE-520
The effect of β2-α2 surface loop deletion on thermal stability of β-1,3-1,4 endoglucanase from *Thermotoga maritima*

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Thermostable endoglucanases are of great importance for industrial applications. In this study, we cloned and purified the β-1,3-1,4 endoglucanase (EC 3.2.1.73) from *Thermotoga maritima*. We hypothesized that the β2-α2 surface loop could have an effect on thermal stability of the enzyme. We generated an enzyme mutant lacking that loop. Catalytic parameters and thermal stability of the enzyme mutant was compared with those of wild-type enzyme. We showed that deletion of the β2-α2 surface loop decreased the thermal stability of β-1,3-1,4 endoglucanase. Although it is common that deleting or shortening of loops increase thermal stability of proteins, our results showed that the surface loop contributed to the thermal stability.

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Keywords: site-directed mutagenesis, Thermostable endoglucanases, *Thermotoga maritima*.

TUE-521
The effects of boric acid on antioxidant enzymes

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Oxidative stress is caused by the production of reactive oxygen species (ROS) or free radicals. They are formed by oxygen or other molecules. Excessive amounts of free radicals may damage lipids, proteins, and nucleic acids and may cause carcinogenesis. Boric acid is a form of boron mineral released into the environment and it is used in pharmaceuticals and industrial materials. For example, in pesticide industry it is used as food preservative against plant fungicides. Boric acid can strengthen the antioxidant defenses with an unknown mechanism that may involve changes in oxidative metabolism.

In this study, red blood cells obtained from human were exposed to different concentrations of boric acid and the effects of boric acid on antioxidant enzyme activities were investigated *in vitro*. Catalase (CAT), superoxide dismutase (SOD), glutathione-*S*-transferase (GST), glutathione reductase (GSH), glutathione peroxidase (GSH-Px) and glucose-6-phosphate dehydrogenase (G-6-PDH) which are the antioxidant enzymes that have important roles in cell defense against ROS were examined. Boric acid

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showed neither an activator nor an inhibitor effect on these antioxidant enzymes. The results of this study led us to conclude that there was no significant effect ($p > 0.05$) on antioxidant enzyme activities of the boric acid in the studied concentration ranges.
Keywords: Antioxidant enzymes, Boric acid, Oxidative stress.

TUE-522

The effects of fluoxetine on circulating oxidative damage parameters in rats exposed to aortic ischemia-reperfusion

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Background: Oxidative stress and reperfusion injury may develop in different ischemia-reperfusion (IR) models. Growing evidence links altered lipid protein redox-homeostasis with IR. The effect of fluoxetine (FLX; N-methyl-3-[4-(trifluoromethyl) phenoxy] benzenepropanamine), on the lipid protein redox-homeostasis mechanisms in the rats exposed to aortic IR is unclear. In the current study, we aimed to investigate the effects of FLX on circulating protein oxidation and lipid peroxidation parameters, such as ischemia modified albumin (IMA), lipid hydroperoxide (LOOH), pro-oxidant-antioxidant balance (PAB), erythrocyte glutathione (GSH), CuZn-superoxide dismutase (CuZn-SOD), ferric reducing antioxidant power (FRAP), as potential IR biomarkers.
Methods: Wistar rats were randomised into three groups ($n = 7$/group): 1) Control (sham laparotomy); 2) IR without FLX, (60 min ischemia and 120 min reperfusion); 3) IR with FLX (FLX + IR) (FLX 20 mg/kg/day, i.p. for three day before surgery). All of the aforementioned parameters (IMA, LOOH, PAB, GSH, CuZn-SOD, FRAP) were measured spectrophotometrically.
Results: IMA, LOOH, and PAB levels in IR group were significantly higher than the control ($p < 0.01$ respectively) and fluoxetine groups ($p < 0.01$, $p < 0.01$, $p < 0.05$ respectively), whereas CuZn-SOD activities, GSH and FRAP levels were significantly lower in IR groups. In addition, fluoxetine group significantly reduced IMA levels when compared to IR group ($p < 0.001$) and control group ($p < 0.01$).
Conclusion: With respect to IMA, LOOH and PAB, impaired redox homeostasis is substantially more prominent in aortic IR. The antidepressant FLX has profitable effects on circulating redox status in rats exposed to aortic IR. FLX administration before IR might decrease the surgery-enhanced free radical production; taken together, the antioxidant effects of FLX supplementation should be considered in future studies.
Keywords: Fluoxetine, aortic ischemia-reperfusion, ischemia modified albumin, lipid hydroperoxide, pro-oxidant-antioxidant balance, glutathione.

TUE-524

The effects of *Salvia absconditiflora* water extract on HepG2 liver cell line

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Salvia absconditiflora is one of the endemic Salvia species grown in Central Anatolia, Turkey, which is consumed as herbal tea. There are around 900 species of Salvia, which contain high amounts of polyphenolic/ bioactive materials and they are used traditionally to treat several diseases. Ninety five of them are present in Turkey. Salvia species have lots of phenolic and flavanoid components including Rosmarinic acid-which is the main phenolic component, catechin, caffeic acid, vanillic acid, ferulic acid, rutin, apigenin, quercetin, and luteolin.

In vitro antioxidant activity of *S. absconditiflora* leaves were determined using DPPH radical scavenging activity assay. Total phenolic and flavanoid contents of *Salvia absconditiflora* water extracts were measured spectrophotometrically and cytotoxic effects on HepG2 hepatacellular carcinoma cell lines were examined via XTT colorimetric and Trypan Dye Exclusion cell viability assay. LC-MS/MS analyses revealed the presence of rosmaniric and caffeic acid, luteolin rutin and coumaric acid. Rosmarinic acid and caffeic acid content of *S. absconditiflora* water extract was determined by using RP- HPLC.

IC_{50} value for DPPH radical scavenging activities of water extracts of *Salvia absconditiflora* leaves collected in 3 months (April-May-June) were calculated as 1071 mg/ml, 1137 mg/ml and 0.999 mg/ml respectively. Rosmarinic acid and caffeic acid content of the these extracts (April-May-June) were determined as 23.69 mg RA/g extract- 2938 mgCA/g extract; 20 395 mg RA/g extract– 2386 mgCA/g extract; 19 635 mg RA/g extract-1718 mgCA/g extract, respectively. IC_{50} value for 48 h incubation of water extracts were 3.13 mg/ml for April, 3.95 mg/ml for May and 3.57 mg/ml for June and IC_{50} value for 72 h incubation of water extracts were found as 2.87 mg/ml for April, 2.1 mg/ml for May and 2.42 mg/ml for June. IC_{50} value of water extracts for all three months were calculated as 3.55 mg/ml for 48 h incubation, 2.57 mg/ml for 72 h incubation.

Effects of *S. Absconditiflora* water extract on the expression of CYP 3A4 and CYP 1A2 in HepG2 cells were investigated with qRT-PCR technique. Treatment of HepG2 cells with different concentrations of water extract increased the expression of both CYP 3A4 and CYP 1A2 genes.
Keywords: Antioxidants, HepG2 Cell Line, Salvia absconditiflora.

TUE-525

The extracellular free purine bases in blood of patients with chronic renal failure

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The aim of the work was to study the free purine bases in blood plasma of patients with chronic renal failure depended on initial clinical form. All patients were treating with hemodialysis. Patients were divided into 2 groups. 28 patients with chronic renal failure of terminal stage (initial disease – pyelonephritis) were included in first group. 16 patients with chronic renal failure of terminal stage (initial disease – glomerulonephritis) were included in second group. The control group consisted of 32 healthy subjects. All patients and healthy subjects had received the full information on probable inconveniences at the blood sampling before giving their written informed consent.

The detection of free purine bases took place in plasma of all subjects following the protocol of Oreshnikov et al. (2008) between of hemodialysis procedures.

The tendency to an augment of free purine bases (guanine, adenine), intermediates of purine catabolism (hypoxanthine, xanthine, uric acid) concentrations in plasma of first group patients was observed. The significant increasing of free purine bases (by 2.8 times, $p < 0.001$), hypoxanthine (by 3 times, $p < 0.001$), xan-

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thine (by 2.2 times, $p < 0.001$) and uric acid (by 1.9 times, $p < 0.001$) was determined in blood plasma in patients of the second group in comparison with indicators of healthy subjects.

Taken together the results obtained demonstrated significant differences between free purine bases and intermediates of purine catabolism in blood of patients with chronic renal failure depended on initial clinical form.

Keywords: purine bases, blood.

TUE-526

The formation of intramolecular complex between N-terminal domain and the main protein body of L-type pyruvate kinase can be responsible for the change of catalytic properties of enzyme

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Pyruvate kinases catalyse the final step of glycolysis, transferring the phosphoryl group of phosphoenolpyruvate (PEP) to adenosine diphosphate (ADP) and producing pyruvate and ATP. In liver tissue both glycolysis and glyconeogenesis can occur, and therefore coordination and reciprocal regulation of these metabolic pathways is necessary.

L-PK can be activated homotropically by PEP and heterotropically by fructose-1,6-bisphosphate (FBP), and both of these effects depend on phosphorylation of the N-terminal domain of this enzyme. Kinetic measurements revealed that the non-phosphorylated L-PK followed common hyperbolic Michaelis-Menten plot and the activity of the enzyme was not regulated by FBP. Phosphorylation of the protein by cAMP-dependent protein kinase switched on the cooperativity of the enzyme toward PEP (Faustova, I. *et al.*, 2010).

The regulatory phosphorylation occurs on the 12th serine residue of N-terminal domain. So it was checked whether redistribution and/or introduction of new ionic groups around the phosphorylation site can mimic the effect of phosphorylation. Some of these mutations reduced the effectiveness of PEP binding, but differently of phosphorylation, the cooperativity of the enzyme was not switched on by these mutations (Faustova, I *et al.*, 2012).

It was suggested (Fenton & Tang 2009) that the flexible N-domain may interact with the main body of the enzyme affecting substrate binding, and this interaction can be interfered by phosphorylation. Following this suggestion the computational blind docking approach was used to identify putative binding sites in L-PK subunit for peptides RRASVA and the phosphorylated derivative RRAS(Pi)VA. These peptides mimic the phosphorylatable N-terminal regulatory domain of the enzyme. Also the same docking analysis was done for both L-PK substrates: PEP, ADP, and for the allosteric activator FBP. The docking site of the peptide RRASVA was found in the enzyme active centre, while docking of the phosphorylated analogue RRAS(Pi)VA occurred preferably in the C domain of the L-PK molecule, in a site overlapping with the allosteric site for FBP binding (Kuznetsov, A *et al.*, 2013).

The formation of intramolecular complexes between N-terminal domain and enzyme body may change the equilibrium between active R-state and less active T-state of the enzyme. At the same time the induction of cooperativity by the N-domain phosphorylation could be connected with phosphopeptide intramolecular binding with the enzyme allosteric site.

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4. Kuznetsov A, Faustova I, Järv J (2014) *Comp Biol&Chem, 48,* 40

Keywords: Pyruvate kinase, allostery, phosphorylation.

TUE-527

The influence of *N*-stearoylethanolamine on fatty acid composition in rat pancreas under alimentary obesity-induced insulin resistance

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The progression of alimentary obesity triggers the overaccumulation of fat in pancreatic islets that provokes the b-cell failure and diabetes type 2. Therefore, it is of a current interest to find a cytoprotective drug compound that could correct pancreatic dyslipidemia under obesity-induced insulin resistance (IR) conditions. We suggest that one of these compounds could be N-stearoylethanolamine (NSE) with its membrane stabilizing and protective properties. Thus, the aim of our study was to investigate the effect of NSE on pancreas fatty acid composition from rats with IR.

For the experiment, we took a rat model of prolonged high-fat diet induced IR (58% of fat). Control rats were given regular chow (4% of fat). The existence of IR was estimated by results of glucosotolerance test and plasma blood insulin content. Rats with IR were divided into 2 groups: «IR» and rats that were given per os water suspension of NSE (50 mg/kg daily) for 2 weeks («IR + NSE»). The fatty acid (FA) composition was investigated by thin-layer and gas-liquid chromatography and performed as a percentage of the total FA content. The estimated fatty acid desaturase (D) activities were calculated, using product-to-precursor indexes.

The lipid assay of IR rat pancreas showed considerable changes in the individual FA composition of phospholipid (PL), triglyceride (TG) and free fatty acid (FFA) fractions. According to the results, in "IR" rats the 16:0 content was lower in all fractions, whereas 18:0 was more than two times higher in TG and FFA fraction over intact values. The PL 18:1, FFA 18:1 and FFA 18:3, TG 18:3 content considerably increased in IR animals over controls as a possible consequence of enhanced Δ9-D and Δ6-D activities. The low level of 18:2 in all fractions was associated with up-regulation of Δ6-D (synthesizes 18:3). The TG 20:4 and FFA 20:4 level was reduced, while in PL fraction it was significantly increased. Taking into account the fact that 18:0 is toxic to β-cells under fat overload, the protective effect of NSE was in a reduction of 18:0 level. First, NSE reduced convention of 16:0 into 18:0, thus increasing 16:0 FA level and second, NSE enhanced Δ9-D activity, increasing 18:1 content in all lipid fractions from rats with IR. The NSE triggered a reduction in PL 20:4, TG 20:4, and FFA 20:4 level under IR conditions. Moreover, 18:3 (a precursor for 20:4 synthesis) was decreased in FFA and TG fractions under NSE action in comparison with "IR" rats that was associated with down-regulation of Δ6-D.

The NSE administration normalized the redistribution of the main fatty acids between lipid fractions in pancreas of rats under IR conditions. This corrective effect of NSE on FA metabolism may be through the regulation of acyl-CoA-D activity.

Keywords: experimental insulin resistance, lipid metabolism, N-stearoylethanolamine.

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TUE-528

The interaction of *E. coli* thymidine phosphorylase with therapeutically important azido nucleosides

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Thymidine phosphorylases (TP) belong to the enzymes of nucleosides metabolism. They catalyze the reversible phosphorolysis of glycosidic bond in thymidine and 2'-deoxy uridine as well as transference of deoxyribosyl moiety from one pyrimidine base to another. TP participate in the salvage pathway of the nucleosides biosynthesis and are involved in the angiogenesis in tumour cells. So the search of selective inhibitors of TP activity is of special interest.

In this work it is found that according to kinetic measurements 3'-azido-3'-deoxythymidine (AZT) which is used widely for the treatment of human acquired immunodeficiency syndrom and 3'-azido-2'-fluoro-2', 3'-dideoxyuridine (N_3FddU) are the reversible inhibitors of *E. coli* TP.

The crystalline complexes of TP with both ligands have been prepared and the crystal structures of *E. coli* TP complexed with N_3FddU and with AZT were solved by molecular replacement method and refined correspondingly to 1.50 and to 1.55 Å resolution.

Both azido nucleosides were located with full occupancy and in the similar orientation in the nucleoside binding pockets of the corresponding TP complexes. The comparison of the position of both ligands with position of substrate (thymidine) in homologous *St. aureus* thymidine phosphorylase revealed the essential difference in the arrangement of substrate and inhibitors. Although the positions of pyrimidine rings in the substrate and in the azido nucleosides are overlapped, the pyrimidine rings of both azido nucleosides are rotated by 180° relatively thymidine pyrimidine ring around the axis connecting the atoms N3 and C6 of the pyrimidines. As a result the azido ribosyl rings of both inhibitors and ribosyl ring of the substrate are removed from each other. Besides that a new hydrophobic pocket, which comprises the amino acid residues, surrounding 3'-azido group is formed in the TP active site. This finding can be used for the search of new types of selective inhibitors of thymidine phosphorylases. The results of this work conform also that 3'-azidotymidine can interact with various enzymes of the nucleoside metabolism.

This work was supported by Central Scientific Research Institute of Machine-Building of the Russian Federal Space Agency (Roscosmos), RFBR grant 13-04-01100 and Program 9 of Division of Chemistry and Material Sciences RAS (OChNM-9).
Keywords: Crystal structure, enzyme activity, X-ray crystallography.

TUE-529

The investigation of oxidant and antioxidant effect of melatonin on rat liver in fructose induced-metabolic syndrome

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Background: A close relationship between the incidence of metabolic syndrome and increased fructose consumption was found in both human and animal studies. In this study, we aimed to evaluate the effect of melatonin on total oxidant status (TOS), paraoxonase (PON), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) levels in fructose-administered rats.

Method: We used 24 'Sprague-Dawley' breed of male rats and distributed equally into four groups; control (group1), fructose (group2), fructose and melatonin (group3), melatonin (group4). Fructose administration was accomplished by giving daily prepared 20% D-fructose solution in tap water, and 20 mg/kg/day melatonin by oral gavage during 8 week. Insulin resistance was evaluated by the homeostasis model assessment index. We measured TOS level, PON, GPx, SOD and CAT activities.

Results: In our study, TOS level in the liver comparison to group1 statistically significantly increased in all groups ($p < 0.05$). TOS levels were significantly higher in group2 ($p = 0.04$) than in group3 ($p = 0.01$) and the group4 ($p = 0.04$). PON activities in all groups decreased significantly when compared with the group1 ($p < 0.05$). PON activities were significantly lower in group2 ($p = 0.04$) than in group3 ($p = 0.03$) and the group4 ($p = 0.5$). GPx activities in all groups increased when compared with the group1 ($p < 0.05$). GPx activities were significantly higher in group3 ($p = 0.03$) than in group2 ($p = 0.173$) and the group4 ($p = 0.2$). SOD activities in group 2,3 and 4 increased when compared with the group1($p > 0.05$). SOD activities were significantly higher in group2 ($p = 0.03$) and 4 ($p = 0.008$). Liver CAT activities comparison to group1 showed this was not statistically significant in all groups ($p > 0.05$).

Conclusion: Metabolic syndrome model was successfully demonstrated. Fructose diet did not cause clear oxidative stress in liver. TOS level in the liver comparison to group1 statistically significantly increased in all groups. It significantly increased PON, GPx and SOD activities but no chanced CAT activities in liver tissue. Although melatonin treatment showed prooxidant effect on some parameters and has not positive effect on atherogenic lipid profile, it prevented increases in systolic blood pressure caused by high-fructose diet.

Keywords: liver, melatonin, metabolic syndrome.

TUE-530

The investigation of oxidative-antioxidative homeostasis in the blood of men with giardiasis

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Background: The disturbance in the system of antioxidant protection (AOP) is associated with the insufficient activity of one or more enzymes, which develops to destabilization cytomembranes and enhance the lipid peroxidation (LPO). The indicatorsofLPO-AOPusedascriteriaofconditionof the body in diseases of different genesis, according to references.

Purpose of the study was toevaluate the state of activity of antioxidant enzymes and purine metabolism in the blood of men with giardiasis.

Methods: Wedetectedthecatalase (CAT) usingthemethodofM.A. Koroluketall.(1988).It isenzymesofAOP. Absorbance was measured on spectrophotometer at a wavelength of 410 nm, relative to the mixture consisting of the water and the lysate.

The determination of the activity of glutathione peroxidase (GPO) was defined according to Vlasov V.N. et al (1990). The content of GPOwas registered at a wavelength of 260 nm against water on spectrophotometer.

Wedetectedtheactivity of the enzyme of purine metabolism. Itwas adenosine deaminase (ADA), weusedthemethodofI.B.Nemechek (1993). The content of ADAwas registered at a wavelength

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of 265 nm against potassium phosphate buffer on spectrophotometer.

Data were processed by conventional methods of variation statistics, we calculated the arithmetic mean (M) and standard dispersion (m).t-test (t) was used to assess differences.

Results: Our study found the significant increase in the activity of all enzymes AOP in the blood of men with giardiasis compared with the control group.The greatest increase was observed in the activity of catalase in the blood of men with giardiasis. Contentof catalase was significantly higher than in control group in 5.8 times ($p < 0.05$).

The increase of activity of GPO was detected in 1.5 times in the blood of men with giardiasisvs control group. The increase of activity of ADA was detected. The main enzyme of purine metabolism, required for the biosynthesis of nucleic acids and cell proliferation was in 3.7 times higher in the blood of men with giardiasisvs control group ($p < 0.05$).

Conclusion: Thus, the mechanisms of oxidation- antioxidant homeostasis were inducted in the blood of men with giardiasis. This is confirmed by increasing the activity of enzymes of AOP and purine metabolism compared with the control group.

Keywords: enzymes of antioxidant protection, giardiasis, blood.

TUE-531
The mechanism of calcium induced inhibition of gestational diabetic fructose 1,6-bisphosphate aldolase

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An important part of proof has shown that glycolytic enzymes in many kind of cells may form metabolically active macromolecular complexes whose stability is regulated directly and indirectly by calcium ions and glycolytic intermediates. Such association not only changes the regulatory characteristics and the kinetics of glycolytic enzymes, but may also simplify the channeling of substrates between metabolically sequential enzymes increasing the velocity of the glycolytic pathway. Calcium (Ca^{2+}) is of critical importance for many biochemical processes: Ca^{2+} controls muscle contraction and relaxation and the rhythm of the heart, the formation of enzymes and hormones; also the DNA formation in chromosomes; regulates the blood clotting, urine filtration. Ca^{2+} is the main buffer used in the body to neutralize acids and maintain the proper the pH.

Gestational diabetes mellitus is defined by glucose intolerance of variable severity with onset of first recognition during pregnancy. The placenta is a temporary established organ that operates exclusively for the time of pregnancy. In diabetes, the placenta undergoes a variety of structural and functional changes.

Fructose–1,6-bisphosphate aldolase (FBPA) plays an effective role in glucose metabolism and gluconeogenetic pathway and reversibly catalyzes the split of fructose 1,6-bisphosphate into the triose phosphates D-glyceraldehyde phosphate and dihydroxyacetone phosphate. Aldolase has 160 kDa molecular weight and three tissue specific isozymes.

A partition equilibrium study has shown calcium ion to be a noncompetitive inhibitor of aldolase adsorption by placental muscle. This inhibition is interpreted quantitatively in terms of aproximately 10-fold decrease in the intrinsic association constant for the aldolase-myofibril interaction upon Ca^{2+} binding to either or both of the low-affinity troponin sites associated with regulation of muscle contraction.

In our study, we investigated inhibition effects of Ca^{2+} ion on FBPA, and the interaction of human placental aldolase with bivalent Ca^{2+} and diabetic complications. It was defined that Ca^{2+} is noncompetitive inhibitor of human placental FBPA. Ki values of diabetic human placental aldolase for Ca^{2+} was determined 1.77 ± 0.38 mM.

Keywords: Calcium, Fructose 1,6 bis-phosphate aldolase, Inhibition.

TUE-532
The relationship between respiratory burst, serum pancreatic elastase concentration and mean platelet volume, in childhood obesity

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Increased serum pancreatic elastase activity, high mean platelet volume (MPV) and increased oxidative stress are risk factors for atherothrombosis. In vitro, serum pancreatic elastase increases superoxide production by phorbol myristate acetate stimulated neutrophils.

The aim for this study is to investigate the monocyte NADPH oxidase activity (respiratory burst), the MPV value and serum pancreatic elastase concentration, and to find correlations between these parameters in childhood obesity.

Sixty obese children (9–16 years) and thirty age and sex matched lean children were involved. Chemiluminescence for respiratory burst, spectrophotometry for serum pancreatic elastase concentration were used and the MPV value was obtained from hemogram. Albumin/globulin ratio was calculated as a surrogate marker of inflammation. Pearson correlations were used for linear regression.

In the obese children versus the lean ones, the activity for monocyte NADPH oxidase (0.62 versus 0.4 RLU, $p < 0.01$), the concentration for serum pancreatic elastase (0.7 versus 0.58 ng/ml, $p < 0.04$) and the value for MPV (8.93 versus 8.13 fl, $p < 0.001$) were higher. In the obese children, serum pancreatic elastase concentration was positively correlated with MPV ($r = 0.32$, $p < 0.05$) and with respiratory burst ($r = 0.26$, $p < 0.05$). The monocyte cells number and the albumin/globulin ratio were positively correlated with MPV ($r = 0.29$ and $r = 0.4$, respectively, $p < 0.05$) and with serum pancreatic elastase concentration ($r = 0.33$ and $r = 0.39$, respectively, $p < 0.05$).

In conclusion, in the obese children there is a cluster of athrerothrombosis risk factors: the mean platelet volume, the respiratory burst and the serum pancreatic elastase concentration are higher than in the lean children.

Keywords: atherothrombosis risk, childhood obesity, respiratory burst.

TUE-533
The resin from *Protium heptaphyllum* inhibits adipogenesis *in vivo* and *in vitro*

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Introduction: *Protium heptaphyllum* March (Burseraceae), popularly known as almecegueira is a medicinal plant that grows abundantly in Amazon region and in various other parts of Brazil. The resinous exudate collected from the trunk wood of this plant in its natural form is a reputed folk remedy with anti-inflammatory, analgesic, insect repellant, expectorant and wound healing actions.

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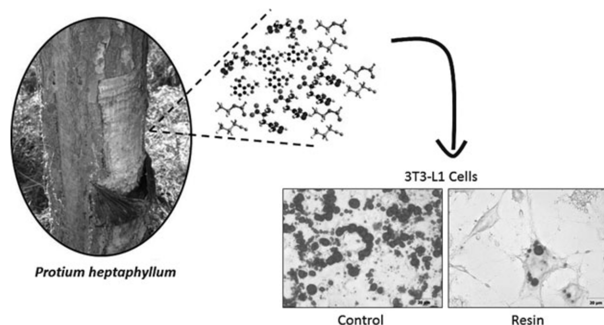

Fig. 1.

Objective: The aim of this study was to investigate the antiadipogenic effect *in vivo* and *in vitro* of the resin obtained from *Protium heptaphyllum* (RPH).

Methods: Five groups of male Swiss mice weighting 25–30 g were treated with normal diet (ND), high-fat diet (HFD, control), HFD + RPH (10 mg/kg, p.o.), HFD + RPH (20 mg/kg, p.o.) or HFD + Sibutramine (SIB, 10 mg/kg, p.o.) for 15 weeks. At the end of this period, animals were starved for 6 h and then sacrificed. The liver and abdominal adipose tissues (epididymal and parametrical) were dissected and weighed. Tissue samples of hepatic and epididymal adipose tissue were fixed and stained with hematoxylin and eosin, examined under light microscopy, and the adipocytes area was measured using the Image J software. The measures of 100 cells for each mouse (n = 6/group) were used to calculate the average cell surface area (mm^2). The cytotoxicity of the RPH was tested against the preadipocyte cell line 3T3-L1 by MTT assay, after 72 h of incubation. The differentiation of the 3T3-L1 cells to adipocytes was induced using prodifferentiative agents. Simultaneously, the cells were treated with the RPH at concentrations of 6.25; 12.5; 25 and 50 μg/ml. The results were observed by Oil-red-O stain. Results and Discussion

The RPH showed no cytotoxic effects ($IC_{50} > 50$ μg/ml) although a reduction in the adipogenesis process was observed in the 3T3-L1 cells treated with RPH. Furthermore the RPH was able to reduce the gain in body mass in RPH-treated animals compared to HFD-fed controls. Besides, RPH decreased the abdominal fat accumulation and the gain in liver weights promoted by HFD.

Conclusions: Our findings suggest that the resin obtained from *Protium heptaphyllum* has the antiadipogenic potential with no significant cytotoxicity and therefore may be a promising candidate in the development of newer antiobesity drugs. Supported by: CNPq, CAPES, FUNCAP.

Keywords: Adipogenesis

TUE-534
The role of alcohol and aldehyde dehydrogenases in the control of mammalian gene activity

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Methanol (MeOH) is poisonous to humans because the enzyme alcohol dehydrogenase (ADH) converts MeOH in toxic formaldehyde (FA), which is then converted into formic acid by aldehyde dehydrogenase (AlDH). The detection of MeOH in the blood of healthy mammals suggests that MeOH could be a substance with specific functions and not simply a waste product of cell metabolism. Here, we directly demonstrate that an increase in the MeOH concentration in mouse and rat blood led to a change in the accumulation of mRNAs from genes primarily involved in detoxification processes and the regulation of the ADH/AlDH gene cluster. To test the role of ADH in the maintenance of low MeOH levels in the plasma, we used the specific ADH inhibitor 4-methylpyrazole (4-MP) and showed that intraperitoneal administration of 4-MP resulted in a significant increase in the plasma concentrations of MeOH, ethanol and FA. Removal of the intestine significantly decreased the rate of MeOH accumulation in the plasma, suggesting that the gut flora may be involved in the endogenous production of MeOH. However, data from the rat liver perfusion system did not support the hypothesis that this organ participates in the generation of endogenous MeOH. Nevertheless, liver ADH and AlDH were identified as the primary enzymes for metabolising MeOH because an increase in the MeOH and ethanol levels in the liver homogenate was observed after 4-MP administration into the portal vein. Quantification of the liver mRNA levels showed changes in the accumulation of mRNAs from genes involved in cell signalling and detoxification processes. We also confirmed the involvement of alpha lipoic acid in the detoxification process of ethanol and MeOH. Alpha lipoic acid has the potential to boost the accumulation of AlDH mRNA and mitochondrial AlDH-2 activity, both of which accelerate the conversion of FA to formic acid. We hypothesised that endogenous MeOH acts as a regulator of homeostasis by controlling mRNA synthesis. Although MeOH may be exhaled into the air as a waste product (similar to carbon dioxide), this chemical may also influence biochemical processes important for cellular function.

Keywords: methanol, alcohol dehydrogenase, intestinal microflora.

TUE-535
The role of vitamin D3 in prevention of prednisolone induced dysfunction of hepatocytes

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Background and Aims: Growing evidence suggests that glucocorticoid therapy associated side effects could be ascribed to inhibition of vitamin D_3 turnover, particularly due to abnormal processes of hydroxylation and 25OHD3 formation in hepatocytes. Changes in cholecalciferol hydroxylation can be linked to prednisolone induced impairments of structural and functional state of hepatocytes. Therefore, we investigated prednisolone induced impairments of vitamin D_3 25-hydroxylating systems and hepatocytes function and estimated the efficacy of vitamin D_3 treatment.

Methods: Female Wistar rats received prednisolone (5 mg per kg of b. w.) with or without 100 IU of D_3 (for 30 days). The contents of $25OHD_3$ in serum were measured by ELISA. Vitamin D_3 25-hydroxylase activity was assayed in vitro in isolated hepatocytes by method of radio-competitive binding of [^{3}H]-$25OHD_3$. The levels of CYP27A1, CYP2R1 and poly(ADP-ribose) polymerase 1 (PARP-1), poly-ADP-ribosylated, nitrated proteins in liver tissue were measured by Western-blot analysis. Expression of Bax and Bcl-2 in hepatocytes was assessed by immunocytochemistry. ROS and RNS production in isolated hepatocytes and cell viability were determined by flow cytometry with DCF-DA and propidium iodide respecively.

Results: It was shown that prednisolone administration lowered the level of $25OHD_3$ (by 70%) in serum and inhibited two-fold the total activity of vitamin D_3 25-hydroxylase in hepatocytes vs. control. Prednisolone reduced the content of CYP27A1 and

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CYP2R1 isoforms of 25-hydroxylase by 78% and 27% respectively. Administration of the GC led to disruption of the integrity of hepatocytes triggering destructive changes in these cells and thus reducing the number of functionally active hepatocytes. These cytological changes were further confirmed by significant increase in the number of hepatocytes capable to accumulate PI that is associated with necrotic cell death. In contrary, apoptotic index Bax/Bcl-2 was found to be markedly reduced suggesting pro-apoptotic signaling down-regulation. Prednisolone administration led to increased oxidative nitrosative stress as is evident from enhanced ROS and RNS generation in liver tissue vs. control. These alterations are most likely responsible for DNA damage-mediated activation of PARP-1 since a marked 1.77-fold rise in the level of poly-ADP-ribosylated proteins was established. Moreover, prednisolone administration also resulted in more than 1.63-fold increase in the level of 89 kDa cleaved fragment of PARP-1, indicative of apoptosis occurring in parallel with necrotic death. Normalization of vitamin D_3 availability might be considered to be perspective in reducing the negative effects of GC on function of hepatocytes.

Keywords: glucocorticoid, Hepatocyte, Vitamin D.

TUE-537

The significance of HbA1c, fasting glucose, insulin sensitivity indices, fasting insulin and glucose/insulin ratio in the diagnosis of gestational diabetes mellitus

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Gestational Diabetes Mellitus (GDM) is defined as glucose intolerance first diagnosed in pregnancy. OGTT, although recommended as the gold standard test, is a cumbersome procedure and could be subjected to interferences. Therefore, use of any other test, such as the non-fasting HbA1c with low intraindividual variability and good predictive value in determining the complications of diabetes, would be a significant progress. Insulin sensitivity indices such as HOMA, HOMA-1%β and QUICKI for the evaluation of insulin resistance have also been excessively addressed in recent studies.

For this purpose, 260 pregnant women (24–28 gestation weeks), not previously diagnosed with diabetes, completed a 2-h 75 g OGTT and were assigned to groups (GDM and Control) according to their OGTT results using the ADA criteria. Insulin sensitivity indices and glucose/insulin ratios were calculated from their fasting glucose and insulin measurements. The diagnostic performances were evaluated using MedCalc11.3.3.0 and SPSS for Windows 17.0.

As a result, glucose was observed to be the parameter with the most significant difference among the groups ($p < 0.001$). HOMA, QUICKI, HbA1c, fasting insulin and HOMA-1%β followed. Considering the AUC values, fasting glucose (AUC=0.771) provided the best discriminatory test and the best overall accuracy for the diagnosis (sensitivity 80%, specificity 63.35%). HOMA and QUICKI (AUC=0.701) were also sensitive diagnostic tests for GDM showing higher specificity than fasting glucose (82.20%) but lower sensitivity. However, HbA1c (AUC=0.678) when compared to HOMA and QUICKI, demonstrated lower discriminatory values. Although fasting insulin demonstrated some diagnostic value, HOMA-1%β and glucose /insulin ratios provided no discriminatory values. Evaluating all of the statistical data, it was observed that HbA1c have not yet met the required criteria for being an ideal diagnostic test.

In conclusion, there is still not enough data including this study for these tests to be used as alternatives to OGTT. Although promising, fasting glucose, HOMA and QUICKI indices along with HbA1c to be used as alternatives to OGTT, could only be possible with evidence of their strong correlation with glucose levels in pregnancy and pregnancy outcomes and complications.

Keywords: GDM, HbA1c, HOMA.

TUE-539

Tissue-specific regulation of triacylglycerol metabolism during fasting and re-feeding in medaka

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Fish utilize triacylglycerol (TAG) as a main energy source. They store TAGs in several sites, such as adipose tissues, liver and muscle. Fasting is known to be a natural occurrence in fish life. During fasting, TAGs stored in muscles are considered to play as one of the important energy sources for muscle functions. In addition, liver is well known as the main site of intermediate metabolism. The aim of this study was to investigate the effects of fasting and re-feeding on the TAG stores and regulation of related genes in muscle and liver of medaka (*Oryzias latipes*).

Four groups of medaka were acclimated for 3 weeks. One group of medaka was used as the control, and two groups were fasted for 4 and 8 days, while the remaining one group was re-fed for 4 days after the 8-day fasting. Fasting reduced hepatic TAG levels, followed by their recovery during re-feeding. In contrast, muscle TAG levels were increased during 4-day fasting, whereas these were decreased during 8-day fasting. Re-feeding raised the TAG levels in muscle again.

To investigate the underlying molecular mechanisms, comprehensive analysis of gene expression patterns were performed using next generation sequencing technique. The changes in expression values of the genes involved in TAG metabolism and fatty acid (FA) degradation implied that the enhanced hydrolysis of the hepatic TAGs by hepatic triacylglycerol lipase followed by β-oxidation resulted in the decreased TAG levels in liver. In contrast, the β-oxidation in muscle would be suppressed by the down-regulations of acyl-CoA synthetase and oxidase during the early fasting stage. However, the induced β-oxidation and lipolysis of intramuscular TAG pools during 8-day fasting would cause the decrease in TAG levels. Re-feeding significantly reduced the TAG hydrolysis and β-oxidation in both tissues, whereas the remarkable gene expressions related to TAG synthesis was not found.

Due to the limited capacity of muscle FA synthesis, FA transport among the tissues regulated by lipoprotein lipase (LPL) has a large effect on muscle TAG levels. In this study, LPL mRNA levels were further determined by quantitative real-time PCR. The positive correlations of LPL mRNA levels with those of TAGs in both tissues implied the potential FA transport between liver and muscle during fasting.

In conclusion, the observations in this study suggest that tissue-specific TAG accumulation is associated with the coordinated regulation of the genes involved in TAG hydrolysis as well as FA degradation and transport between liver and muscle. We will also investigate protein expression levels in order to further disclose the functions of the related genes.

Keywords: Fasting, Global gene expression patterns, Triacylglycerol metabolism.

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TUE-540
TLR4 deficiency increases peroxisomal β-oxidation in LDL knockout mice livers

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Purpose/Objetive: Non-alcoholic fatty liver disease (NAFLD) is now regarded as the most common liver illness in the developed world, affecting up 30% of the population. It comprises a spectrum of disorders ranging from simple steatosis without inflammation, to NASH, progressing to fibrosis and cirrhosis. It is closely linked to visceral adiposity, insulin resistance, dyslipidemia and type two diabetes. A growing body of evidence suggests that toll like receptors, especially TLR4, have a key role in the pathogenesis of chronic inflammatory liver diseases. Our goal was to investigate if TLR4 activation could modulate metabolic lipid pathways and alter the onset of NAFLD.

Material and Methods: We used LDL receptor-deficient mice (LDLrKO) fed with Western-style atherogenic diet as a model. The role of TLR4 activation was evaluated by crossing LDLrKO mice with the TLR4 knockout mice. Animals were fed for 12 weeks with high fat high cholesterol diet (HFHC) containing 18% saturated fat and 1.25% cholesterol. Plasma lipid levels and liver lipid content was determined with commercial kits. Gene expression of enzymes related to triglycerides synthesis and degradation were evaluated by real time PCR.

Results: LDLrKO/TLR4KO mice presented lower triglyceride levels when compared to LDLrKO, despite the type of diet ingested. High fat diet induced triglyceride and cholesterol accumulation in the liver of all mice genotypes studied, but LDLrKO/TLR4KO liver lipid content was not different from LDLrKO mice. Gene expression of ApoB100, GPAT1 e DGAT2 was not differentially altered in LDLrKO/TLR4KO and LDLrKO mice. On the other hand, TLR4 deficiency enhanced the expression of several enzymes involved in β-oxidation of fatty acids, as follows: acyl-CoA oxidase, carnitine palmitoyl transferase 1, mitochondrial trifunctional protein A and B, peroxisomal bifunctional enzyme (PBE), 3-ketoacyl-CoA thiolase A and B.

Conclusion: TLR4 can regulate directly the expression of enzymes related to lipid synthesis and degradation in the liver.

Financial support: FAPESP (#2012/07957-6).

Keywords: beta-oxidation, Fatty liver disease, Toll-like receptor 4.

TUE-541
TNF-α and insulin resistance in normal pregnancy

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Objective: The purpose of this study was to evaluate the role of resistin and tumor necrosis factor- α (TNF- α) in insulin resistance during pregnancy.

Study design: Serum resistin and TNF-α concentrations were measured by ELISA in 86 healthy pregnant women (26, 23 and 37 of them in the 1st, 2nd and 3rd trimesters, respectively) and in 21 healthy non pregnant women in a cross sectional study.

Results: Resistin concentration was significantly higher in the third trimester (9.5 ± 3.3 ng/ml) as compared with non pregnant women (7 ± 3.3 ng/ml). Serum TNF-α level were also significantly increase in pregnant women (2.6 ± 1.9 pg/ml) as compared with maternal healthy controls (0.8 ± 0.7 pg/ml). There were significant correlation between gestational age and BMI ($r = 0.28$, $p = 0.01$), resistin ($r = 0.36$, $p = 0.002$) and TNF-α ($r = -0.44$, $p < 0.0001$). There was not significant correlation between gestational age and insulin resistance (IR). We also did not found correlation between IR and resistin as well as between IR and TNF-α in pregnant women.

Keywords: IR, pregnancy, TNF.

TUE-542
Tumor metabolism and Docetaxel resistance in prostate cancer

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Drug resistance of cancer cells is recognized as the primary cause of failure of chemotherapeutic treatment in most human cancers. Mounting evidences supports the idea that deregulated cellular metabolism is linked to drug resistance in cancer therapy. Indeed, both components of the glycolytic pathways and mitochondria are involved in altered metabolism linked to chemoresistance of several cancers [1,2]. Our aim is to evaluate the metabolic adaptations induced by the drug able to confer advantages for docetaxel-resistant PC3 cells compared to sensitive ones.

We found that docetaxel-resistant PC3 cells acquire a pro-invasive behavior and activate an EMT program and a pro-metastatic phenotype with respect to sensitive cells. Moreover, DoceRes cells show a decrease of intracellular ROS and proliferation compared to sensitive cells. These features are not linked to an induction of the pentose phosphate pathway, but are associated with an enhancement in the antioxidant response, mainly driven by increased expression of the transcription factor Nrf2 (Nuclear factor erythroid 2 related factor 2). Metabolic analysis reveals a greater utilization of glucose, glutamine and lactate by mitochondrial respiration in resistant than sensitive cells. In agreement, metformin, impairing mitochondrial complex I function, selectively decreases proliferation and invasiveness of resistant cells. Furthermore, stromal fibroblasts, which cause a "reverse Warburg" phenotype in prostate cancer cells [3], can protect sensitive and resistant cell lines to docetaxel toxicity. In keeping, an approach based on re-expression of a microRNA, the miR205, able to shift the mitochondrial respiration to a Warburg metabolism induces an increase of docetaxel toxicity in prostate cancer cells.

Taken together, these findings suggest that chemoresistance to docetaxel induces an escape from Warburg metabolism with a potential involvement of mitochondrial respiration to confer a metabolic advantage to these cells and this could be attractive as potential therapeutic target.

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Keywords: Metabolism chemotherapy cancer.

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TUE-544
Ubiquinone lowers cholesterol level and increases expression of LDL receptor in isolated primary hepatocytes

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Introduction: Cholesterol is a component of cell membranes, precursor of steroid hormones and bile acids. Cholesterol biosynthesis occurs by Mevalonate (MVA) pathway involving intermediates farnesyl-pyrophosphate that is converted to Cholesterol, Dolichol and Ubiquinone.

Coenzyme Q (UQ) is an energy production component of the electron transport chain. Formation of the UQ molecule involves phenylalanine as precursor of the phenyl ring of UQ and Isoprene units derived from MVA pathway. Many reports showed a decrease in UQ level with age, whereas cholesterol increases.

Hypothesis: We postulate UQ as regulator of cholesterol pathway. This will be addressed by investigating the effect of UQ1 and UQ10 on:

1- Cholesterol level.

2- LDL receptor.

Methods: a. Effect of UQ1 and UQ10 on the cholesterol synthesis.

Isolated hepatocytes were pre-treated with UQ1/UQ10 and/or HMGCoA reductase inhibitors, Farnesyl Transferase inhibitors, and isoprenyl transferase inhibitors for 1 h. The level of cholesterol, lanosterol and Squalene following treatment with ^{14}C-MVA, lipid extraction, TLC was determined.

b. Quantitative and qualitative analysis for LDL receptor expression by:

LDL receptor expression of the above treatment was qualitatively and quantitatively determined by immune staining and western blotting.

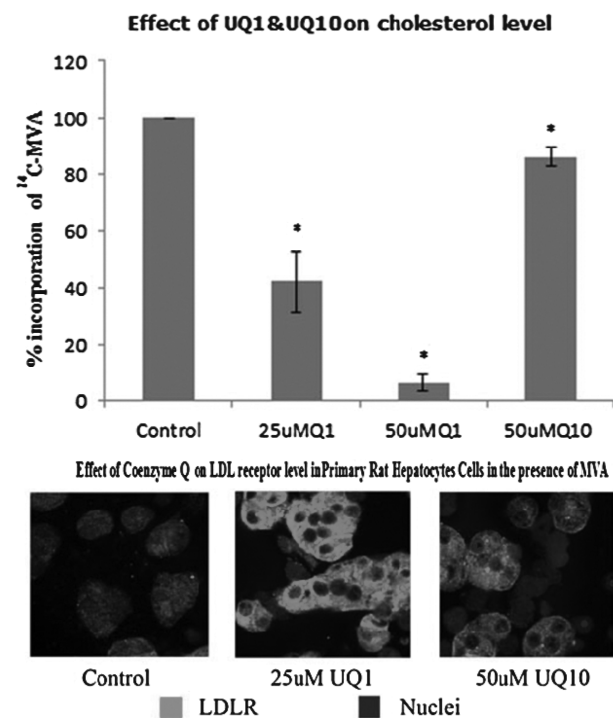

Fig. 1.

Results (Figure): - Both UQ1 and UQ10 lowered cholesterol level by 65% and 15% respectively in primary hepatocytes

- Lanosterol and Squalene were increased in UQ1 treated primary cells

- Western blotting showed 2 fold increase in the LDLR in UQ treated cells which were confirmed qualitatively by western blotting.

Conclusions: We provide evidence on the role of ubiquinone (natural product) in regulating cholesterol and up-regulating LDLR. The possible use of UQ as alternative medicine to statin, with known side effects, requires further investigation.

Keywords: cholesterol, LDL-receptor, Ubiquinone.

TUE-545
Vascular, ischemic and oxidative effects of alpha lipoic acid on experimental NSAID induced gastropathy

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Administration of nonsteroid anti-inflammatory arachidonate analogues, ubiquitously prescribed to treat pain, is associated with a wide range of side effects. NSAID induced gastropathy, beside usage as an experimental disease modelling, is one of the most frequent and clinically important adverse effect. We aimed to search possible effects of a strong amphipathic anti-oxidant molecule, alpha lipoic acid (LA), in NSAID induced gastropathy, on the basis of altered redox homeostasis, endothelial microcirculation and angiogenesis.

Three groups of Sprague-Dawley male rats were randomly categorized as Grup I-C (n = 7, control, omeprazole 30 mg/kg) Grup II-A (n = 8, 50 mg/kg, 8 weeks alpha lipoic acid + indomethazine 30 mg/kg) and Grup III-S (n = 8, 8 weeks serum physiologic + indomethazine 30 mg/kg). Malondialdehyde (MDA), lipid hydroperoxides (L-OOH), advanced oxidation protein products (AOPP), trans-trans cis-trans conjugated dienes (CD), albumin, uric acid, thiol fractions (T-SH, NP-SH, P-SH) and Cu,Zn-superoxide dismutase (Cu,Zn-SOD) activity parameters are measured for the assessment of redox status, together with ischemia modified albumin, vascular endothelial growth factor and prostaglandin E2(PGE2) levels to evaluate perfusion and angiogenesis. Stomach tissues of the rats were also analyzed by histopathologic examination.

Albumin ($p < 0.001$), uric acid ($p < 0.05$), serum total thiols ($p < 0.001$), tissue T-SH ($p < 0.05$), serum P-SH ($p < 0.01$), tissue P-SH ($p < 0.05$), serum and tissue NP-SH ($p < 0.05$), Cu, Zn-SOD activity ($p < 0.01$) and PGE2 levels were also significantly lower ($p < 0.05$) in A and S groups when compared to group C. Serum and tissue AOPP ($p < 0.001$), IMA albumin index ($p < 0.001$), serum L-OOH ($p < 0.01$), tissue L-OOH ($p < 0.05$), serum MDA ($p < 0.01$), tissue CD ($p < 0.05$) and VEGF-A ($p < 0.05$) levels were significantly higher in groups A and S, when compared to group C. Albumin ($p < 0.05$), serum T-SH ($p < 0.01$), serum P-SH ($p < 0.001$), serum NP-SH ($p < 0.001$) and PGE2 ($p < 0.05$) levels were significantly lower in group S, when compared to group A. Mucosal damage score correlation between those parameters were highest in serum L-OOH (r:0.70) and P-SH (r: −0.82) levels.

LA can be counted as an oxidative stress and agiogenetic stabilizing agent in NSAID induced gastropathy.

Keywords: alpha lipoic acid, NSAID, oxidative stress.

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TUE-546
Vitamin B3-derived ribosides are authentic intermediates of human NAD metabolism

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Nicotinamide adenine dinucleotide (NAD) is known as a coenzyme of redox reactions in central metabolic pathways, in which it functions as carrier of electrons and hydrogen ions. Moreover, NAD also serves as substrate of several families of regulatory proteins such as protein deacetylases (Sirtuins), ADP-ribosyltransferases and Poly-ADP-ribosyl polymerases, which govern vital processes including gene expression, progression of the cell cycle, insulin secretion, DNA repair, apoptosis, aging and many others. The proper regulation of these NAD-dependent metabolic and signaling processes depends on how efficiently cells can maintain their NAD levels. Human cells generally replenish their NAD contents through NAD biosynthesis using the vitamin B3 precursors delivered with food: nicotinamide and nicotinic acid as well as nicotinamide riboside (NR) and nicotinic acid riboside (NAR). Nicotinamide and nicotinic acid can be directly converted to the corresponding mononucleotides (NMN and NAMN) by phosphoribosyltransferases.

In this work we have tested the hypothesis that, besides being supplied with the diet, the ribosides NR and NAR are also authentic intracellular intermediates and thereby represent an integral part of NAD metabolism in humans. We found that the known human cytosolic 5′-nucleotidases CN-II and CN-III can dephosphorylate the mononucleotides NMN and NAMN and thus produce NR and NAR *in vitro*. Our results indicate that 5′-nucleotidases require high (millimolar) concentrations of nucleotides for efficient catalysis suggesting that NAMN and NMN, at their physiological concentrations, are unlikely to be substantially dephosphorylated in cells. Only under conditions when NMN or NAMN concentrations rise, the 5′-nucleotidases would be activated. In line with this assumption, overexpression of FLAG-tagged CN-IA, CN-II and CN-III in human cells resulted in the riboside NAR formation. However, NAR formation was only detectable under conditions that lead to increased NAMN production from nicotinic acid. Taken together, our observations establish human 5′-nucleotidases as physiologically important components of NAD metabolism, namely, to generate nucleosides (NAR and NR) by dephosphorylation of the corresponding mononucleotides.

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Keywords: None.

TUE-547
Whole cell regioselective hydroxylation of *N*-heteroaromatic compounds using *Burkholderia* sp. MAK1

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Hydroxylated pyridines are commercially desirable compounds because of their wide application in the production of herbicides, insecticides, fungicides and plant growth regulators. Moreover, the pyridine derivatives are in great demand as synthons for pharmaceutical products. Currently, pyridines are used either as biologically active substances or as building blocks for polymers with unique physical properties. Selective hydroxylation of the *N*-heteroaromatic ring is still a very challenging task in organic synthesis, therefore biocatalysis is an attractive tool.

In this study, the hydroxylation of pyridine and its derivatives by *Burkholderia* sp. MAK1 cells has been investigated. The 2-hydroxypyridine-degrading *Burkholderia* sp. MAK1 has been isolated from soil. This whole cell biocatalyst oxidized 73 of the 110 tested aromatic compounds, mostly pyridines, pyrazines and pyrimidines. UV-VIS and HPLC-MS analyses revealed that hydroxylation occurs at 5-position of the ring of 2-hydroxy- and 2-aminopyridines containing methyl-, chloro-, bromo- and other substitutions. In order to prove the position of regioselctive hydroxylation several products have been purified and identified by 1H and 13C NMR spectroscopy. Biosynthesis of aminopyridinols has been shown for the first time. In addition, it has been discovered that *Burkholderia* sp. MAK1 is capable of oxidizing pyridine, pyrazine and their methylated derivatives to corresponding *N*-oxides.

Fig. 1. Regioselective hydroxylation of pyridine derivatives by whole cells of *Burkholderia* sp. MAK1.

Keywords: biocatalysis, pyridinols, regioselective hydroxylation.

TUE-548
X-ray structure analysis of *Acinetobacter* sp. L-ribose isomerase for metabolizing non-natural sugar L-ribose

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L-Ribose is non-natural sugar, so-called rare sugar, and is not generally used in metabolic pathway as a carbon source. An enzyme, L-ribose isomerase from *Acinetobacter* sp. strain DL28 was reported as a new enzyme nearly a couple of decades ago, which can constitutively produce L-ribose isomerase [1]. L-Ribose isomerase catalyzes the reversible aldose-ketose isomerization between L-ribose and L-ribulose. L-Ribulose could be an intermediate toward D-xylulose 5-phosphate in pentose phosphate pathway by utilizing L-ribulokinase and L-ribulose-5-phosphate 4-eipimerase. The strain, DL28 was isolated as a mutant strain from *Acinetobacter* sp. strain LR-7c that could utilize D-lyxose as a carbon source and produce inductive L-ribose isomerase. The cloned gene of *Acinetobacter* sp. DL28 L-ribose isomerase (L-RI) had no significant amino acid sequence similarity to known protein structures [2,3]. Since L-RI uses L-ribose as the most favorable substrate for isomerization reaction while it uses D-lyxose with almost 50% for L-ribose. *Acinetobacter* sp. DL28 may have a novel metabolic pathway to assimilate L-ribose as a carbon source.

L-ribose has a potential usage as a precursor for the synthesis of L-nucleoside analogues, which are widely used as pharmaceutical compounds such as antiviral and anticancer drugs. An interest in the industrial production of L-ribose is increasing, and effective enzymatic production of L-ribose is quite expected. Therefore, an enzyme like L-RI, which is capable of recognizing and catalyzing L-ribose, is attractive.

So far, we have determined the crystal structure of L-RI (tetramer) and found that the subunit structure of L-RI showing cupin-type barrel fold is similar to that of D-lyxose isomerase (dimer) from the pathogenic *E. coli* O157:H7 (D-LI) by Dali search, though their sequence identity and r.m.s.d. are 18% and

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2.8, respectively. In this study, to further understand the difference of favorable substrate recognition, we determined the crystal structures of L-RI in complex with L-ribose, L-ribulose, or ribitol, and an inactive mutant form (E204Q) in complex with L-ribose or L-ribulose. Based on these complex structures, we propose the catalytic reaction mechanism including ring-opening of substrate in L-RI. In addition, site-directed mutagenesis study of L-RI showed that E211 and R243 are involved in substrate recognition of L-ribose and D-lyxose.

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Keywords: Rare sugar, Sugar isomerase, X-ray structure.

TUE-549
X-ray studies of the phosphopantetheine adenylyltransferase from *M. tuberculosis*

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Phosphopantetheine adenylyltransferase from *Mycobacterium tuberculosis* (PPAT *Mt*) is involved in the coenzyme A (CoA) biosynthesis, catalyzing the penultimate step of the process, resulting in the formation of the dephosphocoenzyme A (dPCoA) from 4′phosphopantetheine (PhP) and ATP. Reduction of the intracellular level of CoA prevents the bacterium growth. Therefore PPAT that catalyses the key step of CoA biosynthesis is suitable therapeutic target for the rational drug design.

In the presented study, the crystal structures of recombinant PPAT *Mt* in two crystal modification of apo form and in complex with ATP, CoA, and dPCoA were determined using the crystals grown in the microgravity by counter-diffusion method [1,2]. The peculiarities of the arrangement of the ligands in the active site cavity of PPAT *Mt* are described. The conformational states of the PPAT molecule at the consequent steps of the catalyzed reaction in apo enzyme, enzyme-substrate, enzyme-product complexes are characterized. It is shown that the ATP and dPCoA binding induces the rearrangement of the short part of polypeptide chain restricting the active site cavity in the subunits of the hexameric enzyme molecule. The changes in the quaternary structure caused by this rearrangement are accompanied by the variation of the size of the inner water-filled channel which crosses the PPAT molecule along the three-fold axis of the hexamer. The molecular mechanism of the observed changes have been described. The crystal packing and its influence on the conformation of the enzyme molecule have been studied through the comparison of different crystal forms of apo form of enzyme.

This work was supported by RFBR grant 14-02-31110-mol_a, and Central Scientific Research Institute of Machine-Building of the Russian Federal Space Agency (Roscosmos).

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Keywords: drug design, Protein structure, Tuberculosis.

TUE-550
Zinc-dependent estrogenic effect and high apoptotic activity in frog *Rana ridibunda*

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Despite well-known vulnerability of amphibians to the chemical impacts that is related to endocrine disruption (ED), the molecular reasons for this phenomenon aren't clear. Particular instability and easy loss of zinc (Zn) by Zn-sequestering intracellular protein metallothionein (MT) in frog was shown recently (Falfushynska et al., 2008, 2010). Hypothetically it could be of concern for the dysfunction of Zn-dependent signaling, including the induction of vitellogenesis and apoptotic activity. To study the possibility of this dependence, male frogs, *Rana ridibunda* were exposed to Zn^{2+} (Zn, 3.1 μM), nano zinc oxide (nZnO, 3.1 μM), Ca-channel blocker nifedipine (Nfd, 10 μM) and combination of nZnO and Nfd (nZnO + NFD) for 14 days. MTs from the liver were estimated from coordinated metals (MT-Me (Zn, Cu, Cd)), thiols (MT-SH) and by *ELISA Kit* (MTi). ED effects were characterised from the level of vitellogenin-like protein (Vtg-LP), and thyrotropin (TSH) levels in serum, activity of Zn-dependent 5-deiodinase in the liver. Cellular injury was assessed in the liver by measuring level of oxiradicals, CYP1A1-dependent ethoxyresorufin-*O*-deethylase (EROD) activity, DNA fragmentation, and activity of the main effector enzymes in the apoptotic cascades, caspase-3 and cathepsin D (total and free).

Common responses for all exposed groups were detected as an increase in the levels of MT-SH (up to 2 times) and caspase-3 activity (in 6 times in ZnO + Nfd-group). The comparison of MT-SH, MTi and MT-Me levels shown their simultaneous increase in Zn- and ZnO + Nfd groups (with the increase of MTi by ~ 50%) and discrepancy in Nfd group (the decrease of MTi by 40%) and consequently, the increase in MTs unsaturated form, apothionein. Importantly, concentrations of Vtg-LP and deiodinase activity were increased only in the exposures to Zn-contained compounds. nZnO caused particular responses: decreased level of cathepsin D (total and free), whereas it was increased in all other exposed groups, and the absence of differences in the levels of TSH and EROD with control group, whereas in all other groups they were increased. Antioxidant potential of Zn- and ZnO and prooxidant effect of Nfd were detected by the evaluation of oxiradical level. Hence, Zn-contained compounds affected specifically estrogenic activity in male specimens (vitellogenesis) and thyroid activity in the liver of frog. Particular effect of nZnO on apoptosis was related lysosome protease cathepsin D, whereas well-known anti-apoptotic effect of Zn on caspase-3 was not realized.

Keywords: metallothionein, vitellogenesis, zinc.

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CSIV-04 – Modelling biological processes

WED-001
A fully enzymatic method for site-directed spin labeling of long RNA: a tool to investigate structural and dynamical properties of complexes involved in biological processes

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The elucidation of the mechanisms of recognition between RNAs and their interacting partners remains a major objective in molecular and structural biology. Nuclear Magnetic Resonance (NMR) provides an efficient method to unravel both structural and dynamical aspects of RNAs and their complexes. However, the study of large RNA molecules by NMR remains challenging, due the combined effect of efficient relaxation and spectral crowding. One promising route to the application of NMR methodologies to larger RNA systems relies on the incorporation of paramagnetic probes, coupled to the measurement of the induced effects on nuclear spins (1). At the same time, Electronic Paramagnetic Resonance (EPR) has rapidly expanded to study structure and dynamics of macromolecules (2–4). During recent years, several spin labeling techniques of RNA relying on partial or complete solid-phase chemical synthesis methods were developed (5–11).

We propose here a fully enzymatic method, which allows the insertion of a paramagnetic center at a specific position in an RNA molecule. The technique is based on a segmental approach using a ligation protocol with T4 RNA ligase 2. One transcribed acceptor RNA is ligated to a donor RNA in which a thio-modified nucleotide is introduced at its 5′-end by *in vitro* transcription with T7 RNA polymerase. The efficiency of the coupling reaction and the quality of the resulting spin-labeled RNA were assessed by Mass Spectrometry, EPR and NMR. This method enables various combinations of isotopic segmental labeling and spin labeling schemes, a strategy that will be of particular interest to investigate, by NMR and EPR, the structural and dynamical properties of large RNA complexes involved in biological processes.

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Keywords: EPR spectroscopy, NMR Spectroscopy, RNA.

WED-002
A general model for fibrinogen cleavage by thrombin

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Thrombin is a key enzyme in the blood coagulation cascade. The thrombin hydrolyzes fibrinogen into fibrin, the later specifically associates into the fibers which finally build up a thrombus scaffold. This multistep process could be described as a set of stepwise reactions and needs a complete and detail kinetic portrait. The earlier kinetic models were focused on some particular parts of the process, for example either on the mechanism of enzyme action itself, or on the kinetic of formation of fibrin associates.The study provides a general model of fibrinogen cleavage by thrombin, taking into account some peculiarities as the dimeric nature of fibrinogen, association of desAA-fibrin, and stepwise removal of fibrinopeptides as well as application of the quasi-equilibrium approach. The model could be easily updated with forthcoming data on fibrinogen hydrolysis and fibrin association. The research provides an example of thorough description of the set of reactions with several intermediates. The developed algorithm could be applied to model some sophisticated systems with branch points and feedbacks: the blood coagulation cascade and signal transduction.
Keywords: None.

WED-003
A model of the multilayer structure of GBM and low-oxygen induced invasion: implications for tumor growth and invasion during anti-angiogenic therapy

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The recent use of anti-angiogenesis (AA) drugs for the treatment of glioblastoma multiforme (GBM) has uncovered unusual tumor responses. Here, we derive a new mathematical model that takes into account the ability of proliferative cells to become invasive under hypoxic conditions; model simulations generate the multilayer structure of GBM, namely proliferation, brain invasion, and necrosis. The model replicates key features of GBM including a proliferative rim, necrotic center, and an an invasice. The model is interrogated to derive fundamental insights on the dynamics of GBM and on the clinical and biological effects of AA drugs. Invasive cells promote tumor growth; furthermore, AA drugs increase the fraction of invasive cells leading to rebound rapid growth when the tumor becomes resistant to AA therapy. These results justify the lack of efficacy of Bevacizumab on overall survival times.
Keywords: angiogenesis, Glioblastoma Multiforme, invasion.

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WED-004
A novel proteomic assay for identification of amyloid-forming proteins

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Amyloids are ordered fibrillar protein aggregates with characteristic cross-beta structure. Prions are specific subgroup of amyloids possessing infectious properties. These protein aggregates are currently extensively studied not only due to their pathological features (about 40 incurable human amyloid-related diseases are known), but also due to important functional roles, which have recently been discovered. One of the central problems for amyloid studies is the lack of universal methods that could provide efficient identification of novel amyloids. Thus, identification of each novel amyloid remains a notable event in biology. We developed a method, which allows identification of a wide range of amyloid-forming proteins in cells or tissues of different organisms. This method is based on the unusual resistance of amyloids to ionic detergents and has very high resolution due to of the use of 2D-DIGE technology. We validated our method by identification of reference amyloids (PrP, Aβ, Htt, Bgl2p, as well as prions of Sup35 and Rnq1). In addition, we identified a set of novel proteins that are capable of forming detergent-resistant polymers in *Saccharomyces cerevisiae*, and can be considered as candidates for novel functional amyloids. The amyloids of Sup35 were found to possess resistance to chaotropic agents (urea and thiourea) and formic acid.

Overall, the method developed in this study opens wide opportunities for characterization of novel functional and pathological amyloids in different organisms from prokaryotes to human.

Keywords: Amyloid, Mass-spectrometry, Prion.

WED-005
A quantitative model of nucleocytoplasmic dynamics and turnover of mRNA

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Eukaryotic mRNA undergoes a procedure of transcription, nuclear export, translation and decay. Although studies have provided important insights into these fundamental processes, we still lack accurate quantitative models of the chain of events that determines the abundance and nucleoplasmic distribution of mRNA for each individual gene.

Here, we measured the genome-wide dynamics and nucleocytoplasmic distribution of mRNA in Drosophila cells by 4-thiouridine labeling and fractionation. Through mathematical modelling, we obtained rates of transcription, nucleocytoplasmic permeability and cytoplasmic decay for ~6500 genes.

Studying relationships between kinetic steps, we found physiologically meaningful correlations along the whole chain of gene expression: globally, genes that are highly transcribed tend to produce mRNAs that are more efficiently transported and degraded less quickly. This is particularly true for all genes that encode cytoplasmic ribosomal proteins. We are currently investigating whether this 'kinetic consistency' is an optimization outcome of evolution and/or due to a dedicated molecular imprinting mechanism that coordinates the three consecutive steps. Overall, we found that cytoplasmic decay explains ~20% of abundance variance, which is markedly more than previously estimated.

To considerable degree, transcript length correlates negatively with transcription rate but positively with cytoplasmic decay rate. The latter observation revives a 'stochastic decay model' that involves the random activity of endonucleases, a historically proposed but still unconfirmed mechanism.

We also tested whether the mRNA kinetics parameters are linked to the chromatin type in which the genes are embedded. As expected, transcription rates are low in known repressive types of chromatin, and high in euchromatin. Surprisingly, nucleocytoplasmic transport of mRNA also correlates with the chromatin state. We found that transcripts from HP1-containing chromatin tend to be more readily transported, while those from Polycomb-marked chromatin tend to be much less. We speculate that some chromatin protein complexes, of which some have been reported to have RNA-binding activity, could function to either facilitate nucleocytoplasmic export or retain transcripts in the nucleus.

Keywords: chromatin proteins, mRNA dynamics, Systems biology.

WED-006
A RNA binding score for predicting RNA binding probabilities of protein residues

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The understanding of the recognition principles of RNA binding to proteins is necessary to predict the binding interfaces. In the past decade, tens of computational prediction algorithms have been developed to predict RNA binding sites based on either protein sequences or protein structures. Most of the state-of-art methods depend on machine learning approach based on PSSM and other residue propensities, ranging from SVM, neural network to random forest and Naive Bayes. However, in order to discriminate RNA binding sites from non-binding sites, the existing programs are all-or-none classifications. Here, we propose a simple score to predict RNA binding probabilities based on a combination of protein sequences and structures.

The prediction score is based on physico-chemical and evolutionary principles. As amply demonstrated, RNA binding residues are accessible on protein surface, tend to be positively charged and are highly conserved in sequence. The derived score is a combination of residue accessibility surface, electrostatics potential and conservation entropy.

Importantly, the prediction score avoids comparison of all RNA binding residues and non-binding residues of different proteins together. Instead, it maximizes the prediction accuracy for each protein separately. It achieves similar or even better accuracy than the other best prediction programs.

Further, we have found that the RNA binding residues, when defined by commonly used distance cut-off, even for the same protein can vary in different PDB complexes. We define the residues that are always considered as RNA binding residues in different PDB complexes as conserved RNA binding residues. And the prediction score is also useful in predicting such residues.

With the prediction score, the residues around the RNA binding position can be plotted as an energy funnel: residues farther away from the binding position are scored lower. Therefore, it helps in the localization of the most central RNA binding region in a protein.

Keywords: Bioinformatics, prediction, RNA-protein interaction.

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WED-007
An encyclopaedia of protein complexes

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Understanding the role and make-up of protein complexes is integral to understanding biological processes. While the scientific literature contains a wealth of information on the role and function of protein complexes no central resources existed for searching and depositing information about macromolecular complexes. The IntAct molecular interaction database now includes a new resource, the Complex Portal (www.ebi.ac.uk/intact/complex), through which an encyclopaedia of protein complexes from major model organisms is available for search, viewing and download. Each entry contains information about the participating molecules (including small molecules and nucleic acids integral to the complex), their stoichiometry, topology and structural assembly. Complexes are annotated with details about their function, properties and complex-specific Gene Ontology (GO) terms. Consistent nomenclature is used throughout the resource with systemic names, recommended names and a list of synonyms all provided. Complexes are extensively cross-referenced, e.g. to PDB, Reactome, ChEMBL and OMIM. The use of the Evidence Code Ontology allows us to indicate for which entries direct experimental evidence is available in a protein-protein interaction databases such as IntAct (www.ebi.ac.uk/intact) or if the complex has been inferred based on homology or orthology. The data is searchable using (lists of) standard identifiers such as UniProt, ChEBI and GO IDs, protein, gene and complex names or synonyms as well as complex cross-references (e.g. PDB IDs) and species IDs and names. Data is available in the community standard interaction format (PSI-MI XML) and downloadable from our ftp site. Other formats can be made available on request. This reference resource will be maintained and grow to encompass an increasing number or organisms. Input from the representative model organism communities is welcome.

Keywords: database, protein complex, systems biology.

WED-008
Analysis of binding of porphyrins to heme proteins by molecular docking method

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The method of molecular modeling, docking, which an aim is to find the most accurate orientation and conformation of the ligand in the binding center of the target protein, in bioinformatics is one of the important tools. Porphyrins are low molecular weight natural compounds which are included in the structure and plays a key role in the function of various proteins. On the other hand free exogenous porphyrins are essential elements of the energy transfer to oxygen and the formation of singlet oxygen in photodynamic therapy of tumors (PDT) [1]. Study of complexes formation of porphyrins with carrier proteins and their delivery into malignant cells are important tasks for PDT [2]. The recognized carrier proteins are serum albumin, lipoproteins, and hemoglobin, such a function the least investigated for hemoglobin.

To study the binding sites of cationic porphyrins on macromolecule of protein as a model was chosen model of molecule of human hemoglobin (Hb). To construct a model of a molecule of Hb we were use the web site of Protein Data Bank www.pdb.org (file ID: 1KOY.pdb). Study of the binding sites on the hemoglobin were carried out with cationic porphyrins containing various peripheral functional groups with different hydrophobicity and the content of hydroxyl groups (TOE4PyP, TBut4PyP and TAll4-PyP), as well as with the most important fatty acids of blood plasma (palmitic and stearic).

By method of molecular docking has been shown that:

1. From the comparison of the minimum binding energy for porphyrins and fatty acids follows that binding of all three porphyrins with the interior of the macromolecule Hb significantly preferable than for fatty acids.

2. When embedding of complexes [porphyrin + fatty acid] in the internal cavity of the macromolecule Hb they bind to a macromolecule Hb significantly stronger than porphyrins or fatty acids separately, as evidenced by lower values of minimum binding energies for the complexes [porphyrin + fatty acid] with the macromolecule Hb, than for porphyrins or fatty acids.

It follows a very important conclusion:

-when adding the fatty acids in the hemoglobin solution with porphyrins can form complexes [porphyrin + fatty acid] which binds significantly more probabilities with Hb, than with porphyrins and thus complexes [porphyrin + fatty acid] can displace porphyrins from the interior of the macromolecule Hb.

Thus, the molecular docking method can give much new information about the possibilities of binding the porphyrin, fatty acids, and their complexes with hemoglobin and complement the NMR and X-ray structural studies.

1. R. Bonnett. Chem. Soc. Rev. 24, 19–33, (1995).

2. R. Hudson and R. Boyle. J. Porphyrins & Phthalocyanines 8, 954–975 (2004).

Keywords: molecular docking, porphyrins, proteins.

WED-009
Atomistic molecular dynamics simulations of the Shiga toxin B subunit binding to its receptor in a lipid bilayer

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Atomistic molecular dynamics simulations of the DOPC-Gb3 and DOPC-Gb3-Shiga toxin B subunit (STxB) systems are performed to investigate the structure of the complexes.

The results for DOPC-Gb3 lipid bilayer show a strong tendency for the Gb3 with a saturated acyl chain to phase separate into a domain enriched in Gb3, while for the Gb3 with an unsaturated acyl chain de mixing with a slow rate is seen.

Also, increasing the Gb3 concentration, results in ordering of both the DOPC and Gb3 lipid chains and a dramatic reduction of these diffusion constants.

Simulations of the DOPC-Gb3-STxB complex show that Gb3 in a lipid bilayer can not fully bind to all STxB binding sites,

due to the hight difference of the STxB binding sites and the two dimensional structure of the lipid bilayer.

As well, we show that hight difference between the STxB binding sites is a prerequisite for curvature generation.

Keywords: None.

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WED-010
Biochemical and immunological blood parameters in dynamics under esophageal acid burn model of 2nd degree

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Exogenous poisoning with alkalis takes the leading position among causes of acute poisoning. About 75% of the affected persons are children. There are many pathologies and complications following esophageal burns: scar stricture, esophageal deformations, corrosive esophagitis, gastroesophageal reflux and others. Nowadays there are not enough adequate experimental models of esophageal acid burns for investigation of this pathogenesis. Thereby the burn disease is an important issue that needs solution immediately.

The purpose of the work was to reproduce the model of an esophageal acid burn of 2nd degree experimentally in rats and to characterize the biochemical and immunological blood parameters in dynamics.

In experiments, we used nonlinear white mature rats (weight 90 ± 10 g). The animals were administered with CH3COOH (30% solutions) to induce esophageal burn. Rat blood samples were obtained after 1, 3 and 7 days after alkali administration.

The biochemical parameters were determined with analyzer Humalyser 3000. The level of the blood antibodies was assessed by ELISA analysis which was performed in 96-well microplates (Dynatech, Sweden).The level of circulating immune complexes (CIC) in serum was determined by precipitation with 4.5% solution of polyethylene glycol-6000.

After 2nd degree acid burn simulation the level of investigated biochemical parameters (content of total protein, albumin) statistically decreased on the 1st – up to 7th day. The level of urea, creatinine and potassium concentrations were increased during all research duration. The most significant changes of biochemical parameters were observed on the 1st day after 2nd degree acid burn simulation. It was confirmed serum blood ALAT and ASAT activities increasing, as well as increasing of IgG antibodies and CIC levels the 1st- up to 7th day compare to the control datas. In that way the most pronounced pathological process development was observed on the 1st day of acid treatment.

So in the experiments, we reproduced an acid esophageal burn model of 2nd degree in rats. The changes of basic blood biochemical and immunological parameters in experimental animals with acid burn of esophagus were established. Obtained results allow us to conclude that the acid model used is adequate for human esophageal burns research. Our approach can be used for study molecular mechanisms of acid esophageal burn pathogenesis of 2nd degree.

Keywords: burn, biochemical parameters.

WED-011
Breaking down DNA allostery: a protein-DNA binding computational study

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The accessibility of the genetic information stored in DNA is dependent on the flexibility and conformational constraints of the macromolecule as well as on its energy landscape. The view that DNA acts as an inert template onto which proteins assemble to replicate or transcribe genes has evolved to acknowledge an active role for the DNA through its capacity to undergo conformational changes in response to protein binding.

Novel evidence from a single-molecule study reopened the issue of DNA alostery (Kim S et al, 2013). However, mechanistically understanding how this ranged cooperative protein binding takes place is a challenge experimentally, but it is at last possible by molecular dynamics (MD) techniques. Up to this point atomistic MD were not employed for the study of allosteric effects and simplified models were used in order to circumvent the big amount of calculations needed.

Using state-of-the-art force fields, MD gives the most precise computational reproduction of DNA-protein-solvent interactions at near physiological conditions. We performed full-scale detailed simulations at large enough time scales to encompass the characteristic times of structural distortions and information transfer upon protein binding to DNA. By analyzing the induced perturbations, we were able to explain the propagation of medium and long-range conformational transitions that modulate the affinity and specificity of subsequent protein-DNA binding events. We chose a variety of systems based on the length and sequence of the DNA and studied the binding of BamHI, a type II major groove binding endonuclease, as well as the relaxation upon removal of the protein. We characterized the spatial and temporal propagation of information along DNA, in terms of both energetics and structural deformations. The results lead to the description of an information transfer process as a proposed operating mechanism for allostery. We distinguished between the nature of perturbation propagation along different special sequences of DNA depending on their intrinsic flexibility properties.

It was found that the flexibility pattern and base pair correlation decay length of protein bound DNA is oscillatory with a periodicity of approximately 10 base pairs. We also concluded that the induced distortions in the DNA conformation measured by deviations in the major groove width propagate through the backbone and they affect the ability of DNA to bind to a second protein. The results confer significant agreement with the experimental data while providing new valuable insight into the functional basis of the process and validatind the efficiency of an all-atom molecular dynamics simulation approach to the meaningful issue of DNA allostery.

Keywords: DNA allostery, information transfer mechanism, molecular dynamics.

WED-012
Channel-forming activity of amyliod peptides is affected by phloretin

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Amyloid precursor protein (APP) is an integral membrane protein expressed in many tissues and concentrated in the synapses of neurons. Its primary function is not known, though it has been implicated as a regulator of synapse formation [Priller et al., Neurosci., 2006], neural plasticity [Turner et al., Neurobiol., 2003] and iron export [Duce et al., Cell, 2010]. APP is best known as the precursor molecule whose proteolysis generates amyloid β-peptides (Aβ), amino acid peptides whose amyloid fibrillar form is the primary component of amyloid plaques found in the brains of Alzheimer's disease patients. The aim of our study was the investigation of the influence of the membrane active agents, flavonoids and styryl dyes, on the channel-forming

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activity of amyliod β-peptide fragment 25–35 (Aβ25-35) in planar lipid bilayers. Virtually solvent-free bilayer lipid membranes were prepared from binary mixtures of dioleylphosphoserine and dioleylphosphoethanolamine in 0.1 M KCl (pH 7.4) using monolayer-opposition technique. Aβ25-35 was added to aqueous solution at one side of the bilayer to obtain a final concentration ranging from 1/10 μM. We found that both-side addition of 20 μM of phloretin to the bilayer bathing solution which decrease the membrane dipole potential on 90 ± 10 mV, was accompanied by more than 10-fold increase in the Aβ25-35-induced steady-state transmembrane current (Iop). The introduction of 20 μM of other flavonoids, phloridzin, quercetin, genistein or 2′,4′,6′-trihydroxy-acetophenone, reducing the membrane dipole potential in the different rates, did not change the Iop. At the same time, increasing the membrane dipole potential more than on 80 ± 10 mV due to the introduction of 5 μM RH 421 or RH 237 also did not affect the Aβ25-35-induced steady-state transmembrane current. Comparing the results of measurements of the dipole potential of membranes and the influence of dipole modifiers (flavonoids and styryl dyes) on the channel-forming activity of Aβ25-35 one can conclude that the observed effect of phloretin is not caused by the changes in the membrane dipole potential. The possible mechanisms of phloretin action on the channel-forming activity of Aβ25-35 comprising the influence of phloretin on the oligomerization/aggregation of amyloid peptides were discussed. The study was supported in part by RFBR (#14-04-31738), the Program of the RAS «MCB» and SS-1721.2014.4.
Keywords: amyliod peptides, phloretin, planar lipid bilayer.

WED-013
Cold-denaturation of a protein dimer monitored at atomic resolution

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Protein folding and unfolding are crucial for a range of biological phenomena and human diseases. Defining the structural properties of the involved transient species is therefore of prime interest. Using a combination of cold-denaturation with nuclear magnetic resonance spectroscopy we reveal detailed insight into the unfolding of the homodimeric repressor protein CylR2. Seven three-dimensional structures of CylR2 at temperatures from 25°C to -16°C reveal a progressive dissociation of the dimeric protein into a native-like monomeric intermediate followed by transition into a highly dynamic, partially folded state [1]. The core of the partially folded state appears critical for biological function and misfolding.

[1]. Jaremko, M., Jaremko, L., Kim, H.-Y., Cho, M.-K., Schwieters, C. D., Giller, K., Becker, S., Zweckstetter, M. (**2013**) Unfolding of a protein dimer at atomic resolution, *Nat. Chem. Biol.*, **9**, 264–270.

Keywords: cold denaturation, Protein structure, protein unfolding.

WED-014
Combined in vitro and *in silico* evolution of the organophosphate metabolizing reactibody A17

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Reactibody A17 was selected from Griffin library against arylphosphonate. The deep structural and functional analysis has revealed the architecture of its active center to have a cholinesterase-like anion binding site, hydrophobic pocket, and reactive Tyr residue. Reactibody A17 showed high reactivity with parent substrate, an aryl-phosphonate. As expected, the reaction was non-catalytic. We found that reactibody A17 does not react with echothiophate and p-nitrophenyl phosphocholine, but does interact with DFP, AEBSF and paraoxon. Moreover we observed paraoxon hydrolysis by A17 and showed that this process involves covalent intermediate formation. Although our selection method was designed primarily to provide covalent binding, our general task remains to obtain catalytic antibodies. To that end, we have identified one substrate which is hydrolyzed with catalytic turnover. We have established that the for initial non-covalent binding and proper orientation, the positive amino acids is preferred, the hydrolysis require the negative charged amino acids for stabilization of hydroxyl ion and decreasing of proton transfer. To engineer an abzyme based on the A17 reactibody to turn over organophosphorus substrates we used two approaches of active center evolution. We created the sub-library of reactibody active center and developed the computational maturation to provide best driving forces for paraoxon hydrolyzing antibody. We confirmed that arginine mutant showed high reactivity with paraoxon. It was two orders of magnitude more compared to wild type. But no catalysis was observed. Thus our results were in line with our computed predictions. A histidine-35 mutant improved covalent binding with paraoxon and retained same catalytic activity. Glutamic acid-35 mutant blocked interaction with both phosphonate and paraoxon molecules.

Keywords: combinatorial library, QM/MM calculations, reactibody.

WED-015
Comparison of assembly efficiency of cognate and artificial *Potexvirus ribonucleoproteins*

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Potato virus X (PVX) (helical filamentous plant virus, genus *Potexvirus*, family *Alphaflexiviridae)* has the possibility of reversible dissociation of virions into coat proteins (CP) and RNA followed by the *in vitro* self-assembly of viral particles which save the structure and biological activity of the virus. Previously we showed that *in vitro* incubation of PVX CP with the RNAs of various plant and animal viruses results in the viral ribonucleoproteins (vRNP) formation (Arkhipenko *et al.*, 2011). The helical structure, morphology and translational properties of these artificial vRNP were identical to cognate vRNP assembled from PVX RNA and PVX CP and to PVX virions. However, the widely used methods do not allow us to estimate the efficiency of assem-

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bly of artificial vRNPs formed by PVX CP and different plant and animal RNAs. To obtain data about the particles size (hydrodynamic diameter) and concentration the method of Nanoparticle Tracking Analysis (NTA) was used. PVX CP was incubated with PVX RNA and different viral RNAs. Formed cognate and artificial vRNP were analyzed by NTA. This method based on laser-illuminated optical microscopy enables to detect the RNPs assembly in real-time in liquid. The detected number of formed particles indicates the assembly efficiency. For the first time the information about efficiency of the potato virus X particles assembly was obtained by NTA. The assembly efficiency of RNPs formed by PVX CP with PVX RNA and heterologous viruses RNA was compared. The proposed method allows studying the mechanisms of initiation and elongation of the viral ribonucleoproteins.

Reference

1. Arkhipenko *et al., Acta naturae* 2011, 3, 3(10), 40–46.

Keywords: assembly, plant virus, ribonucleoprotein.

WED-016
Computer simulation method to generate the variant sequences of influenza viruses using the time series substitution pattern

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Since the outbreak of novel influenza pandemic in 2009, various kinds of simulation methods have been developed. Most of the developments, however, are focused on the determination of the spreading patterns of influenza viruses over time. In this study, we introduce a novel approach to predict the possible variants of influenza viruses using the empirical data of nucleotide sequences determined by experimental methods. We developed a simulation tool named SimFlu (simulation tool for influenza virus) to predict possible future variants of influenza viruses. SimFlu can create variants from a seed nucleotide sequence of influenza A virus using the codon variation parameters included in the SimFlu package. The SimFlu's library provides pre-calculated codon variation parameters for H1N1, H3N2 and H5N1 subtypes of influenza A virus isolated from 2000 to 2011. All the source sequences (56 328 sequences) of the SimFlu's library were collected from the Influenza Virus Resources at NCBI. The SimFlu supports 3 types of operating systems, including Windows, Linux, and Mac OS X, and it also provides a command option-based version to run on a Linux queuing system. SimFlu is publicly available at http://lcbb.snu.ac.kr/simflu. Moreover, we also developed an analytical tool for calculating the codon substitution patterns named SimFluVar to support the researchers who want to use their own variation parameters. SimFluVar provides precise patterns of co-don variations between 2 viral groups, especially for the influenza virus groups. SimFluVar also provides the useful functions, such as editing and visualization of the result matrix. SimFluVar is in C++, and Java RCP is used for distribution package. Documentation, examples, results and source code are available at http://lcbb.snu.ac.kr/simfluvar.

Keywords: codon variation, computer simulation, INFLUENZA VIRUS.

WED-017
Conformational flexibility and domain binding interfaces in human tyrosyl-tRNA synthetase studied by molecular dynamics simulations

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Human tyrosyl-tRNA synthetase (*Hs*TyrRS) is a key enzyme of protein biosynthesis, which catalyzes the aminoacylation of cognate $tRNA^{Tyr}$ and also reveals non-canonical cytokine activities. After proteolytic cleavage *Hs*TyrRS reveals both IL8-like activity of its N-terminal catalytic module and EMAP II-like activity of non-catalytic C-terminal domain (Wakasugi and Schimmel, 1999; Kornelyuk et al., 1999). Earlier, it was found that the ELR-motif (E91, L92, R93) in *Hs*TyrRS is responsible for IL8-like cytokine activity (Wakasugi and Schimmel, 1999), but its structural state in full-length human TyrRS was unknown.

In this work we constructed the model of the full-length *Hs*TyrRS structure and studied its putative compactization by all-atom molecular dynamics simulations. All MD computations were performed using grid services of the MolDynGrid virtual laboratory (http://moldyngrid.org). 3D structure of *Hs*TyrRS was constructed in Modeller 9.7 using structure templates (1N3L, 1NTG, and 1OPL for interdomain linker). Six independent 100 ns MD trajectories of *Hs*TyrRS were computed using GROMACS 4.0.5 software in G43a1 force field. The Contacts Analyzer Script (CAS) and the tRMSF tool of the Pteros molecular modeling library (http://pteros.sourceforge.net) were used for MD simulation data analysis. The Distributed Analyzer Script was used for analytical tools automation (Savytskyi et al., 2011).

Our MD simulations revealed the strong binding energy of ~1000 kJ/mol for C-module binding to mini-TyrRS dimer. Antiparallel β-sheet formation at the Ala355-Val363 region of interdomain linker was observed at 3–100 ns time interval (~85% of time). Also, the β-turn formation at the Pro365-Arg367 region was revealed for 40–90 ns time interval (~50% of time).

During 100 ns MD simulations the H-bonds formation between R93 residue of ELR cytokine motif and A340 and E479 residues of C-module was observed. These findings support the idea that the full-length TyrRS lacks its cytokine activity due to the direct interactions between N-terminal and the C-terminal modules, which protect the ELR cytokine motif.

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Keywords: tyrosyl-tRNA synthetase; cytokine; ELR motif; molecular dynamics; interdomain linker; GROMACS.

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WED-019
Cytostatic and proapoptotic effect of n-hydroxy-4-({[(e)-2-phenylethenyl]sulfonyl} amino) butanamide on tumor cells

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Hydroxamic acids possess high biological activity due to their ability to form stable complexes with metal ions which play an important role in the metabolic processes of living organisms. Polifunctional hydroxamic acids coordinating with enzyme protein fragmentare able to bind metal ions in their active site in complexes. Therefore, many hydroxamic acids are inhibitors of metal-containing enzymes. In particular, they can inhibit zinc-containing enzymes histone deacetylases and metalloproteases, thereby have a role in regulating gene expression, proliferation and cell migration, angiogenesis. Histone deacetylases inhibitors cause accumulation of acetylated forms of proteins which can alter their structure and function, induce different phenotypes in various transformed cells, including growth arrest, apoptosis and mitotic cell death. This mechanism of action of hydroxamic acids determines their high antitumor and antibacterial activity and the selectivity of the effect, because normal cells are resistant to the action of histone deacetylases inhibitors.

We found thatN-Hydroxy-4-({[(E)-2-phenylethenyl]sulfonyl} amino) butanamide (here in after compound 1) has cytostatic and apoptotic effect with respect to cells of the cervix carcinoma line Hela. The compound 1 at concentration 2.5 mkM-2.5 mM increased the number of apoptotic cells in 6 times, and shifted the distribution of cells in phases of the cell cycle. Last was manifested in the increase of cell percentage inG_0/G_1 phase in 1.2 times and reduction in S phase of the cell cycle in 2 times, compared with the control.

We have also identified changes in the morphology of the cell culture under the action of the compound 1. Hela cells acquire the phenotype of normal epithelium â�� polygonal shape with characteristic processus, elongated nucleus after co-culturing with the compound.

Thus, N-Hydroxy-4-({[(E)-2-phenylethenyl]sulfonyl}amino) butanamide can be a promising compound for cancer therapy.

Keywords: Hydroxamic acids, proliferation.

WED-020
Development of a double sandwich enzyme immunoassay for quantitation of Moroccan snakes venom after envenomation

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Snake envenomations remain an important yet neglected public health problem in tropical and subtropical countries. In morocco, viper species (*Cerastes cerastes* and *Macrovipera mauretanica*), and the Moroccan Cobra specie (*Naja haje legionis*) are among the common causes of severe snakebite accidents. In this work, a double sandwich enzyme-linked immunosorbent assay (ELISA) was developed for the first time in Morocco and validated to detect and to quantify snakes venom in biological samples.

The method proved to be simple, specific, reproducible, sensitive (detection limit = 0.5 ng/ml) and the calibration plot was based on linear regression analysis ($r^2 = 0.980$) between 0.9 and 1000 ng/ml of venom concentration, with a lower limit of quantification of 1.58 ng/ml. The intra- and interassay coefficient of variation ranged from 2.02 to 4.62% and 5.29 to 7.40%, respectively.

The specificity of the assay was tested using vipers, cobra and scorpion venom. This method detected venom from all viper species tested without significant cross reactivity with other venoms in the concentration range of 0.9–1000 ng/ml.

This ELISA may prove to be helpful to establish a rationale approach of specific antivenom therapy, as well as it is a promising test for identification of envenomations by species of vipers found in most of North Africa. It can also be used to study the toxicokinetics of the vipers venom in experimental envenomations.

Keywords: Moroccan snakes venom, Sandwich ELISA, validation.

WED-022
Effect of charge-stabilized silver nanoparticles with various surface properties on physiological state of seedlings of *Triticum aestivum*

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The widespread use of silver nanoparticles causes that they can be transferred to the environment in an uncontrolled manner. This poses a serious problem because some studies show that silver nanoparticles can be toxic in environment. Although the mechanism of silver nanoparticle action is still not precisely elucidated, it is assumed that it can be affected by their surface properties, especially by stabilizing agents of nanoparticles which were used during the synthesis.

Therefore, the aim of this work was to determine the influence of charge-stabilized silver nanoparticles of similar size but various surface properties on *Triticum aestivum* seedlings. Three types of silver nanoparticles used in our experiments were synthesized using trisodium citrate, tannic acid and sodium hexametaphosphate as stabilizing agents.

The morphological changes in roots and shoots of *T. aestivum* treated by suspensions showed that the silver nanoparticles stabilized by sodium hexametaphosphate exhibit the most adverse action on seedlings whereas the influence of citrate-stabilized nanoparticles was rather indifferent. Moreover, taking into account that silver nanoparticles interact with the cellular components which results in the formation of reactive oxygen species (ROS), the changes in catalase (CAT) and superoxide dismutase (SOD) activity were examined. It was found that CAT activity increase significantly in roots of plants treated by tannic acid-stabilized nanoparticles. On the other hand, SOD activity increases for tannic-acid and phosphate-stabilized nanoparticles. The strongest impact on membrane permeability in both leaves and roots was caused by phosphate-stabilized nanoparticles and by silver nitrate. Additionally, it was shown that all suspensions as well as silver nitrate caused the increase of quantum efficiency of energy trapping in PS II reaction center compared to the control.

This results unequivocally shown that the surface properties of nanoparticles of similar size can affect wheat seedlings growth in various ways. The most profound effect is exerted by silver nanoparticles stabilized by sodium hexametaphosphate.

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Keywords: phytotoxicity, plant metabolism, Silver nanoparticles.

WED-023
Effect of serotonin on membranes properties studied by molecular dynamics simulations

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Extending the life of transfused red blood cells (RBC) has major implications in the treatment of several diseases (anemia, sickle cell disease) that require repeated transfusions. Recently, it has been demonstrated that serotonin (5-hy- droxytryptamine), a monoamine neurotransmitter implicated in the central nervous system, has a protective role on red blood cells and is crucial to ensure normal erythropoiesis and red blood cells survival in mice. Without serotonin, the half-life of RBC is reduced both in vivo and in vitro. Among the mechanisms responsible for this effect, oxidation cascade plays a significant role. Serotonin could act as an antioxidant agent to prevent destabilization of the membrane and lipid peroxidation. Different hypotheses are advanced to investigate its protecting effect: How does serotonin prevent destabilization of membranes? What are the mechanisms in which serotonin interplays with lipid peroxidation?

We present results related to the first question, i.e. how does serotonin interact with membranes. We performed molecular dynamics simulations with a protonated serotonin molecule initially placed in the solvent compartment of a fully hydrated bilayer of POPC. We observed that serotonin rapidly locates at the membrane interface. The key interaction is a salt bridge between the amine group of serotonin and the phosphate group of lipids with the aromatic group of serotonin pointing inward the bilayer. Simulations with several serotonins show no aggregation of the latter at the interface. Examination of different membrane properties shows a slight increase of the order parameter for the unsaturated chain. Impact on membranes composed of lipids with different alkyl chain unsaturation is also examined. Analogs of serotonin molecules, which have increased or decreased protective effects compared to serotonin, are currently underway in similar conditions.

Keywords: insaturation, membrane, simulation.

WED-024
Effect of vitrification on expression level of DNA methyltransferase genes

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Background: Ovarian tissue cryopreservation is a feasible method to preserve female reproductive potential, especially in young patients with cancer or in women at risk of premature ovarian failure. Vitrification has recently emerged as a new trend for biological specimen preservation. However, this technique has not been optimized. On the other hand, gene expression changes during vitrification can influence oocyte maturation and need to be studied. Methylation of mammalian DNA is a major epigenetic regulatory mechanism that play a special role in the initiation of chromatin remodeling and gene expression regulation. The aim of the present study was to evaluate the effects of vitrification on mRNA expression levels of DNA methyltransferase (DNMTs) genes *Dnmt1, Dnmt2, Dnmt3a, Dnmt3b* and *Dnmt3 l*

Material &Method: Ovaries of 4- to 6-week old NMRI female mice were placed in two control and needle immersed (NIV) vitrification groups. In vitrification groups, ovaries were transferred in to equilibration and vitrification medium, then they immersed in liquid nitrogen after loading by acupuncture needle. Parallel to vitrification process, morphology of ovarian tissues in control and vitrification group were analyzed and compared by using hematoxylin & eosin staining. Then, the expression of *Dnmts* genes were investigated by real-time PCR.

Result: In morphological analysis, ovarian tissue integrity was well preserved in vitrification group and was similar to the control group. However the results of this study showed that the expression levels of all candidate genes in vitrification group were increased in comparison with control group; although this increase was only significant for *Dnmt1*, *Dnmt3b* and *Dnmt3 l* genes ($p < 0.05$).

In conclusion: In general we can conclude that despite normal morphology of ovarian tissue after vitrification, this process may induce changes at the genetic/epigenetic level of cryopreserved tissues.

Keywords: DNA methylation, ovary, Vitrification.

WED-025
Free radical destruction of amine-containing biomolecules

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Amine-containing compounds such as amino acids and amino sugars are of great biological importance. Serine (Ser) and threonine (Thr) are building blocks of proteins, being also essential parts of active sites of many important enzymes (serine proteases, serine integrases, cytochromes P450 etc.) and receptors (protein kinases substrates). Glucosamine (GlcN) and its derivatives are the monomeric units of mucopolysaccharides. It is also well known that reactive oxygen and chlorine species (ROS and RCS), in particular hydroxyl radicals (•OH) and hypochlorous acid (HOCl), generated in biosystems via various enzymatic and non-enzymatic pathways, can cause damage to biomolecules.

Using γ-irradiation model for •OH generation as well as chromatographic techniques coupled with UV-visible, fluorescence and mass spectrometry detectors, we have shown that GlcN, Ser, Thr, Thr-Val, Ser-Ala and similar dipeptides undergo •OH-induced carbon skeleton destruction in aqueous solutions. The free radical mechanism of the destruction has been proposed, which includes formation of N-centered radicals and their further fragmentation. It has been proven experimentally that the presence of 2-aminoethanol moiety as well as an unsubstituted and non-protonated amine-group in the biomolecules is necessary for the realization of this type of fragmentation. For instance, it was shown that in case of Ser homolog homoserine, which contains hydroxyl-group in γ- rather than in β-position to amine-group the proposed scheme of conversion was inhibited. It is interesting to note that the non-enzymatic free radical Ser conversion according to the proposed scheme, resulting in the formation of glycine and formaldehyde, is similar to the Ser bioconversion driven by serine hydroxymethyltransferase. This bioconversion has recently been revealed to be important for cancer metabolism [1]. Since cancer cells exhibit increased intrinsic ROS, the non-enzymatic Ser conversion can also be supposed.

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It is known that HOCl, formed in living organism mainly due to myeloperoxidase activity, reacts with a wide variety of biological molecules [2] and causes tissue damage. It was established that HOCl can initiate carbon skeleton destruction of Ser, Thr and GlcN according to the proposed scheme.

Therefore the discovered conversion mechanism is recommended to take into consideration during investigation of ROS/RCS-induced pathophysiological processes involving biomolecules with 2-aminoethanol moiety, such as Ser, Thr and the corresponding peptides as well as GlcN, and, possibly, their complex macromolecular derivatives.

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Keywords: amino acids, amino sugars, free radical destruction.

WED-026
Functional proteomic analysis of the mechanisms of proteotoxicity in cardiac AL amyloidosis

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Introduction: In AL amyloidosis, widespread deposition of immunoglobulin light chains (LCs) as amyloid fibrils translates into organ dysfunction. In vivo and in vitro evidence indicates that soluble amyloidogenic LCs possess intrinsic toxicity towards cells. The mechanisms of damage are not yet clarified and may involve abnormal interactions between toxic LCs and cell components. We developed a system to investigate LC-protein interactions in cardiac cells, potentially leading to functional perturbation and genesis of proteotoxicity.

Methods. Monoclonal LCs that are amyloidogenic and cardiotoxic in vivo, along with non-amyloidogenic ones (controls), were purified by chromatography from urines of clinically characterized patients (with AL cardiomyopathy and multiple myeloma, respectively), and assessed for purity and aggregation in vitro. Direct and inverse co-immunoprecipitation (co-IP) approaches, combined with mass spectrometry and immunoblotting, were used to identify and confirm the protein species interacting in vitro with each light chain. The lists of interactors were analyzed by bioinformatics. Selected interactions were validated by imaging approaches in cultured human cardiac cells.

Results. Our functional proteomics approach showed that the soluble LCs that are cardiotoxic for patients interact with a common subset of cellular proteins in vitro, mainly possessing mitochondrial localization and involved in key viability and metabolic functions. No evidence of in vitro formation of light chains amyloid fibrils was documented during the analysis time. Imaging studies, in human cardiac fibroblasts (hCF) and cardiomyocytes (hCMC), showed that LCs are internalized. Validation of proteomic data by co-localization analyses, in hCF, confirmed that LC-protein interactions, specific for the analyzed cardiotoxic LCs, occur within live cells.

Discussion. Our data indicate that toxic LCs establish interactions with intracellular species, affecting proteins involved in viability and metabolism. This study opens new perspectives on the mechanisms of LC proteotoxicity and AL cardiomyopathy and on potential therapeutic strategies.

Acknowledgements: This work was supported by grant GR-2010-2317596 from the Italian Ministry of Health
Keywords: Amyloid, cardiotoxicity, Functional proteomics.

WED-027
GeroScope: a new method for *in silico* screening of candidate geroprotective drugs using gene expression data

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While there is an urgent need to find new drugs that suppress aging and age-related diseases, in vivo studies evaluating drug efficiency in higher mammals may take decades. An intelligent process for predicting the activity and ranking the geroprotective activity of various factors and strengthening the prediction in rapid and cost-effective studies on cell cultures and model organisms may help increase the longevity dividend of these studies. We developed a method and a software package termed GeroScope for *in silico* screening and ranking of drugs and other factors that act on the many signaling pathways implicated in aging and longevity by calculating their ability to minimize the difference between signaling pathway activation patterns in cells of young and old patients. We applied GeroScope on publically available gene expression datasets, computed pathway activation profiles of various tissues and selected potential geroprotective drugs to be tested *in vivo* and *in vitro* to narrow down the list of preventative medicine choices for clinical trials.

Keywords: Aging, Drug discovery, Transcriptomics.

WED-028
HOCl-induced sphingolipid free radical destruction resulting in bioactive 2-hexadecenal

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It was found that the action of hypochloric acid (HOCl) on aqueous lysosphingolipids dispersions produces 2-hexadecenal. For the realization of such process, the presence of a free amino group in the sphingolipid molecule is required to ensure formation of N-centered radicals from the substrate on its interaction with reactive oxygen and chlorine species.

The obtained data allows to conclude that hypochlorous acid chlorinate amino group of sphingosine, sphingosine-1-phosphate, sphingosine-1-phosphocholine followed by rapid C–C bond rupture and accumulation of 2-hexadecenal.

Analysis of the effect of HOCl on transplantable rat glioma C6 cells indicates 2-hexadecenal to be formed. We developed method based on high performance liquid chromatography using fluorescence detection to quantify the sphingolipid destruction product 2-

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hexadecenal. The obtained results suggest the described mechanism of free radical fragmentation of sphingolipids may be implemented on cell culture under stress of reactive chlorinating species.

We demonstrated that 2-hexadecenal causes cytoskeletal reorganization during neutrophil adhesion which is revealed by increasing of both F-actin content and the cells' size. 2-Hexadecenal can regulate the redox state of neutrophils which stimulated by chemotactic peptide fMLP, redistributing contribution of myeloperoxidase, phospholipase A_2 and enzymes of arachidonic acid metabolism in the formation of reactive oxygen and halogen species.

These findings evidence the existence of non-enzymatic pathway of sphingolipid destruction leading to formation of 2-hexadecenal, which possesses a wide spectrum of biological activity. Therefore, any change in the sphingolipid/2-hexadecenal ratio in a cell should play an important role in the signaling way switch-over mechanisms and, as a consequence, in functioning of the cell. Hence, realization of non-enzymatic reactions leading to formation of 2-hexadecenal in living organisms may influence functions of the latter.

Keywords: 2-hexadecenal, reactive chlorinating species, sphingolipids.

WED-029

How can we quantify ligand sensitivity for single-cell heterogenous dynamical responses?

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All biological organisms need to sense and response to their environment. At the level of single cells, surface receptors convert extracellular cues into activation of transcription factors that control cellular decisions.

A considerable unresolved issue is how information about ligand binding is encoded into nuclear activity of the transcription factors. A growing number of studies supports the hypothesis that this is achieved by temporal regulation of their activities. The current challenge is to recognise the features of temporal activity profile that represent information about a given stimulus.

A natural strategy to decipher this temporal coding is to scan cellular responses across a range of considered stimuli and identify most sensitive features of temporal profiles. Methods however to quantify sensitivity at the single cell level, where stochastic effects play a major role, are virtually missing. We have developed a statistical framework to measure sensitivity of cellular outcomes from time-resolved, single cell, heterogeneous responses. The method allow us to quantify changes in response to stimuli despite substantial heterogeneity of single cell behaviours.

We use the method to analyse nuclear translocation of the NF-kB transcription factor upon TNF stimulation in mouse embryonic fibroblasts. We identified how the sensitivity the system changes with TNF concentration and indicate the essential features of the nuclear NF-kB temporal profile that are most sensitive to TNF changes. Our method provides essential methodological advancement needed to gain understanding how temporal activity profiles encode information about a given stimulus.

Keywords: Information Processing, Sensitivity Analysis, Signal Transduction.

WED-030

How do peptidic tree-like molecules fold?

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Peptide dendrimers are tree-like molecules formed by alternating functional amino acids with branching diamino acids such as lysine [1]. Dendrimers of this kind have already rendered several models for different applications, such as vitamin B12 transporters, antimicrobial agents and catalytic systems.

Unfortunately these molecules have not yet yield to experimental structural characterization, hampering the possibility of constructing truly tailor-made peptide dendrimers and improve the existing ones. Computational methods seem to be an adequate tool to address these issues.

Herein we present a comprehensive set of computational studies using molecular dynamics simulation methods, including stochastic titration constant-pH simulations, to explore the conformational behavior of these molecules and the key determinants to such behavior [2,3].

Moreover, we unravel the first hints on the influence of pH in the folding of these molecules, and the role played by dendrimer-substrate interactions during dendrimer-catalyzed ester hydrolysis.

We used several conformational analysis procedures (clustering, energy landscapes and multivariate analysis) to analyze conformational changes that can be correlated with particular structural trends.

Our results show that peptide dendrimers exhibit a remarkable structural plasticity which is crucial for their activities.

This work provides new insights into the atomic-level structures of these systems and might, in a near future, contribute to the development of novel knowledge-based dendritic systems with enhanced functionalities.

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Keywords: Molecular Dynamics simulations, Peptide dendrimers.

WED-031

IKKα regulates hair follicle morphogenesis and hair growth cycle

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The IKKα protein is mainly known by its essential function in epithelial physiology. It is also recognized by its role in non-melanoma skin cancer (NMSC) tumor development. To gain insight into IKKα function in adult skin as well as in skin appendages such as hair follicles- considered the place of origin of NMSC-, we have generated IKKα-siRNA transgenic mice. Here, we compare the skin of these mice with that of IKKa+/- mice expressing reduced levels of IKKα.

Both types of transgenic mice show similar external phenotypic features consisting in scarce and ruffled hair. This hair coat phenotype was observed in the first hair growth cycle. After depilation, hair regrowth was delayed in IKKa-siRNA transgenic mice and

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there were apparent anomalies in the histology of the new hair follicles, consistent in distorted shapes and follicle degeneration, accompanied by dermal deposition of melanin and local infiltration of mononuclear cells around the follicular debris. In addition, transgenic mice presented morphologically distorted hair shafts, which did not correspond to any of the usual hair types found in control mice—guard, awl, auchene, and zig-zag hair. In aged IKK+/- mice, in addition to hair alterations, we have observed alopecia and histological alterations of the intrafollicular epidermis, including foci of parakeratosis, basal hyperplasia and atypia that occasionally lead to the development of spontaneous papillomas and *in situ* carcinomas. We have not analyzed aged IKKα-siRNA mice.

The study of the skin and hair follicles of both, IKKα-siRNA and IKKa+/- transgenic mice show that IKKα is required for the control of hair follicle homeostasis. The alterations of these appendices are similar in both types of mice. These results and previous work of our group suggest that the expression of IKKα must be strictly regulated since both the decreased and increased levels of IKKα lead to the predisposition to skin malignancy.

Keywords: None.

WED-032
Interplay of crystallography, Raman microspectroscopy and electrospray mass spectrometry for studying the reaction between ruthenium complexes and proteins

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Ruthenium complexes are evaluated as prospective pharmaceutical agents and have emerged as promising alternatives to Platinum compounds for anticancer chemotherapy. Due to the large interest of this class of compounds for bioinorganic chemistry and biomedical applications, it is demanding to investigate their reactivity with proteins. The reaction between NAMI-A and AziRu, two structurally related Ru(III) compounds with antitumor activity, and the model proteins hen egg white lysozyme, HEWL, and bovine pancreatic ribonuclease, RNase A, was investigated through electrospray ionization mass spectrometry, Raman microscopy, UV-visible absorption spectroscopy and X-ray crystallography [1–3]. In all cases, formation of stable metal-protein adducts was unambiguously demonstrated. Similarly, the product of the reaction between the paddle-wheel tetrakis (acetato)chlorido diruthenium (II,III) complex, $[Ru_2(\mu\text{-}O_2CCH_3)_4Cl]$, and HEWL has been characterized [4]. Taken together, the results of these works suggest that the combined used of X-ray crystallography, Raman microspectroscopy and electrospray mass spectrometry is a valuable tool for examining in detail the protein metalation process.

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Keywords: metallodrug-protein interactions, metallodrugs, ruthenium compounds.

WED-033
Investigation of interactions between anti-tumor antibiotics and biomolecules by molecular modeling

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Objective: Chemotherapy is the treatment of cancer with natural or synthetic chemicals, biological agents and hormones which selectively kill especially, rapidly dividing cells as an important part of cancer therapy. A glycopeptide antibiotic bleomycin and an antracycline antibiotic doxorubicin with anti-tumor activity are produced by the bacterium Streptomyces verticillus and Streptomyces peucetius, respectively. These anti-tumor antibiotics targeted to DNA by intercalation. The structure of bleomycin is composed of mainly 4 domains; metal-binding domain, bithiazole tail (DNA-binding domain), linker region and carbohydrate domain. The spectroscopic studies and crystal structure models of bleomycin have been used to understand the function of metal binding domain of bleomycin. The modes of intercalation with DNA, minor groove binding, full or partial intercalations have been contraversial issues for both drugs. For this reason, we will study the interactions of bithiazole tail of bleomycin and doxorubicin with the DNA by using quantum mechanical methods.

Material and Methods: For this purpose, conformational analyses of bithiazole domain of bleomycin and doxorubicin were performed with Spartan 08 software. In order to identify the appropriate conformer of bithiazole domain and doxorubicin, optimizations were performed with Spartan 08 software. Geometry optimizations and frequency analyses were performed for each conformer using density functional theory (DFT), at B3LYP/6-31G (d,p) level using Gaussian 09 software.

Results: 798 conformers for bleomycin and 422 conformers for doxorubicin were determined by conformational analyses. In order to find the most stable structure (i.e. lowest-energy structure), geometry optimization was performed. After the frequency analyses the most stable structures for bithiazole domain of bleomycin and doxorubicin were determined.

Conclusion: In this study, it was shown that bithiazole domain of bleomycin molecule can be found in 798 different conformations and doxorubicin molecule can be found in 422 different conformations. The most stable structures of these conformers were determined by optimization analyses. In order to clarify the effects of these drugs on DNA,

these structures will be used to study interactions of bithiazole domain and doxorubicin with the DNA.

Keywords: Bleomycin, doxorubicine, molecular modeling.

WED-034
Iron in *Mycobacterium smegmatis* Dps: defining its pathway, a role for flexible aspartate residues in its channeling and charge neutralization by chloride ions

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Iron plays a vital role in the biology of living organisms. It acts as a co-factor in many redox processes and is found in the active site of many enzymes. But the acquisition and storage of iron poses a problem for aerobic organisms as it is easily oxidised to its insoluble form Fe^{3+} which is non bioavailable. Free iron

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generates toxic byproducts through a process called Fenton reaction, harmful to organisms. Ferritins are a family of iron storage proteins present in vertebrates, invertebrates, plants and microorganisms, and has the function of storing iron as a non-toxic and bioavailable form in their hollow protein shell. The 12-meric compartments are called DNA binding proteins under starvation or miniferritins, since they protect DNA by direct binding, as well as indirectly from free-radicals by sequestering iron in the core. *Mycobacterium smegmatis* has two Dps proteins, namely the MsDps1 and MsDps2. MsDps1 was discovered in starved mycobacterial cultures, and MsDps2 based on a BLAST search from the TIGR database.

By studying the effect of substitutions at conserved sites near ferroxidation center, we attempt to arrive at a pathway which iron atoms take to reach ferroxidation site. This pathway is the shortest route for iron from the entry site, and is lined by negatively charged residues. The two regions, the ferroxidation and iron entry sites, which are highly electronegative are linked by Arg73 which is placed at the junction and extends a number of inter-subunit interactions. When Arg73 is substituted with a glutamate, we find that the dodecamer dissociates upon the addition of iron. This instability in the presence of iron suggests that there is a direct interaction of iron with the residues which are part of the cluster. Also, by crystallization of proteins loaded with varying amounts of iron, we tried to map the changes in the protein structure in the presence of it's ligand. The protein does not show any detectable iron clusters inside the dodecamer even on saturation conditions of iron. In the presence of iron a number of residues were found to exhibit alternate conformations. Iron was detected at the entry site of the protein, and the chloride ion present at the Dps-like trimeric interface was found to dissociate at high amounts of iron, suggesting an anion channel at this interface for MsDps2.

Keywords: Crystal structure, iron metabolism, Mycobacteria.

WED-035
Live-cell measurements with fluorescence cross-correlation spectrometry reveal a potential role in competitive bindings for in vivo dissociation constants

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The EGFR-Ras-ERK MAP kinase pathway plays a pleiotropic role in many cellular functions such as proliferation, differentiation, survival, and tumorigenesis. Computer-assisted simulation is a promising approach for clarifying such complicated signaling networks; however, this approach is currently limited by a deficiency of reliable kinetic parameters. Moreover, even when such parameters are available, they are often determined by in vitro experiments, which may not be applicable to computer simulation of intracellular signal transduction. To overcome this problem, we applied fluorescence correlation spectrometry (FCS) and fluorescence cross-correlation spectrometry (FCCS) to measure dissociation constants of signaling molecule complexes in living cells (*in vivo* K_d). Among several pairs of fluorescent molecules tested, that of mEGFP and HaloTag-tetramethylrhodamine was most suitable for the measurement of *in vivo* K_d by FCS and FCCS. Using this pair, we determined more than 20 *in vivo* K_d values of signaling molecule complexes comprising EGFR-Ras-ERK MAP kinase pathway. With these parameters, we developed a kinetic simulation model of the EGFR-Ras-ERK MAP kinase pathway, and revealed a potential role played by stoichiometry in Shc binding to pEGFR during the peak activations of Ras, MEK and ERK. This multiple binding o Shc proteins to pEGFR was validated by quantitative imaging, showing that approximately 3 Shc molecules associated with an pEGFR molecule. Intriguingly, most of the *in vivo* K_d values determined in this study were higher than the *in vitro* K_d values reported previously. This data implicated the importance of competitive bindings inside cells, but not macromolecular crowding, which reduced K_d values. These *in vivo* K_d values will provide an important basis for the quantitative understanding of signal transduction.

Keywords: dissociation constant, FCCS, live-cell measurements.

WED-036
Low resolution structure of the pNth1:Bmh1 complex

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Neutral trehalase (Nth1, EC 3.2.1.28) from *Saccharomyces cerevisiae* hydrolyses disaccharide trehalose amassed by yeast as a storage carbohydrate and a universal stress protectant. Nth1 activation is mediated by PKA-phosphorylation, Ca^{2+} and the yeast 14-3-3 protein (Bmh1) binding [1, 2]. Bmh1 modulates the structure of both the catalytic domain of Nth1 and the region containing the EF-hand like motif which is conserved among many Ca^{2+} binding proteins [3]. The structure of the pNth1: Bmh1 complex and the importance of the EF-hand like motif were investigated using site-directed mutagenesis, enzyme kinetic measurements, hydrogen deuterium exchange coupled to mass spectrometry, chemical cross-linking and small angle X-ray scattering (SAXS) [4]. We proved that structural integrity of the EF-hand like motif is essential for the Bmh1 mediated activation of Nth1 and that Ca^{2+} binding facilitates this process by affecting Nth1 structure. The low resolution structural views of Nth1 alone and the pNth1:Bmh1 complex (see Fig.) show that the Bmh1 binding induces a significant structural rearrangement of the whole Nth1 molecule. We revealed that the EF-hand like motif-containing region forms a separate domain that interacts with both Bmh1 protein and the catalytic trehalase domain. Thus EF-hand like motif functions as the intermediary through which Bmh1 modulates the activity of Nth1. Our study is important not only for better understanding the mechanism of the yeast stress response and trehalose metabolism but also as an example of structural study of complex of 14-3-3 protein with fully active enzyme.

This work was supported by the Czech Science Foundation (Project P207/11/0455) and by Grant Agency of Charles University (Grant 644313).

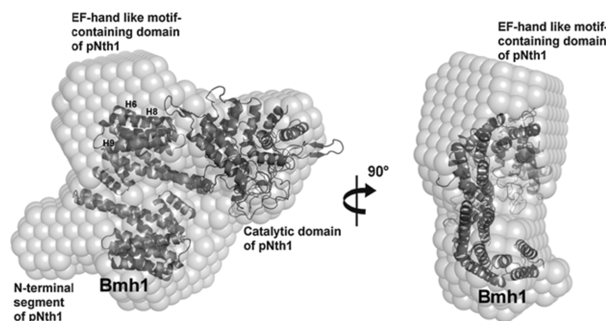

Fig. 1.

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Keywords: 14-3-3, Bmh1, neutral trehalase.

WED-037
Lysozyme adsorption at SiO2 and mica surfaces: atomistic molecular dynamics and experiments

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Lysozyme (LYS) is a model macromolecule in the study of protein adsorption on surfaces. We combine fully atomistic Molecular Dynamics (MD) simulations with experimental measurements to gain new insight into LYS adsorption at a silica surface. We describe the LYS adsorption mechanism at SiO_2 in detail: we elucidate the adsorption driving force, list the most important residues anchoring the protein at the surface, and describe the protein structure alteration required for the adsorption. Comparison of LYS adsorption at SiO_2 and a model mica surface reveals no substantial differences in the general adsorption mechanism.

Experimental work has shown that both the surface properties and the protein itself influence the effectiveness of adsorption. We performed analysis of conformational changes of proteins by quartz crystal microbalance (QCM-D) and surface plasmon resonance (MP-SPR). Furthermore, QCM-D can be used to study the adsorption behavior (kinetics, adsorbed amount and the thickness of protein film layer). Effect of solution concentration, pH and electrolyte type was studied to elucidate the nature of the association processes. Effect of contact time and applied load was related to the viscoelastic properties of the protein matrix on the surface, formed under different conditions. MP-SPR was used to study structural effects such as changes in the thickness/density of the protein layer. Such protein layer structures are expected to possess viscoelastic properties, giving information on molecular conformation, which can be confirmed by QCM-D.

The experimental methods give the average signal from the entire adsorption surface. In contrast, using MD we observe changes during adsorption of a single protein. From the atomistic resolution of the MD results on the one hand, and good agreement with experimental data on the other, the nature of the protein interactions with the solid surface can be understood in detail.

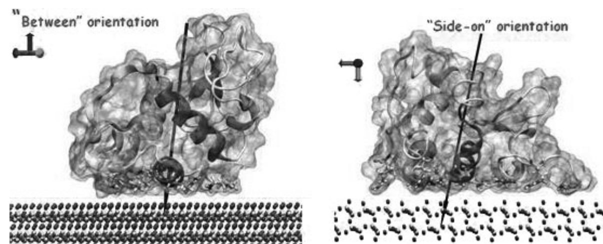

Fig. 1. Sample LSZ orientation at the SiO2 surface. The arrow shows the protein dipole moment. LSZ orientation can be "side-on" and "between".

Keywords: Lysozyme Adsorption, Quartz Crystal Microbalance with Dissipation Monitoring, Surface Plasmon Resonance.

WED-038
Metabolic network reconstruction and modelling of nitrogen fixation in *Azotobacter paspali*

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Azotobacter is an aerobic, free living N fixing bacterium. Most Azotobacter species can utilize nitrate as a nitrogen source by reduction to nitrite and then to ammonium. Azotobacteria is used on the behalf of studying nitrogen fixation and inoculation of plants because it has rapid growth and high level of nitrogen fixation (Jagnow, 1987; Gouri and Jagasnnatathan, 1995; Maltseva et al., 1995; Mrkovaki et al., 1996a). The nitrogen fixation bacterium *Azotobacter paspali* was first described by Dobereiner (1966) and has only been isolated from the rhizosphere of Paspalum notatum, a tetraploid subtropical grass widely distributed in South America (Dobereiner and Campelo, 1971).

Our strategy for bacterial nitrogen fixation consisted of three steps; metabolic reconstruction for *A.paspali*, *in silico* modelling of nitrogen fixation, and evaluation of computational predictions and experimental results. The microbial genome sequence was the first step to attain analysis of gene-protein associations and metabolic reconstruction in this systems biology research. The genome sequence of *Azotobacter vinelandi* was used for this process due to its phylogenetic similarity with *A.paspali*. Through an iterative reconstruction process, the metabolic network was constructed based on the genomic, biochemical and physiological information on *A.paspali*.

In this work we present a genome scale metabolic reconstruction for *A.paspali*, which includes 380 metabolic and transport reactions across 26 metabolic pathways. This model was used to analyze the physiological capabilities of *A. paspali* during stages of nitrogen fixation. To study the physiological capacities *in silico*, an objective function was determined to conduct simulations of nitrogen fixation. After flux balance analysis (FBA) performed, it was observed that predicted active metabolic pathways agreed with experimental data. In addition to being a useful guide for identification and filling of knowledge gaps, the reconstructed metabolic network will accelerate the research on this bacteria towards application of systems biology approaches and design of metabolic engineering strategies to enhance nitrogen fixation.

Keywords: Metabolism, Modelling metabolic reconstruction, Nitrogen fixation.

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WED-039
Microtubule catastrophe as a multi-step sequence of reversible events

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Microtubules are cytoskeletal filaments, essential for cellular structure, transport and motility. They are dynamically unstable, i.e. exhibit distinct phases of growth and shortening. Transitions between growth and shortening are called catastrophes. Catastrophes determine the lengths of microtubules in the cells and affect a variety of key cellular processes including cell division, cell migration and others. Experiments in vitro and in vivo have demonstrated that the frequency of microtubule catastrophes increases during microtubule polymerization, manifesting in "microtubule aging". The molecular nature of the "microtubule aging" and the mechanism of microtubule catastrophe remain unclear. Two recent models have been proposed to explain these phenomena. One model postulates that catastrophes result from irreversible accumulation of three catastrophe-promoting features in the microtubule lattice. Another model finds the origin of the "microtubule aging" in progressive tapering of the microtubule tip, leading to gradual microtubule destabilization. To examine these hypotheses, we have created a detailed model of the microtubule based on a combination of Brownian dynamics and kinetic approaches. This quantitative model successfully recapitulates available structural and kinetic data about microtubule dynamics, including the phenomenology of the "microtubule aging". The model however does not involve accumulation of irreversible catastrophe-promoting features nor does it show any gross changes in the tip structure during MT growth. Thus, we propose an alternative hypothesis to explain the "microtubule aging". We argue that the microtubule catastrophe is a statistical phenomenon that results from rare combinations of multiple short lived reversible molecular events taking place at the microtubule tip. Our data indicate that these reversible destabilizing molecular events likely represent a combination of stochastic GTP hydrolysis and formation of curved protofilaments. Altogether, our study provides novel insights into the microtubule catastrophe and offers important predictions about the mechanisms of action of proteins and drugs, regulating microtubule dynamics.
Keywords: catastrophe, microtubule, modeling.

WED-041
Modelling of *Saccharomyces cerevisiae* K2 toxin entry and response of the host cell

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The production of antimycotic killer toxins (mycocins) has been detected in several yeast genera and has been proved to be a widely distributed phenomenon. The toxins of the yeast origin confer a growth advantage to their hosts by increasing survival in clinical, environmental, and industrial ecosystems. They find application in food protection from spoiling, wine production, phytopathogen control, creation of a new generation vaccines and antibiotics, development of antifungal immunotherapy. The characterization of such toxins has consistently provided significant insights into the basic mechanisms of self-defence, virus-host cell interactions and toxin entry into target cell.

In order to identify gene products modulating the sensitivity to K2 killer toxin, we performed high-throughput genome-wide screen in *S. cerevisiae* and identified 332 genes affecting resistance formation process. To get further insights into the cellular activities involved in the biology of K2 killer toxin, we analysed discovered effectors and calculated the enrichment of 'biological process' and 'cellular component' gene ontology (GO) terms associated with pronounced K2 resistant and sensitive phenotypes. We built interaction networks and demonstrated physical and functional interaction of such gene products inside the cell. Also, we assigned several unknown factors to cell wall biogenesis, mitochondrial function, and stress signalling pathways. Based on the established data, we performed the modelling of the entry of K2 toxin into the susceptible yeast cell and response of the target cell by activating cell wall integrity and high osmolarity glycerol pathways.
Keywords: host effectors, *Saccharomyces cerevisiae*, toxin.

WED-043
Molecular dynamics simulations of oncogenic mutations of the EGFR ectodomain reveal an unexpected activation intermediate

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Conformational changes are central to protein function, but in spite of their robust character, they can be sensitive to perturbations at critical regions such as mutations. In particular, interdomain regions can be important to orchestrate large-scale rearrangements of multidomain structures. Here we analyze the role of interdomain interactions in the conformational dynamics of the extracellular domain of the Epidermal Growth Factor Receptor (EGFR). EGFR activation is triggered by a ligand-favored transition of the ectodomain from a closed, self-inhibited tethered monomer, to an open untethered state, which exposes a loop required for dimerization and activation. A preliminary coarse-grained analysis shows that cancer-mutations tend to cluster at conserved interdomain interfaces and loops important for large-scale movements. Molecular Dynamics simulations of two muta-tions targeting one of the detected hot spots reveals a dramatic conformational shift towards a transient untethered intermediate different from known crystal structures, but in agreement with several experimental evidences. The present findings point to the existence of conformational states other than the open and closed, and to differential processes driving ligand-dependent and independent untethering in EGFR. In a wider

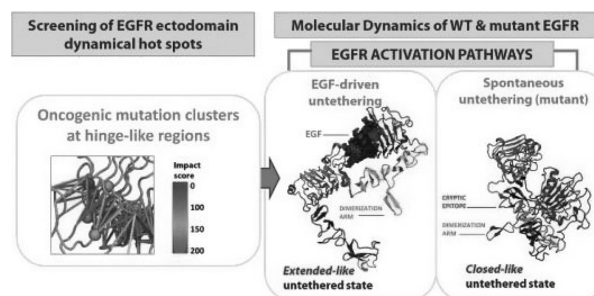

Fig. 1.

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context, our results suggest a novel oncogenic and evolutive mechanism based on the perturbation of the native motions at the interfaces of structural elements.

Keywords: EGFR, glioma mutations, molecular dynamics.

WED-044
Molecular mechanism of fibrosis – proteomic and transcriptomic analysis

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The epithelial-mesenchymal transition (EMT) plays a pivotal role in embryonic development, wound healing, tissue regeneration, organ fibrosis and cancer progression. This process is initiated by Transforming Growth Factor-β1 (TGF- β1) which induces potent stimulation of the expression of numerous genes participating in fibrosis.

Endothelial to mesenchymal transition (EndMT) might be an important source of the mesenchymal cells participating in the fibrotic process. To establish profiles of the endothelial cell genes involved in the early stages of fibrosis HMEC-1 cells (human microvascular endothelial cell-1) were stimulated with TGF- β1 at the concentration of 5 ng/ml for 48 h.

The treatment of HMEC-1 with TGF- β1 leads to increase in the level of Snail1, Vimentin and decrease in the level of Claudin1. Differential proteomics to perform a global quantitative comparison of two proteomes with Orbitrap Velos mass spectrometers and iTRAQ – a labeling method analysis of HMEC-1 displayed a total number of 5522 proteins among those only 17 were overexpressed and 27 were down-regulated. Transcriptomic analysis of 57716 genes demonstrated up-regulation of 264 genes and down-regulation of 211 genes. These small changes at the molecular level give rise to the formation of EndMT-dependent fibrosis.

Keywords: EndMT, fibrosis, TGF-β1.

WED-045
Mutations close to the central residues of the structural network of the beta glucosidase Sfbglu affect its thermostability

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The structure of proteins can be represented as a network in which the amino acids are the nodes and their non-covalent interactions are the connections. The presence of central nodes contributes to existence of shorter paths (sets of connections) between different nodes on the network. Thus, it has been suggested that central residues are determinant for several enzymatic properties, as stability, allostery and catalytic activity. Besides that it is proposed that mutations directed to the central residues or their proximity could be easily propagated and cause significant modifications in the protein structure. The goal of this project is to test this hypothesis, that is, to evaluate the relevance of the central residues in the propagation of mutational effects through the structural network of the beta-glucosidase of *Spodoptera frugiperda* (Sfbglu) changing its thermostability.

The mutant Sfbglu were produced as recombinant protein in *E. coli* BL21 gold (DE3) using the vector pET46. After that they were purified using affinity chromatography (Ni-NTA). The homogeneity of these samples was verified by SDSPAGE. To assess whether the mutants structures were properly folded, tryptophan fluorescence spectra were collected. The spectra of the mutants did not show any change with respect to the WT Sfbglu. Furthermore, experiments of tryptophan fluorescence suppression by acrylamide were performed. The K_{sv} parameters of the mutant Sfbglu were equal to the WT Sfbglu. Circular dichroism spectra were also collected for the mutant Sfbglu, of which only two, A264F and D84A, showed slight differences from the wild-type protein. So these experiments indicated that mutant Sfbglu were properly folded. To evaluate the thermal stability of the WT and mutant Sfbglu, T_m were calculated using two different techniques, circular dichroism and thermal shift assay. Mutations D260A, A263F, L335A, V336F and S337F, which were directed to close neighbors of the central residue F251, decreased the T_m from 45°C (WT Sfbglu) to values between 30 and 41°C. Moreover mutations A264F (connected with the central residue F251) and D84A (indirectly connected with central residue R97) abolished the thermal transition. Conversely mutations E261A, M262F and E265A at 3 distant neighbors of the residue F251 caused no change in the T_m. In agreement mutations V319A, L389A,Y390A and N391A, which are close to the non-central residue F280, caused no significant change of the T_m. Therefore the experimental data suggest that the propagation of the mutational effect altering the thermostability of Sfbglu correlates with the closeness between the mutated and the central residues.

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Keywords: beta-glucosidase, structural network, thermostability.

WED-046
Neutral and anionic forms of aspartic and glutamic acids interacting with RNA and DNA nucleotides

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Asp and Glu amino acids are usually depicted under their negatively charged form involving a carboxylate group. In this respect, they form anion–nucleic acid interactions [1,2]. Yet, they might also interact with nucleic acids in their unfamiliar neutral form that involves a carboxyl group. We report here, along with a classification of regular Asp/Glu binding sites, several surprising instances of close contacts between Asp/Glu side chains and hydrogen bond acceptors in nucleic acid systems. Favored nucleobase binding sites emerged; more specifically, guanine Watson-Crick sites were observed to dominate over other possibilities without excluding less obvious interaction patterns and contacts with backbone phosphates. The adenine/glutamic acid pair that involves the neutral form of the amino acid illustrates this point (see figure). Such counterintuitive contacts involving Asp/Glu residues generally considered as negatively charged and nucleic acid electronegative atoms can only be understood by considering the neutral forms of these amino acids. To confirm that these interactions associated with neutral Asp and Glu forms are not anecdotal, a survey of the PDB was performed. This survey collected a large array of short hydrogen bond contacts between Asp and Glu residues with themselves and with other electronegative atoms, therefore stressing that their neutral interacting form is more frequent than previously thought.

The purpose of this study is to emphasize the implications of such unexpected hydrogen bond networks in our current understanding of protein/nucleic acid interactions. It is anticipated that the neutral Asp and Glu amino acids will have to be included into an expanded bestiary of interacting residues leading to novel recognition patterns. These interaction motifs will certainly have

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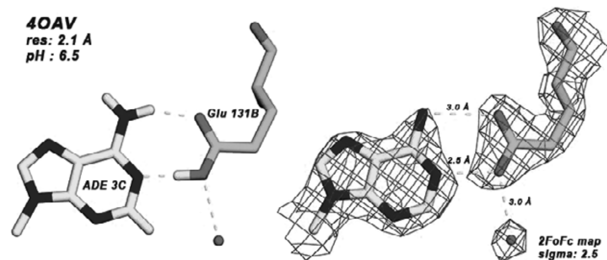

Fig. 1.

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Keywords: None.

WED-047

New control mechanisms of water transport identified in AQP1 by combining molecular dynamics simulations and experiments

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Aquaporin family gathers membrane proteins that are identified as selective water channels. The transport of water is bidirectional, extremely rapid and is driven by the osmotic pressure. High-resolution 3D structures have brought valuable information to characterize the translocation channel. However, a better understanding of the protein function requires a dynamic view of the transport process. Interestingly, the transport rate is compatible with current molecular dynamic (MD) simulations time-scale. Accordingly, they were extensively used to catch the details of the water transport mechanisms. In particular, simulations helped in identifying two filter regions, one located in the central part of the channel and the other facing the extracellular medium.

In the present work, we revisited the transport of water in AQP1 using long MD simulations. We characterize two new gates located on the extracellular and cytoplasmic sides respectively. Interestingly, the cytoplasmic exit is controlled by two conserved residues (H74 and E17), which are stabilized by hydrogen bonds. We tested different protonation states for these two residues and we showed how they influence the permeation events.

These results suggest that the intracellular pH (pHi) would play a role in the transport mechanisms. In order to confirm this hypothesis, we measured the Pf (osmotic permeability) at different pHs (pHi=pHe=6.5 or 7.2 or 8.0) by following the fluorescence quenching of the 5 (6) carboxyfluorescein. The low Pf at pH6.5, whatever the mannitol gradient, was increased at pH7.2 and pH8.0. When pHi was fixed to 7.2 but the extracellular pH (pHe) varied, Pf was unchanged.

These results clearly demonstrate the impact of pHi on transport and how combining theoretical studies and experiments help to enrich the spectrum of mechanisms that control AQP1 water transport.

Keywords: Aquaporin, permeability, pH.

WED-048

New control mechanisms of water transport identified in AQP1 with molecular dynamics simulations

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Aquaporin family gathers membrane proteins that are identified as selective water channels. The transport of water is bidirectional, extremely rapid (~3 billion water molecules per second), and is driven by the osmotic pressure. In addition, charged species are excluded from the transport. Aquaporins are present in all organisms and in mammalians, 13 homologous AQPs (AQP0 to AQP12) have been characterized. They also differ by their tissue distribution and the pathologies with which they are associated. High-resolution 3D structures have brought valuable information to identify translocation channel. However, a better understanding of the protein function requires a dynamic view of the transport process. Interestingly, the transport rate is compatible with current molecular dynamic (MD) simulations time-scale. Thus, MD simulations have been extensively used to catch the details of the water transport mechanisms. In particular, simulations helped in identifying two filter regions, the NPA (asparagine/proline/alanine) region located in the central part of the channel and the constricted ar/R (aromatic/arginine filter) region facing the extracellular medium.

In the present work, we revisited the transport of water in AQP1 using long MD simulations. We identified new gates located on the extracellular and cytoplasmic sides respectively. Interestingly, the cytoplasmic exit is controlled by two conserved residues (H74 and E17), which are stabilized by hydrogen bonds and seem sensitive to intracellular pH. We tested different protonation states for these two residues and we showed how they influenced the permeation events. These results, which were confirmed by different experiments, enrich the spectrum of mechanisms that control AQP1 water transport.

Keywords: Aquaporin, Molecular Dynamics simulations, transport.

WED-049

New in DEPPDB – a portal for electrostatic and other physical properties of genome DNA and its elements

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DNA is highly charged and its electrostatic and other physical properties define its shape in the functional space and influence its interactions with different proteins, esp. regulating transcription, in particular RNA-polymerases and transcription factors. DEPPDB was developed to hold and provide all available information on these properties of genome DNA combined with its sequence and annotation of biological and structural properties of genome elements and whole genomes, organized on a taxonomical basis.

The electrostatic potential around the double-helical DNA molecule was calculated by the original method [1] using a program package [2,3]. Calculations of other physical properties are based on the di- and trinucleotide content. Different cross-correlation analysis algorithms are applied.

DEPPDB (deppdb.psn.ru or electrodna.psn.ru) contains all completely sequenced bacterial, viral, mitochondrial, plastids and eukaryotic genomes according to current release of NCBI RefSeq

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[4]. Data for promoters, regulation sites, binding proteins etc. are incorporated from DBs and literature. All data are fully integrated, several tools are provided to support different forms of analysis. Calculation on the fly of the user-provided sequences is available.

DEPPDB can be considered as a portal or collection of databases on the electrostatic and other physical properties of whole genomes and different genome elements in different taxa and organisms: Promoter DB, Regulatory Sites (Transcription Factors, TF) DB, Gene Starts DB, Terminator DB, etc. as well as comprehensive analysis toolbox.

Acknowledgements: The authors are grateful to Saveljeva E. G. for technical support and the Institute of Mathematical Problems of Biology of RAS for hosting the Database.

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Keywords: biological database, DNA electrostatic properties, Genomics.

WED-050
New parameters for describing conformational rearrangements in G-quadruplexes

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Conformational changes in G-quadruplexes (GQs) are important issues for molecular biology considering the profound effects of GQs on genome function. Molecular modeling may complement NMR and XDR data on known GQ structures and is commonly used to get insight into GQ folding or to predict GQ unwinding. For quantitative description of GQ geometry, specific parameters are needed. Ideally, they should allow characterization of various types of GQ topologies in dynamics. Several attempts have been made to introduce such parameters, but no systematic studies have been reported so far.

Reshetnikov et al. used mean square deviations from the initial structure to characterize dynamics in models of the thrombin binding aptamer [Biochemistry (Moscow), 2010, 75(8); J. Chem. Theory Comput., 2010, 6(10)]. The distance between the centers of mass (COMs) of G-quartet guanines was used to monitor “in plane” GQ motions (fluctuations in a quartet plane), and the distance between COMs of the two tetragons (the outer one formed by the N9 atoms and the inner one formed by the O6 atoms) was used to monitor “from plane” GQ motions (the tendency of a quartet to form a tepee structure). Some additional characteristics have been proposed to illustrate GQ rigidity and twisting.

Although the aforementioned parameters seem applicable for assessing certain conformational changes (GQ disruption, ion capture, etc.), they are far from exclusive and may not be optimal in the case of some complex rearrangements related to the recently reported new types of GQs with defects (i.e. bulging of the mismatching nucleosides from G-quartets).

We present novel parameters that enable comprehensive and systematic description of GQ geometry in dynamics and compare them with the previously reported parameters. We used a telomeric GQ rearrangement to illustrate our approach to monitoring GQ topological changes. In this approach, G-quartet planarity is characterized by the angles between the normals to guanine planes. An alternative planarity-related parameter determined for each particular guanine in a quartet is N1 atom deviation from the plane created by other N1 atoms. The new parameters were shown to be more informative and representative than the “in plane” and “from plane” motion characteristics discussed above. Interestingly, quartet collapse upon GQ rearrangement or deviation (bulging) of a single nucleoside from the quartet is often followed by its stacking with a neighboring quartet. We used the distances between COMs of particular guanines and COMs of the neighboring quartets to monitor such a process in the telomeric GQ.

In conclusion, we developed a new set of GQ-characterizing parameters and demonstrated its usability for monitoring GQ dynamics.

This work was supported by RSF [14-25-00013].
Keywords: G-quadruplexes, molecular dynamics.

WED-051
Nuclear EP2 receptor mediates the transactivation of epidermal growth factor receptor by PGE2

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The biological actions of PGE2 have been generally attributed to result from its interaction with four membrane G-protein coupled receptors designated EP1, EP2, EP3 and EP4. Our previous work in human renal proximal tubular HK2 cells showed that PGE2 increases the expression of hypoxia-inducible factor-1, a putative renoprotective factor. The mechanism, which also was shared by several cancer cell lines, involved EP receptor-dependent transactivation of epidermal growth factor receptor (EGFR) leading to increased expression of retinoic acid receptor-beta (RARB). PGE2-induced EGFR transactivation was prevented by inhibiting the prostaglandin uptake transporter and EP receptors were ubiquitously located in the cells. Therefore, intracellular EP receptors rather than cell membrane EP receptors seemed to be responsible for the transactivation of EGFR by PGE2. Given that the nucleus contains functional EP receptors, we hypothesized that nuclear EP activated a subset of EGFR which was also located in the nucleus. Here we aimed to confirm this hypothesis. We analyzed in nuclei isolated from HK2 cells: i) expression of EP receptors (immunofluorescence), ii) the effect of PGE2 on the phosphorylation of EGFR and c-src, which may mediate EP-dependent transactivation of EGFR (western-blot), iii) the EP subtype responsible for the PGE2 effects (specific EP agonists), and iv) the effect of PGE2 on the transcription of RARb (in vitro transcription assay).The results showed that PGE2 increased the phosphorylation of EGFR and c-src in the isolated nuclei through EP2 receptor, which resulted in increased expression of RARb mRNA. This is the first time in which has been provided a piece of evidence showing that transactivation of EGFR may proceed in the cell nucleus. The fact that EGFR transactivation was triggered by a nuclear subset of EP receptors,which has its major correlate in the transactivation of cell membrane-located EGFR by G-protein coupled EP receptors, suggests that there might be more cases in which nuclear transactivation of EGFR is induced by nuclear subsets of cell membrane receptors.

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Keywords: EGFR transactivation, human renal proximal tubular HK2 cells, Nuclear Prostaglandin E2 receptors.

WED-052
Oleuropein aglycone: a natural polyphenol with inhibitor effects on toxicity by *Transthyretin fibrillogenesis*

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Transthyretin (TTR) is a plasma protein secreted by hepatocytes into the blood and cerebrospinal fluid, where it transports thyroid hormones, thyroxine (T4) and triiodothyronine (T3) and cotrasport of vitamin A with Retinol Binding Protein (RBP). The TTR is an amyloidogenic protein implicated in diseases such as senile systemic amyloidosis (SSA) and familial amyloid polyneuropathy (FAP), both characterized by extracellular deposition of insoluble amyloid fibrils in heart, peripheral nerves and other organs. In particular, fibrils in FAP patients are composed of single-site mutant TTR and among the numerous pathogenic variants Leu55 → Pro55 (L55P) is the most amyloidogenic and it forms amyloid fibrils in vitro. It is suspected that the single-point mutations accelerate amyloidogenesis by destabilizing the monomeric partially unfolded amyloidogenic intermediate state rather than by altering the tetrameric native state.

TTR fibrils have been considered direct responsible of tissue impairment in FAP and SSA, but the unstable fibril precursors are increasingly considered the main responsible of cell suffering and tissue impairment in amyloid diseases. In particular, the early unstable oligomeric intermediates are highly toxic due to their ability to interact, disassemble and permeabilize cell membranes. Moreover, increasing information on polymorphism of pre-fibrillar and fibrillar assemblies has led to propose that apparently similar fibrils can display different stability and efficiency in generating toxic species. These data suggest the opportunity to search natural or synthetic molecules interfering with amyloid aggregation by stabilizing the TTR native state by hindering the appearance of toxic species, or by favoring the growth of less toxic assemblies. We have recently described a natural compound (oleuropein aglycone) which is protective in Tg animal models of Abeta deposition and cultured cells by stimulating cell autophagy and the endolysosomal path and by modifying the pattern of aggregation of amylin and Abeta peptides skipping the appearance of toxic oligomers and reducing plaque load. Our study is focused on the ability of oleuropein aglycone (the main phenolic component of Mediterranean extra virgin olive oil) to inhibit the toxic effects of both wild-type amyloid TTR and highly amyloidogenic L55P variant on HL-1 cells. Our data offer the possibility to validate and optimize the use of rationally designed and promising drugs that could enter in a clinical experimental phase.

Keywords: Oleuropein,Transthyretin, fibrillogenesis.

WED-054
Pericytes play key role in maintenance of vascular architecture in CADASIL

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), one of the most common small vessel diseases, is a major contributor of vascular dementia in humans. Primarily, it is known to be caused by Notch3 mutation. Recently, ultra-structural changes in pericytes have been shown in CADASIL[1,2]. Pericytes are important component of neurovascular unit that contributes to the integrity of blood brain barrier (BBB). Presence and participation of pericytes in initiation and progression of CADASIL is unknown. In murine model of CADASIL (R169C; Tg88 by overexpression of mutated transgene) 3, we investigate the role of pericytes in neuro-vascular architecture maintenance and vasculopathy of CADASIL.

As previously reported, using *in vivo* immunostaining, we found abnormal Notch3 deposit associated with the brain vessels. Importantly, we found a significant age-dependent decrease in pericyte coverage of cortical micro vessels in CADASIL mice. The decrease in pericyte coverage also correlated with increased Notch3 aggregation and increased BBB permeability. We also addressed the role of neurovascular components such as astrocytes and gap junctions. Along with this functional changes such as cerebral blood flow and neurovascular coupling has been studied. Further, using intravital 2- photon microscopic imaging *in vivo* the pericytes morphology and density will be investigated.

The preliminary results suggest involvement of pericytes in fabricating the neuro vascular architecture in CADASIL.

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Keywords: CADASIL, Neurovascular unit, Pericytes.

WED-055
Predicting protein function from sequence and co-expression: preliminary results for breast cancer molecular markers

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The accurate annotation of protein functions improves understanding of biological processes, but the inherent difficulty and expense of their experimental characterization emphasizes the need for the computational annotation. Since the functional characterization of disease-related proteins can provide new perspective on the disease etiology and therapy, this study focuses on the molecular markers of breast cancer – BRCA1 and BRCA2. These genes are relatively new in evolution, with no orthologs in the yeast, fly or worm, which imply their fundamental role in mammals and specialized functions. These features also make the prediction of their functions difficult for evolutionary-based algorithms. So far, experimental analyses showed BRCA1 and BRCA2 involvement in preserving chromosome structure, DNA repair, recombination, cell cycle control and transcription.

Protein functions of BRCA1 and BRCA2 were predicted using a new algorithm with three steps: selection of similar proteins, co-expression and enrichment analysis, which resulted in the list of Gene Ontology (GO) terms (Fig. 1). The most critical is the

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first step, in which the evolution-independent Informational Spectrum Method (ISM) was used. ISM is the Fourier transform-based pattern recognition method for protein sequence analysis, previously shown to be able to identify proteins involved in the same or similar biological processes. Three ISM approaches were used, with different parameters, although in each of them protein selection was done based on the comparison of informational spectra of BRCA1 and BRCA2 and human proteins in the UniProt database. The final list of the GO terms contained only the terms that were identified in all three ISM approaches.

For BRCA1, 98 GO terms were identified, of which 73 (75%) are already in the BRCA1 GO tree of biological processes. The terms with which BRCA1 has not yet been associated can be clustered as: nucleoside metabolic processes and cytoskeleton and intracellular localization. For BRCA2, 88 GO terms were identified, of which 60 (68%) are associated with this protein in GO. Biological processes not in the BRCA2 GO tree cluster as: processes involved in immune response, response to chemical stimuli, signal transduction and regulation of molecules functions.

The new algorithm for protein function prediction showed precision of 70%, when compared with the known BRCA1 and BRCA2 GO functional annotations. It identified some biological processes in which the involvement of these two proteins should be further investigated. Additionally, this study raises the possibility for the use of the algorithm to guide future protein function experiments.

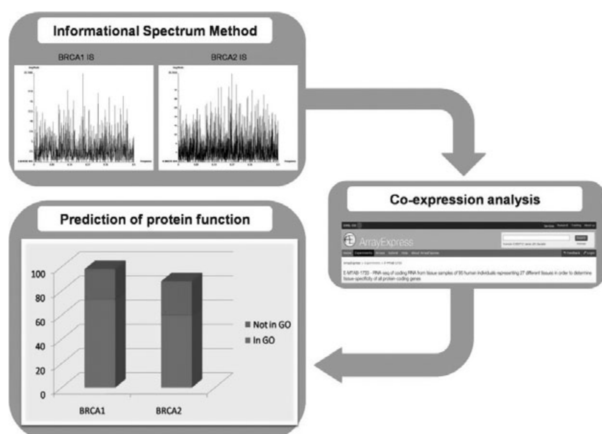

Fig. 1. xxxxx.

Keywords: Breast cancer, Protein function, Sequence analysis.

WED-056
Production of human proteins using well-established and alternative recombinant expression hosts

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A successful production of proteins of interest in a sufficient amount and a satisfactory quality is one of key steps for almost all life science branches, structural biology studies and biotechnology applications. Over the time, a lot of recombinant expression systems have been developed according to the diverse needs of use. In addition to the well-established systems such as *Escherichia coli* or mammalian cells routinely utilized in many laboratories, many alternative expression hosts have been discovered and engineered. And these unconventional systems proved to be often more suitable for protein-of-interest production from many various reasons [1].

In our paper, we demonstrate using several approaches of production of a difficult-obtainable human lectin. It has a low expression yields and it is prone to precipitation. For these reasons, a recombinant form of this protein was prepared and different expression systems were tested to obtain the active protein in a soluble form.

The first attempts were done using production of this protein using the E. coli expression host but this way failed because the protein is produced in the insoluble form only. Hence refolding, *in vitro* denaturation/renaturation and some solubility-enhancing tags were utilized to obtain protein in soluble form. The long-term optimization resulted in low yields of the active protein, which is rather unstable for longer period of time.

A generally better way for production of human proteins is using eukaryotic expression systems. In our case, we utilized the *Leishmania tarentolae* cells because of the combination of a fully eukaryotic protein expression machinery including post-translational modifications and easy *E. coli*-like handling. It appears that the *L. tarentolae* possibilities of the disulfide bond formation and the human-like glycosylation are crucial for a sufficient production of our target protein and it seems to be the very effective system for a routine production of human proteins in general.

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Keywords: *Escherichia coli*, human proteins production, Leishmania tarentolae.

WED-057
PTM-SD: curation of posttranslational modified residues in protein structures

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The residues in a protein can undergo covalent and chemical modifications that are usually called post-translational modifications (PTMs). The concept of PTMs encompasses different types of modifications from a simple addition of atoms group such as the phosphorylation, to the binding of important large groups, in the N-linked Glycosylation. PTMs play important roles in modulating various biological functions by altering the physical and chemical properties, the localization and activity of proteins. They are also associated to major human diseases such as cancer, diabetes, cardiovascular disorders and Alzheimer.

Recent studies have shown that PTMs have significant effects on the protein conformations and on their flexibility (Xin and Radivojac 2012). Current databases contain crucial sequence annotation but do not provide valuable resource on the 3D structure related to these PTMs.

Post-translational modification structural database (PTM-SD) (Craveur, Rebehmed et al. 2014) provides access to structurally solved modified residues which are experimentally annotated as PTMs. It combines different PTM information and annotation gathered from other databases, *e.g*., Protein DataBank (Berman, Westbrook et al. 2000) for the protein structures and dbPTM (Lu, Huang et al. 2013) and PTMCuration (Khoury, Baliban et al. 2011) for fine sequence annotation.

PTM-SD can be browsed by PDB id, UniProt accession number, organism and classic PTM annotation. Advanced queries can also be performed, *i.e*., detailed PTM annotations, amino acid type, secondary structure, SCOP class classification, PDB chain length, and number of PTMs by chain. Statistics and

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analyses can be computed on line on a selected dataset of PTMs. Each PTM entry is detailed in a dedicated page with information on the protein sequence, local conformation with secondary structure and Protein Blocks.

On June 2014, PTM-SD consisted of 10 628 entries. It corresponds to 842 Uniprot AC, 2986 PDB files and 5350 PDB chains containing at least one modified position. 21 different kinds of PTMs were detected, 11 with more than 50 occurrences. The most important one is glycosylation (60.09%), followed by phosphorylation (15.23%) and methylation (8.10%). 206 different organisms are present, with an over-representation of Human (44.27%), and other mammals, e.g., mouse (7.98%), bovine (7.79%), pig (1.64%) and rat (1.52%).

PTM-SD gives valuable information on observed PTMs in protein 3D structure, which are of great interest for studying sequence – structure – function relationships at the light of PTMs, and could provide insights for comparative modelling and PTM predictions protocols.

PTM-SD can be accessed at http://www.dsimb.inserm.fr/dsimb_tools/PTM-SD/.

Keywords: Glycosylation, Post-translational modifications, Protein structure.

WED-058
Pupal weight as suitable indicator for monitoring dengue vectors

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Entomological surveillance of mosquitoes is a prerequisite for prediction of population abundance and thus possibilities of mosquito-borne diseases. Current monitoring strategies for dengue and chikunguniya vectors employ various indices based on sampling of prospective larval habitats. Although these indices provide information sufficient to predict the probable population abundance, the fitness of individual mosquitoes remains obscured limiting projection about the disease transmission potential. The disease transmission potential of dengue vectors are linked to the life history traits, which are dependent on the resources acquired during larval development. Pupal weight provides key information about the probable state of nutrient reserves, adult body size, longevity and fecundity, thereby qualifying as a suitable indicator of fitness of mosquitoes. Based on this proposition, an appraisal of pupal weight as predictor of the life history features of the dengue vectors – *Aedes aegypti* and *Aedes albopictus* was made to validate its use entomological surveillance. Using laboratory reared 1200 F_2-individuals of each species, the correlations of life history traits were evaluated under varying temperature, food and rearing density. Although sexual dimorphism was prominent for each trait, the pupal weight of *Ae. aegypti* and *Ae. albopictus* were significantly correlated with larval development time, adult body weight, nutrient reserves, longevity and fecundity. The strength of correlations varied with the rearing density of larva, ambient temperature and amount of food, suggesting that pupal weight appropriately amplifies the habitat conditions of the developing larvae. Owing to sensitivity to larval habitat conditions, pupal weight qualifies as an indicator of the prospective adult life history traits. Therefore monitoring of larval habitats of dengue vectors should include pupal weight as a parameter to indicate the fitness levels of adult mosquito, thereby facilitating prediction of population characteristics of *Aedes* mosquitoes with higher precision.

Keywords: Life history traits, pupal weight, fitness.

WED-059
Purification and characterization of pineapple (*Ananas comosus*) α-galactosidase by three-phase partitioning

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α-Galactosidases (α-D-galactoside galactohydrolase, EC 3.2.1.22) are the exoglycosidase that catalyses the hydrolysis of α-1,6-galactose bonded D-galactosyl residues of basic and complex oligo- and polysaccharides (raffinose, stachyose, mellibiose, galactomannans). They have been purified with different techniques from plants, animals and various microorganisms. They have great importance in especially sugar industry, elucidation of the biological functions of complex carbohydrates, structural analysis, organic synthesis and medical purpose.

Three phase partitioning (TPP) is a simple and often one-step procedure successfully used for separation and purification of enzymes and proteins in recent years. It involves the addition of a salt to the aqueous solution containing proteins followed by the addition of a water miscible aliphatic alcohol. In less than an hour three phases are formed. The upper solvent phase containing pigments, lipids, hyrophobic materials is separated from lower aqueous phase containing proteins, saccharides and cell debris by an intermediate layer. This protein-rich middle layer generally contains desired enzymes or proteins. TPP process is also simple, inexpensive, scalable and rapid procedure that works at room temperature in comparison to conventional separation and purification processes.

In the present study, we have used three-phase partitioning for direct one-step purification of α-galactosidase from pineapple (*Ananas comosus*) fruit. α-D-galactosidase was first isolated from pineapple with conventional protein extraction methods and then concentrated and purified with TPP. TPP systems were prepared by using ammonium sulfate as a cosmotropic salt and t-butanol as organic solvent. Various system parameters (ammonium sulfate saturation, enzyme/t-butanol ratio and pH) required for the efficient purification of the enzyme were optimized to get highest purity fold and yield. The best α-galactosidase yield (100%) was obtained in the interphase of the TPP system, which consisted of the crude extract to t-butanol ratio of 1.0:0.50 in the presence of 65% (w/v) $(NH_4)_2SO_4$ at pH 6.5. The purified enzyme was also characterized. For this aim, different parameters (temperature, pH and substrat concentration) affecting to the enzyme activity and stability were investigated. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis was also performed to determine the purity and molecular weight of the enzyme. The results showed that, with necessary optimization TPP is a simple, quick, economical and very attractive bioseperation technique for primary purication of α-galactosidases compared to conventional chromatographic protocols.

Keywords: Pineapple, Three-phase partitioning (TPP), α-Galactosidase.

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WED-061
Redox cellular signaling by thiol peroxidase: what are the molecular mechanisms of the specificity of the redox relay H2O2/Gpx3/Yap1 in *S. cerevisiae*?

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Thiol-peroxidases, initially identified as antioxidant enzymes, have been associated with peroxide-dependent cell signaling as sensors and relays of the H_2O_2 mediated signal (1). One of the best documented examples of such a mechanism is the activation of the transcription factor Yap1, a key regulator of the transcriptional peroxide stress response in *Saccharomyces cerevisiae*, which depends on the formation of intramolecular disulfide bonds catalyzed by the thiol peroxidase Gpx3 (2; 3). In this mechanism, it has been proposed that the relay occurs via the oxidation of Gpx3 peroxidasic Cys as a sulfenic acid intermediate (Figure 1: Model of the S. cerevisiae Orp1-Yap1 redox relay) (3). In addition, the Ybp1 protein has been identified as an essential partner for the activation of Yap1 by Gpx3 (4).

The intrinsic reactivity of the sulfenic acid could potentially result in reaction of this intermediate with other thiols in competition with the reaction of activation of Yap1, such as with the regeneration Cys of Gpx3 or with other cellular thiols. This raises the question of the specificity of Yap1 activation by Gpx3. To address this question, and to elucidate the role of Ybp1 in this mechanism, we have used an approach combining (i) the kinetic characterization of the two major reactions in competition: the peroxydasic cycle of Gpx3 and the signal transduction pathway; (ii) the study of the impact of Ybp1 on the evolution of the Gpx3-C_P-SOH intermediate by the two pathways in competition; and (iii) the characterization of the protein-protein interactions between the three partners by spectroscopic and biophysical methods. Our results show that Ybp1 and Yap1 associate and recruit Gpx3, and indicate that within the ternary complex, intramolecular disulfide formation within Gpx3 is slowed down, thus allowing the reaction with Yap1.

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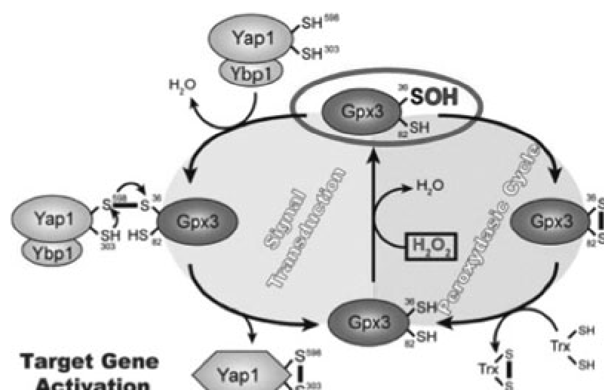

Fig. 1.

Keywords: redox signaling, sulfenic acid, Thiol peroxidase.

WED-062
Regio- and stereospecificity of arachidonic acid oxygenation catalyzed by Leu597 mutants of rabbit 15-lipoxygenase. A quantum mechanics/molecular mechanics study

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Abstract 15-Lipoxygenases catalyze the peroxidation reaction of arachidonic acid (AA) in mammals with a high regio- and stereospecificity. It is known that Leu597 controls the shape and size of the substrate-binding cavity in the corresponding rabbit enzyme (15-rLO), which is the only mammalian 15-LO enzyme with a resolved crystallographic structure. In this paper we have combined quantum mechanics/molecular mechanics calculations with molecular dynamics simulations to show that Leu597 also plays a very important role in the regiospecificity and the stereospecificity of the reaction. We have studied the addition of molecular oxygen to the pentadienyl radical of AA catalyzed by the Leu597Val and Leu597Ala mutants of 15-rLO. In the Leu597Val mutant the O_2 addition to C_{15} of AA is still predominant, although the mutation of Leu597 to a residue with a shorter side chain like Val creates some extra space where the O_2 molecule can be accommodated to form a pre-reactive minimum and to approach easily to C_{11}, so reducing almost ten times the C_{15}/C_{11} product ratio with respect to the wild type. The reaction keeps its S stereochemistry. Very interestingly, mutation to Ala, a residue even shorter than Val, causes just the opposite effects: the regiospecificity favoring addition to C_{15} is somewhat sharper than in the wild type, but the stereochemistry is now R. This is because the extra space created by the mutation to Ala is now big enough for AA to move, so adopting an alternative binding mode, changing the interaction with the different residues and opening new feasible paths for the attack of O_2. So, we have shown that the Leu597Ala mutant 15r-LO works as an aspirin-acetylated cyclooxygenase-2 making 15-(R)-hydroperoxyeicosatetraenoic acid.

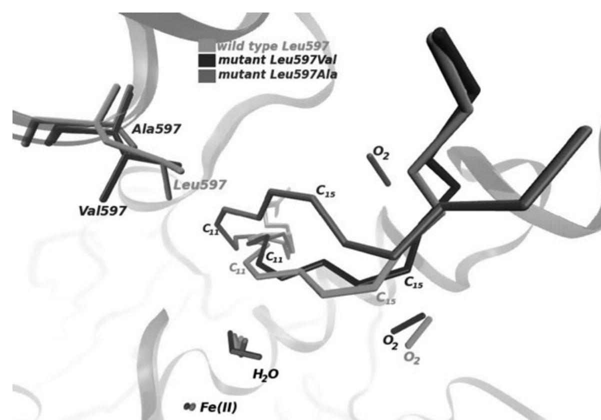

Fig. 1.

Keywords: Enzyme catalysis, Lipoxygenases, QM/MM calculations.

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WED-063
Regulatory action of bile pigments in terms of the native and modified RNA bases interaction with drug carrier protein

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Long period the main product of oxidation heme-containing proteins bile pigment bilirubin was considered to be only as ballast product of its metabolism and toxic agent. However, it was found that bilirubin is able to inhibit free radical reactions, but its regulatory function is still remains unknown. Regulatory function of bilirubin was limited only by a possible inhibition of sphingomyelinase and mediating during the expression activation of one of the cytochromes by ultrasound.

Recent investigations by Japanese researchers (Miyawaki et. al., Nature, 2013) revealed an unususal effect of bilirubin. When the pigment binds with novel expressed UnaG protein, the former is able to activate the latter light emission. Thereby, a new fluorescent protein from eel revolutionizes key clinical assay.

Previously [1], we have established the influence of bilirubin within protein matrix on interaction of meso-tetrakis-(p-sulfophenyl)porphin with albumin. Current investigations are devoted to establish regulatory action of bilirubin in terms of the native and modified RNA bases interaction with drug carrier protein. Several progresses in this field have already been reached [2]. Established, that 5-hydroxy-6-methyluracil more effectively binds with albumin and its complex with bilirubin compared to substituted uracil. Interesting effect is observed when replacing 5-I- to 5-Cl- and 5-F-uracils and thymine. Analysis of binding constant values indicates that practically in all cases bilirubin promotes binding of nucleic acid bases with protein.

Current investigations will help for understanding the pathogenesis of bile pigments and development of new therapeutic treatments for a number of common hyperbilirubinemia diseases.

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Keywords: Bilirubin, Proteins, RNA bases.

WED-064
Revealing of novel affine interactions of NBD-labeled fluorescent steroids with bacterial steroid-converting oxidoreductases using molecular docking

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Previously we have shown that known 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD)-labeled fluorescent 3β-hydroxy-5-en-steroids, trivially mentioned as 22-NBD-cholesterol and 25-NBD-cholesterol, can be converted into their 3-oxo-4-en-derivatives (22-NBD-one & 25-NBD-one, respectively) by bacterial cholesterol oxidase & cholesterol dehydrogenase [1–3]. To predict further metabolism of these compounds by some bacteria, series of rigid docking simulations have been performed using Autodock 4.2 & MGLTools 1.5.6 packages as well as 3D-structures of 3-ketosteroid-1-dehydrogenase (ksd1) from *Rhodococcus erythropolis* (PDB code: 4c3y) & cytochrome P450 CYP125 from *Mycobacterium tuberculosis* (PDB code: 2x5w). It has been shown that 22-NBD-one & 25-NBD-one can be bound affinely into the active site of the ksd1 in the substrate-like manners. The calculated binding energies values (BEs) for the interactions of 22-NBD-one, 25-NBD-one & androst-4-en-3,17-dione (a natural substrate) with the ksd1 have been estimated to be –13.9, –12.9 and –9.7 kcal/mol, respectively. Analogously, BEs for binding of 22-NBD-one, 25-NBD-one & cholest-4-en-3-one into the active site of the CYP125 have been estimated to be –14.7, –15.4 and –12.6 kcal/mol, respectively, however the obtained conformations do not allow to predict definitely if 22-NBD-one & 25-NBD-one can be hydroxylated by the enzyme. Thus, an alternative probable NBD-labeled steroidal substrate for CYP125 has been designed. 3β- as well as 3α-isomer of 3-((NBD)-amino)-cholestane have been computed to be able to bound affinely in the active site of CYP125 (BEs ~ –15 kcal/mol) in the manners, allowing C26 hydroxylation of the both isomers. These novel compounds have been synthesized from cholest-4-en-3-one (15% yield), the isomers have been separated chromatographically & then characterized using fluorimetry ($\lambda_{ex,max}$ 460 nm; $\lambda_{em,max}$ 540 nm) & mass-spectrometry ($[M\text{-}H]^-$ with m/z 549). Analogously, two isomers of 20-((NBD)-amino-pregn-5-en-3β-ol (20NBDP) have been synthesized, characterized ($[M\text{-}H]^-$ with m/z 479; $[M + H]^+$ with m/z 481) & evaluated to be new substrates for cholesterol oxidase (PDB code: 1coy). These findings reveal new perspectives for experimental studies of 22-NBD-one, 25-NBD-one, 20NBDP & 3-((NBD)-amino)-cholestane as novel fluorescent substrates for ksd1, CYP125 as well as related bacterial enzymes for biochemical/biophysical investigations and development of new antimicrobial drugs.

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Keywords: bacterial oxidoreductases; docking & synthesis; fluorescent steroids.

WED-065
Revisiting demand rules for gene regulation

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How do cells chose a particular mode of regulation over the other? Given different topologies to achieve the same task in the

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cell, what determines the choice of one topology over the other. Savageau and coworkers have proposed a set of demand rules that govern this decision – a gene whose product is required by the cell most of the time tends to be regulated positively. The rationale for this was provided by Alon and colleagues in terms of minimization of error in expression. Here, we revisit the question and analyze all transcription factor-target gene interactions in *E. coli*.

Our analysis from *E. coli* and simulation of network topologies suggests that there is a large deviation from the demand rule regarding distribution of regulatory arrangements in *E. coli*. Analysis of carbohydate metaboism genes and amino acid biosynthesis genes in the organism also dictate significant deviations from the demand rules. Combining our analysis and modeling, we propose additional parameters which might plan an important role in choice of regulatory arrangement between a transcription factor and target gene.

Keywords: None.

WED-066
RING MD: gathering time into structures

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Several methods have been developed and tested for molecular dynamics (MD) simulations output analysis. Most of them focus only on Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF). The aim of this work is to provide the scientific community a tool that directly analyses a MD simulation trajectory file, extending and simplifying this step. The tool provides per-residue calculation of H-bonds, salt bridges, disulphide bridges, π-π stacks, cation-π interactions, van der Waals contacts and a comprehensive interaction calculation of the frequencies of occurrence of these interactions. The tool is an extension of the already existing RING (1), which represents proteins and protein interactions as networks, including time as a new dimension. Once the trajectory file is cleaned from all non-protein molecules, the protein is centred on its centre of mass. Subsequently, a network is generated for every frame of the trajectory, and a frequency analysis of a minimal number of representative frames is carried out. The frame number is statistically determined in order to avoid information loosing. Finally, the user is provided of a PDB structure with residues coloured proportionally to the frequency of occurrence (0, no frame-1, all frames) of every type of RING computed interaction, and of a contact map highlighting the most important interactions responsible for protein structural maintenance and function. The submitted frames are then divided into clusters depending on network and structural changes, and a representative structure per cluster will be provided to allow structural analysis of the target. The novel capability of this tool is to depict in a structure the entire behaviour of a MD simulation. RING MD also provides RMSD and RMSF calculations. To validate the tool, three classical MD simulation targets were selected and analysed by means of classical MD analysis, and then compared to RING MD analysis. The targets were Ubiquitin (2), Glutaredoxin (3) and Lysozime (4), which were processed with 50 ns of classical MD simulation each. RING MD provides results that are coherent with classical analysis results.

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GM is an AIRC fellowKeywords
Keywords: Molecular dynamics simulation.

WED-067
RNA-Puzzles Round II: assessment of RNA structure prediction of two large riboswitches

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RNA-Puzzles is a CASP-like collective blind experiment for the evaluation of RNA 3-dimensional structure prediction. The primary aims of *RNA-Puzzles* are to determine the capabilities and limitations of current methods of 3D RNA structure prediction based on sequence, to find whether and how progress has been made, and to illustrate whether there are specific bottlenecks that hold back the field. Ten puzzles have been set up and three assessments are published. Nine groups of modelers around the world participate in this collective effort. We now report a second round focusing on the prediction of two large riboswitches, the adenosylcobalamin and the T-box bound to a tRNA. No homologous structures existed in the databases at the time of the experiment. Although only two targets were selected, these targets provide a wealth of sub-domains (around 10), including both well-known modules like K-turns as well as new ones.

The 168nt adenosylcobalamin riboswitch consists of a ligand-bound structured core and a bent peripheral domain. Although the RMSDs of the prediction models range from 11.7 to 37.5 Å, the topology of the top ranked models are quite similar to the native structure. Top ranked models show much better scores in Deformation Index (DI) and non-Watson-Crick interaction network fidelity (nwc INF) than others, but surprisingly have worse clash scores.

The T-box and tRNA, 96 and 75nt in length respectively, form a large complex. The difficulty in prediction lies mainly in (i) the lack of homologous model for T-box and (ii) the interaction between T-box and tRNA. The RMSD range of the predictions is 6.8–17.4 Å and the top ranked models also have better DI score with worse clash scores.

The Das group performed best in both problems with their models ranked #1 at 14.5 and 7.6 Å, respectively. The Bujnicki group performed well in the second problem with the model

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ranked #1 at 10.2 Å and excellent clash scores with nwc INF around 0.5 like the models of the Das group. Further, the less well predicted models always had worse nwc INF score, demonstrating the importance of identifying non-Watson-Crick pairs and RNA modules.

Keywords: 3D structure prediciton, Bioinformatics, RNA structure prediction.

WED-068
Role of caveolin 1 in the sorting of sphingomyelin

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Sorting of lipids and proteins is a key process allowing eukaryotic cells to execute efficient and accurate intracellular transport and to maintain membrane homeostasis (Van Meer et al. 2008). In contrast to proteins, lipid sorting is still poorly understood.

Our group had previously proposed that if a protein has a high affinity for both curved membranes (such as small vesicles or tubules) and for specific lipids, it can lead to enrichment of these lipids in transport intermediates although they could be normally depleted for physical reasons in the absence of the protein (Safouane et al. 2010). Since caveolin 1 (CAV1) and sphingomyelin have similar trafficking pathways between the Golgi apparatus and the plasma membrane and since CAV1 has a high affinity for sphingomyelin (Haberkant et al. 2008), we hypothesize that sphingomyelin transport can be mediated by their interaction with CAV1.

To test this hypothesis, we use two complementary approaches.

First, we use a powerful in vitro system, the "giant unilamellar vesicles" (GUVs) which has been shown well suited to analyze the effect of a single protein type on the organization of lipid membranes (Sens et al. 2008). As a first step, we have developed an improved purification protocol of CAV1 in E Coli system (Coll.: Marjolaine Noirclerc-Savoye and Michel Thépaut, IBS, Grenoble) and we have reconstituted CAV1 in GUVs for the first time. This will allow us to study caveolin-mediated sphingomyelin sorting in curved structures by pulling membrane nanotubes of controlled diameter from these GUVs.

Secondly, in vivo studies are performed with mouse lung endothelial cells (MLEC) that express (WT) or not CAV1 (KO). We follow by confocal microscopy the distribution of BODIPY-ceramide, a fluorescent analogue of sphingomyelin precursor in order to study the intracellular trafficking of sphingomyelin as a function of the expression of CAV1. In a complementary manner, we quantify the level of sphingomyelin using mass spectrometry (Coll. Justine Bertrand-Michel, MetaToul-Lipodomique, Toulouse) in MLEC WT and KO.

Eventually, this study will contribute to elucidate mechanisms that govern the traffic of lipids in cells.

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Keywords: Caveolin1 reconstitution, Membrane curvature, Sphingomyelin sorting and trafficking.

WED-069
Scanning the role of conserved amino acid in ferritin nanocage

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Ferritins are iron storage proteins composed of 24 subunits, each of one folded in a 4-helical bundle, that, in coming together, form an almost spherical protein shell with 4,3,2 point symmetry (Image). The main feature of the cage is a large cavity, designed to accommodate up to 4500 Fe atoms as iron(III)-oxobiomineral, which communicates with the bulk solvent via six hydrophilic channels at the C3 axes. Other channels lie along the C4 axes, but they are tight and hydrophobic and their function was unknown.

Each catalytically active subunit hosts a ferroxidase site where Fe(II) is oxidized to Fe(III) and then migrates to the biomineralization cavity. The biological significance of that process is related to the very high iron binding capacity of ferritin that concentrates iron in a compact and safe form which can be made readily available when needed. Moreover, the reaction sequesters Fe(II) from Fenton-like reactions in which the spontaneous oxidation to Fe(III) donates single electrons to generate highly toxic radicals.

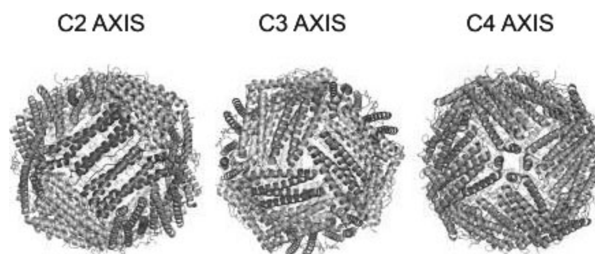

Fig. 1.

Here, we study $2Fe^{2+} + O_2$ protein catalysis and dissolution of ferritin biominerals in variants with altered subunit interfaces at C3 axes (E130I), C2 axes (E88A and D80K), and C4 axes (L165I and H169F). The results extend observations on the functional importance of C3 axes in iron uptake and unravel a functional role of C4 axes. Conserved amino acids localized at C4 axes facilitate dissolution of ferritin-protein-caged iron biominerals. Moreover multiple functions for amino acid along the C2 axis have been delineated; E88A substitution modulates ferritin catalytic activity in agreement with previous cross-linking studies, while a new role for cage self-assembly of some conserved amino acids forming salt bridges at the center of the C2 axis is identified.

Keywords: ferritin, function, mutagenesis.

WED-070
Secondary DNA structures in G/C-rich microsatellites. Investigating the equilibrium between G-quadruplexes, I-motifs and duplexes

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The focus of this work is conformational dynamics in G/C-rich sequences, i.e. the equilibrium between B-DNA and noncanonical

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structures. Recent findings [Dhakal et al. Biophys J. 2012; 102 (11):2575] suggest that in sequences containing four G/C-runs coexistence of G-quadruplexes (GQs) and I-motifs and in unlikely, presumably due to steric hindrance, and GQs or I-motifs are instead opposed by single-stranded loops. We assumed that in the case of microsatellites and other sequences with multiple (>4) G/C-runs, simultaneous formation of GQs and I-motifs might be possible provided that they are mutually shifted, which excludes steric hindrance (Figure 1). To verify our hypothesis and to study the impact of pH and salt concentration on conformational rearrangements in such G/C-rich sequences, we studied a series of DNA fragments from the human genome (chr 18, +66294092 to +66294035): the GQ-forming strand with 6 G-runs $(G_3AT)_5G_3$, its I-motif-forming complement and their truncated analogs containing only 4 G/C runs. We also synthesized elongated oligonucleotides with non-G/C rich flanks to model secondary structures in duplex media and their non-GQ/non-I-motif mutants.

The formation of individual GQs and I-motifs was demonstrated by physicochemical methods (UV-melting and circular dichroism spectroscopy). Noncanonical structures in duplex media were additionally studied by FRET and by monitoring fluorescence intensity of EtBr intercalated in duplex. Fluorescence of EtBr intercalated in GQs/I-motifs or ss-DNA (loops) is insignificant. Thus, decreased fluorescence in the microsatellites concerned in comparison with control B-DNA under GQ/I-motif-favoring conditions may be regarded as an evidence for the existence of non-B secondary structures (pH and salt-dependence of EtBr fluorescence intensity was taken into account).

Our preliminary results confirm the formation of non-B-DNA (presumably I-motif or mutually shifted I-motif and GQ) in native microsatellites. The equilibrium between duplex, I-motif and GQ is highly salt-dependent and may be shifted by the addition of various ligands. Assessment of the duplex:I-motif:GQ: loop ratios under different conditions and visualization of the ‘shifted’ structures by atomic force microscopy is currently underway.

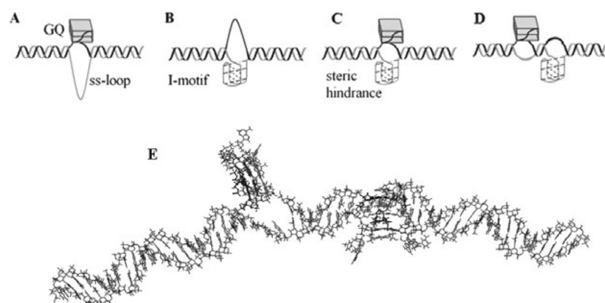

Fig. 1. GQs and I-motifs in duplex media. Possible variants of spatial organization.

Acknowledgements: This work was supported by RSF [14-25-00013].

A, B – Sequences containing four G-runs/ four C-runs. GQs or I-motifs are opposed by single-stranded loops.

C – simultaneous existence of GQ and I-motif opposite each other – the unlikely variant.

D, E – mutally shifted GQ and I-motif (sequences contain >4 G/C-runs).

Keywords: G-quadruplex, I-motif, Secondary DNA structures.

WED-071
Sensitisation in the IFN-α/β,IFN-γ crosstalk reveals mechanisms for enhanced information processing capacity of the STAT1,STAT2 signalling pathway

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Signaling pathways are the main cellular mechanism to transmit information about extracellular cues to the decision-making machinery of posttranslational protein modifications and gene expression processes. In the mammalian immune system processing information about extracellular cytokine concentration is of crucial importance for the tight control of the immune response and efficiency of the defence mechanisms. Malfunction of the signal transduction is tightly related to a variety of disorders ranging from impaired immunity to autoimmune diseases and cancer. Among the most intensely studied pathway is the JAK – STAT mechanism that controls cellular response to the plethora of cytokines, particularly to Interferons α/β and γ. Although interferons have been characterised as having antiproliferatory and apoptotic effect their mechanism of action is significantly more subtle and is a subject of intense investigations. In our work we have theoretically and experimentally examined the crosstalk between IFN-α/β and IFN-γ. The experimental measurements of the STAT1 and STAT2 activation upon stimulation with various combinatorial protocols provided novel insight about the sensitising role of IFN- α/β for subsequent IFN- γ detection. To clarify experimental data we constructed a mathematical model that is capable to explain the sensitisation mechanism, and together with the employed information theoretic methodology explains that prestimulation can enhance information processing capacity of the Jak1/Stat1. Our results provide a detailed mechanistic insight and general design principle that enables information processing in the immune system to be a dynamic process. We explain how adaptation leads to selectivity of information transmission, which is known to be a crucial mechanism for tight control of the immune response.

Keywords: Interferon, Jak/Stat, Stimulation.

WED-073
Stem cells: novel tool for treatment of human rare disoders. Computational modeling of adult stem cells

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Stem cells (SCs) could be considered as relatively new living cells with perspective characteristics and properties. They are a good tool for transplantation, in vitro and in vivo manipulations too. In cancer (leukemia) SCs have been used for surviving of the human organism as well. The aim of the work presented could be formulated as follows: to give some important points omn experiments and theory of stem cells. Also to creat a new computational model based on classical mathematics and mechanics theories for adult stem cells at different environments conditions. Author’ s FORTRAN programs have been designed and used for numerical experiments.

Keywords: None.

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WED-074
Striatin/PP2A complex modulates microtubules dynamics via regulation of MAP2 phosphorylation

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Microtubules (MTs) are components of the eukaryotic cytoskeleton. These tubular polymers of tubulin are responsible for a wide variety of cellular processes including maintenance of the cell shape, motility and intracellular transport. Microtubules are also involved in the formation of mitotic spindle taking a part in a right segregation of chromosomes during the cell division. MTs are highly dynamic structures and their polymerization/depolymerization rate depends on a family of proteins called microtubule-association proteins (MAPs). Phosphorylation of MAPs is the major mechanism of their regulation. The PP2A protein is an enzyme responsible for dephosphorylation of MAP2, the process by which the MAP2-MTs binding is enhanced and, as the result, the MTs are stabilized (Gong Ch-X et al., 2000). PP2A is a serine/threonine phosphatase, composed of the catalytic (C), structural (A) and regulatory (B) subunits. Striatin (STRN), an ubiquitous protein expressed mainly in the central and peripheral nervous system, is one of the regulatory subunits of PP2A (Moreno et al., 2000). The exceptional feature of striatin is the presence of four protein-protein interaction domains. As a consequence, the modular structure of striatin is a molecular scaffold that organizes the large signaling complex (Hwang J. and Pallas D., 2013).

In the course of our studies on the function of striatin in a cell, using immunofluorescent imaging, we have shown that striatin and MTs co-localize in HEK293T cells. In the co-immunoprecipitation experiments we confirmed literature information on the formation of PP2A-STRN complex in HEK293T. Then, we asked a question whether the PP2A-striatin complex is involved in regulation of phosphorylation of MAP2, and thus controls the MTs stability. Co-immunoprecipitation experiments indicated that endogenous MAP2 and STRN form a complex in HEK293T. Subsequently, we found that silencing of striatin, via RNA interference, results in hiperphosphorylation of MAP2. In addition, the decrease in STRN expression caused a modest destabilization of MTs in HEK293T. Taking together all the results we hypothesize that the PP2A/striatin complex modulates the microtubules dynamics via regulation of MAP2 phosphorylation.

The project was financed by Young Researchers Programme for PhD Students of CMMS PAS in Lodz, Poland.
Keywords: Striatin, microtubules, phosphorylation.

WED-075
Structural basis of the sperm-specific glyceraldehyde-3-phosphate dehydrogenase stability: view from Molecular dynamics simulations

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D-Glyceraldehyde-3-phosphate dehydrodenase is an enzyme of glycolysis catalyzed oxidative phosphorylation of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate with reduction of NAD+ to NADH. GAPD is a multifunctional protein it can be directly involved in development of neurodegenerative diseases, apoptosis and many other cellular processes. In mammalian organisms somatic (GAPD) and sperm (GAPDS) enzymes are expressed that encoded by different genes. GAPD appears in all tissues of the organism and places in the cell cytoplasm whereas GAPDS is expressed only in sperms. However, in some cases of pathological disturbances of the cell's functioning, expression of GAPDS in somatic cells was detected.

Two type of the dehydrodenase are very homological but reviled some differences in their properties. In spite of numerous studies of functional and structural properties of GAPDs from different sources understanding of how the small differences in structure and dynamics of the GAPD homologous influence on their properties is still insufficient. In particular there is a lack of information about GAPDS. Here we study structural and dynamics aspects of GAPDS comparing to GAPD and their influence on stability of the tetramers based on MD simulations. Influence of individual amino acids on the stability and dynamics of tetramer is studied by the point mutation of several residues. We believe that detailed comparison of pair interaction energies in GAPD tetramers and elucidation of energetic impact of key residues onto the tetramer stability will provide new information for construction of mutagenic proteins with alternated properties and for development of new inhibitors with indirect influence on catalytic site.

Keywords: D-Glyceraldehyde-3-phosphate dehydrodenase, Molecular dynamics simulations.

WED-076
Switching points in the CYP74 (P450) catalysis

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Unlike most of the cytochromes P450 which are monooxygenases, enzymes of the CYP74 family do not require molecular oxygen nor any redox partners, but use their hydroperoxide substrates as sources for reducing equivalents and as oxygen donors. So their oxygen-binding domain is degenerate and substituted by I-helix central domain (IHCD) which participates in the CYP74 catalytic action. The CYP74 enzymes including allene oxide synthases (AOSs), hydroperoxide lyases (HPLs), divinyl ether synthases (DESs) and epoxy alcohol synthases (EASs) convert fatty acid hydroperoxides into structurally different products: allene oxides, divinyl ethers, hemiacetals and epoxy alcohols. Thus, AOSs and DESs function as dehydrases, whereas HPLs and EASs are isomerases.

Alignment of the CYP74s primary structures revealed several conservative domains; some of them fall into substrate-recognition sites which are typical for all P450s. Number of sites within these domains were chosen, and amino acids at those sites were substituted. The obtained data demonstrate the interconversions of the CYP74 enzymes as a result of site-directed mutagenesis. Three mutations led to complete conversions: AOS into HPL, DES into AOS and HPL into EAS. Some more mutations led to alterations of the CYP74s catalytic activities in different ratios: from partial to almost complete. Moreover, several mutants with dual and trial activities were obtained. On the other hand, no one case of the CYP74s regiospecificity change caused by site-directed mutagenesis was detected. Thus, the results of site-directed mutagenesis revealed primary determinants of the CYP74 catalysis.

The data demonstrate that the catalytic mechanisms of the CYP74 enzymes are closely related. The results of site-directed

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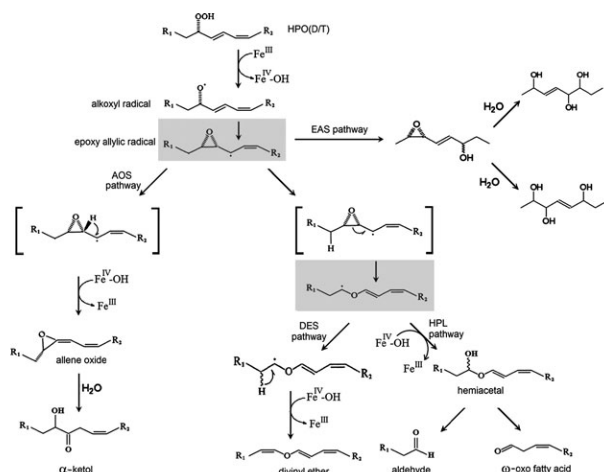

Fig. 1.

mutagenesis indicate that the epoxyallylic radical is the switching point of the CYP74 catalysis (Fig. 1). Depending on primary sequences of conservative domains, the epoxyallylic radical either undergoes the deprotonation to form the allene oxide (AOS pathway), or recombines with hydroxyl radical to form the epoxy alcohol (EAS pathway), or undergoes the rearrangement into the vinyl ether radical, which then is either recombined with hydroxyl radical to afford the hemiacetal (HPL pathway), or loses a hydrogen atom to afford divinyl ether (DES pathway).

This work is partly supported by Russian Foundation of Basic Research (13-04-40103-H, 14-04-01532-A, 12-04-97087-r, 12-04-97059-r), MK-4886.2013.4 and SS-1890.2014.4.

Keywords: Catalytic mechanisms, site-directed mutagenesis, The CYP74 enzymes.

WED-077
Synergistic effect of minerals and low-intensity electromagnetic field on fibroblasts proliferation

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Background: The beneficial actions of warm crystals and low-intensity electromagnetic field (EMF) on human body are well known. A resonance environment that includes at least one mineral / crystal will support the transition of electromagnetic radiation in a favorable frequency range for repairing immediately adjacent tissue. Crystals can transmit vibrational energies that have biological effects. Such a device would be beneficial for facial skin (counteracting effect on wrinkles), creating stability for dental implants and generally by stimulating the regenerative capacity.

The aim of this study was to investigate the influence on cell proliferation of different mineral compositions (aragonite, topaz) placed at the interface between the EMF generating device and outside the culture dish. The beneficial effect is more remarkable as there is no direct contact with the target cells.

Method and results: The cellular model is based on human dermal fibroblasts Hs27; they are the predominant cells to synthesize extracellular matrix, with special importance both in the medical and cosmetic fields.

The electronic apparatus used is based on a device for generating a sinusoidal frequency with precision and stability.

In the action area of EMF, above and below the plate culture were applied various types of mineral powders (topaz and aragonite). On the same culture plate a "control" area was preserved, kept only under the influence of the electromagnetic field. Also, another control culture plate was prepared in the same conditions, but unexposed either to EMF or minerals influences. Fibroblasts were exposed to EMF and minerals for 3 days, 2 h per day. After this, the cells viability was measured using MTS assay. In the case of cells exposed both to minerals and EMF the proliferation rate was higher (7–12%) and extreme statistically significant than in the case of cells exposed only to EMF (5–10%), compared to unexposed control.

Conclusions: Although the beneficial effects of EMF and crystals are well known, official recognition and full acceptance requires more solid experimental data. The proposed method is non-invasive and risk-free because it stimulates the physiological mechanism of healing, opening opportunities for further exploitation in regenerative medicine.

Acknowledgements: The study was supported by a grant of *UEFISCDI*, Romania, ...Innovation voucher" no. 208/2013

Keywords: EMF, fibroblasts, MTS assay.

WED-079
TDP-43 inclusion bodies formed in bacteria are structurally amorphous, non-amyloid and inherently toxic to neuroblastoma cells

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Accumulation of ubiquitin-positive, tau- and a-synuclein-negative intracellular inclusions of TDP-43 in the central nervous system represents the major hallmark correlated to amyotrophic lateral sclerosis and frontotemporal lobar degeneration with ubiquitin-positive inclusions. Such inclusions have variably been described as amorphous aggregates or more structured deposits having an amyloid structure. Following the observations that bacterial inclusion bodies generally consist of amyloid aggregates, we have overexpressed full-length TDP-43 and C-terminal TDP-43 in *E. coli*, purified the resulting full-length and C-terminal TDP-43 containing inclusion bodies (FL and Ct TDP-43 IBs) and subjected them to biophysical analyses to assess their structure/morphology. We show that both FL and Ct TDP-43 aggregates contained in the bacterial IBs do not bind amyloid dyes such as thioflavin T and Congo red, possess a disordered secondary structure, as inferred using circular dichroism and infrared spectroscopies, and are susceptible to proteinase K digestion, thus possessing none of the hallmarks for amyloid. Moreover, atomic force microscopy revealed an irregular structure for both types of TDP-43 IBs and confirmed the absence of amyloid-like species after proteinase K treatment. Cell biology experiments showed that FL TDP-43 IBs were able to impair the viability of cultured neuroblastoma cells when added to their extracellular medium and, more markedly, when transfected into their cytosol, where they are at least in part ubiquitinated and phosphorylated. These data reveal an inherently high propensity of TDP-43 to form amorphous aggregates, which possess, however, an inherently high ability to cause cell dysfunction. This indicates that a gain of toxic function caused by TDP-43 deposits is effective in TDP-43 pathologies, in addition to possible loss of function mechanisms originating from the cellular mistrafficking of the protein.

Keywords: None.

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WED-080
The effect of low frequency electromagnetic field on apoptotic proccesses in rat thymocytes under peroxide induced damage

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Environmental exposure to electromagnetic fields of low frequency (EMF LF) has been steadily increasing, and in the modern world everyone is exposed to a complex mix of weak electric and magnetic fields. EMF LF are known to modulate cells functioning both in physiological and pathological conditions, we sought therefore to clarify the possibility of EMF LF to induce and modulate cell death. It was shown that the influence of EMF LF led to the increased number of apoptic cells. To elucidate the mechanisms of this effect we identified the level of the intracellular calcium concentration $[Ca^{2+}]_i$ in rat thymocytes. It is known that calcium (Ca^{2+}) is a secondary messenger and universal signaling ion that regulates diverse cellular functions and is one of the key elements of the apoptotic signaling pathways. To assess changes of intracellular calcium concentration $[Ca^{2+}]_i$ the dual-wavelength method for detecting changes of fluorescent Indo-1 was used. At early and late stages of apoptosis it was observed from the kinetic curves that the influence of low frequency magnetic fields on the rat thymocytes suspension caused the considerable increase of intracellular calcium concentration both in the presence of H_2O_2, that enhanced the effect, and in solution without H_2O_2. It should be noted that this effect caused by EMF LF could be observed with slight deviation in different pH, and the process of the intracellular calcium concentration augmentation occured with different dynamics. Thus, these data indicate that EMF LF causes an increase in intracellular calcium concentration, both independently and in combination with H_2O_2 (where the effect is greatly enhanced), which in turn can lead to activation of the important mediators of apoptotic processes such as CaMKII and JNK, and thus promotes apoptosis run. At the same time, using the reliable and sensitive method of comet assay the nucleuses DNA damages were studied in the suspension of isolated rat thymocytes after three hour incubation with hydrogen peroxide and exposure to electromagnetic fields, considering both individual and combined effects. We have shown that significant augmentation of DNA damages was observed under the action of hydrogen peroxide, and the effect was enhanced by the EMF LF.

Keywords: Apoptosis, cell damage, electromagnetic fields of low frequency.

WED-081
The effect of *Montivipera raddei* viper venom on human red cell membrane ATPase activites

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We have studied the influence of *Montivipera raddei* venom (MR) (Latoxan, France) on the activity of ATPases. We also have studied influence of MR venom on the erythrocyte ghosts by the fluorescent microscopy. The lyophilized toxin of MR was dissolved in Tris-HCl buffer (pH 7.4); final concentration $3*10^{-5}$ M, 1.1 ml of this solution was added to the sample during ghost visualization. We have shown that in presence MR venom erythrocyte ghosts were shrinking during 58 s.

The addition of MR venom into assay mixture with low, sublethal (0.35 mg/kg approx. 0.5 LD 50 for rat) and lethal concentrations in accordance with LD50, increased Na^+/K^+ ATPase activity in erythrocytes membranes (low concentaraton ~5.28 times, sub –lethal concentration ~ 4.5 times and lethal concentrations ~2.93 times respectively). Under these conditions Ca^{2+} ATPase activity increased at low concentaration ~1.59 times, then decreased at sub –lethal concentration ~2.87 and lethal concentrations ~4.41 times respectively. Mg^{2+} ATPase activity also increased (low concentaration ~12.1 times, sub –lethal concentration ~ 5.98 times and lethal concentrations ~3.05 times respectively).

These results suggest that ATPase activity is very sensitive to venom components and venom influence leads to possible conformation changes in ATPases.

Acknowledgments: Research was performed in the frames of ANSEF project # chemen- 3575.
Keywords: Montivipera raddei, erythrocyte membrane, Na+ K+ ATPase, Ca2+ Mg2+ ATPase.

WED-082
The effect of soloxolone methyl on gene expression profile and its biological activity *in vitro*

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In attempt to find more potent analog of glycyrrhetinic acid we synthesized soloxolone methyl (SM) (2-cyano-3,12-dioxo-11-deoxo-18βH-glycyrrhet-1(2),9(11)-dienoate) [doi: 10.1002/cbic.201000618].

To find the molecular targets of SM we investigated its effect on gene expression profile of KB-3-1 human carcinoma cells using HumanHT-12 v4 Expression BeadChip (Illumina, USA). Incubation of KB-3-1 cells with 1 μM SM significantly alters the expression of 311 genes (Log_2(Fold Change)>1, $p < 0.001$). Gene ontology and promoter analysis was performed using the geneXplain platform. Data show that SM inhibits cell growth and promotes death of cancer cells through the modulation of multiple signaling pathways. Expression of many genes related to apoptosis and cell death, cell cycle and cell proliferation, angiogenesis, metastasis and differentiation is significantly altered upon SM treatment. Master regulator search in TRANSPATH database (BIOBASE GmbH, Germany) let us to identify potential early molecular targets of SM, which are bst2, CEPT1, CRMP2, cxcr4, DDB1, elongin C, FGF-BP, hBre1, insig-1, LAMA5, LT-betaR, Mi2-Beta, MO25, p18INK4c, p22phox, p70S6K2, pkmyt1, SRXN1, SVIL, TMP21, TfR1, UBE2Q2.

Evaluation of biological action of Soloxolone methyl (SM) *in vitro* showed that this compound induces the death of cancer cells by triggering of mitochondrial pathway of apoptosis that confirms obtained bioinformatic data.

We found that SM possesses anti-influenza A activity, causing 10-fold decrease of virus titer relative to control in infected MDCK cells. Time-of-addition studies demonstrated that SM is likely to affect virus penetration into the cell and the early stages of virus replication. It was also shown that SM exhibits the anti-inflammatory activity reducing the IL-6 level upon viral infection.

Additionally, the anti-inflammatory potential of SM was demonstrated in the murine peritoneal macrophages model: SM caused significant reduction of NO production in LPS-activated macrophages.

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Our results show that SM is multifunctional compound with pronounced anticancer, anti-influenza and anti-inflammatory activities.

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Keywords: biological activity, gene profiling, glycyrrhetinic acid derivatives.

WED-083
The effect of substitutions at several sites on the functioning of two CYP74 enzymes (P450 superfamily)

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Oxylipins (fatty acid hydroperoxides, hydroxy-, oxo-, or keto-fatty acids, divinyl ethers, volatile aldehydes, or jasmonic acid) play important roles in plant cell signalling and defense. The key role in biosynthesis of oxylipins belongs to the CYP74 enzymes that are nonclassical cytochromes P450: allene oxide synthases (AOS), hydroperoxide lyases (HPL), divinyl ether synthases (DES). DESs are less characterized than AOSs and HPLs. Earlier we cloned and characterized new member of the CYP74 family from flax (*Linum usitatissimum* L.) – divinyl ether synthase LuDES (GenBank HQ286277.1). It possessed high homology with sequences of the CYP74B enzymes – 13-HPLs. We obtained mutant forms of LuDES and HPL of *Medicago truncatula* (MtHPL) for identifying determinants of the CYP74 catalysis. We substitute two amino acids in the I-helix central domain (IHCD) corresponding to the oxygen-binding domain of classical P450 monooxygenases by site-directed mutagenesis and obtained mutant forms LuDES E292G, A287G and MtHPL G288E. The mutant form LuDES E292G possessed the catalytic activity of 13-AOS, while the DES activity was not detectable. The substitution A287G in LuDES sequence resulted in regiospecificity loss. This mutant produced several isomers of etherolenic acid. The mutant form MtHPL G288E catalyzed conversion of substrate into corresponding epoxy alcohol.This work is partly supported by Russian Foundation of Basic Research (13-04-40103-H, 14-04-01532-a, 12-04-97087-r, 12-04-97059-r), MK-4886.2013.4 and SS-1890.2014.4.
Keywords: divinyl ether synthase, hydroperoxide lyase, site-directed mutagenesis.

WED-084
The molecular dynamics simulation and experimental evaluation of carbon nanotubes – Congo red drug delivery system into cancer cells

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Most of the present anti-cancer drugs have low specificity and devastating side effects. Thus drug delivery systems that combine targeted delivery and controlled release without damaging the healthy tissue are widely investigated. The carrier is expected to have high drug loading capacity and drug release, low toxicity and ensure the effective transport of the carried drugs. The model drug used in this study is doxorubicin (DOX). Single walled carbon nanotubes (SWNTs) are studied as potential transporters of drugs absorbed to their surface. The supramolecular ribbon-like assemblies created by the dyes of the Congo red (CR) type can incorporate by intercalation certain drugs (especially polycyclic, planar molecules like DOX) and thus can also be considered as potential drug carriers. The combination of both systems appears to be promising solution.

The aim of the study is to determine whether it is possible to use CR-functionalized carbon nanotubes for drug delivery into cells and whether CR improves drug-loading properties of SWNTs. Parallel analysis of this drug transporting system was carried out by the molecular dynamics simulation.

Microscopic analysis (AFM, TEM) of the SWNTs-CR complexes showed the increase in the diameter of the SWNTs and change the mechanical properties, suggesting that the CR binds the SWNTs in its supramolecular form. The quantitative analysis of the amount of CR complexed to SWNTs and the release of DOX from these complexes in different pH conditions was studied. The highest rate of DOX release was observed in acidic solutions. Studies concerning the cytotoxicity of the system were conducted using the human glioblastoma U87MG and human fibroblast Hs27 cell lines. The addition of the SWNTs-CR-DOX led to the inhibition of cell proliferation, while cells treated with carriers free of DOX (CR, SWNTs-CR) showed the proliferation at the control level.

The molecular dynamic simulation confirmed the ribbon-like structure of CR micelle and interaction of CR with SWNTs. Using the method of umbrella sampling and weighted histogram analysis of the free energy profiles established that the SWNTs-CR systems are thermodynamically stable.

The results indicate that this type of carrier can potentially improve the pharmacological efficacy and reduce side effects.

Acknowledgements: Anna Jagusiak acknowledges the financial support from the project Interdisciplinary PhD Studies "Molecular sciences for medicine" (co-financed by the European Social Fund within the Human Capital Operational Programme)
Keywords: CR – Congo red, DOX – doxorubicin, SWNTs – single-walled carbon nanotubes.

WED-085
The mRNA exporter GLE1 is essential for embryonic development

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GLE1 was first identified in yeast as a mediator of mRNA export. Since then, several mutations have been identified in *GLE1* across several Finnish families that result in lethal congenital contracture syndrome type 1 (LCCS), a multisystem foetal disorder characterised by prominent motor neuron degeneration in the spinal cord. To determine whether *Gle1* is essential for embryonic development, its role in motor neuron development and the expression profile of *Gle1* during key developmental stages, mice were generated with a targeted disruption of the *Gle1* gene using gene trap technology. The gene trap *consists of a* β*-geo* reporter inserted after the third exon that generates an N-terminal β-galactosidase fusion product that is detectable using X-gal staining. *Gle1* hemizygous mice are phenotypically indistinguishable from their wild-type littermates from early development through to adulthood. We were unable to detect mice homozy-

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gous for the disrupted *Gle1* allele from E8, and our examination of E5.5 embryos and E 3.5 blastocyst cultures suggest that embryonic lethality occurs between E3.5 and E6. X-Gal staining of mouse embryos hemizygous for the disrupted allele showed regionally restricted expression in the brain and other organs. Notably, X-Gal staining was prominent in developing joint cartilages, consistent with the multiple joint contractures seen in LCCS1. X-Gal expression was low or absent in the spinal cord, though prominent in the dorsal root ganglia, suggesting that its role in motor neuron survival may be non-cell autonomous.

Keywords: ANIMAL MODEL, development, Gle1.

WED-086

The quest for the dynamic fingerprint of photo-switching in GFPs: a comparative molecular dynamics study

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Green Fluorescent Proteins (GFPs) are well know for their valuable properties for high-resolution microscopy. In most cases the GFPs used for cell labeling are not the wild type proteins, but mutants which enable faster or slower photo-switching (ps) and thus higher resolution and conditions which are more suitable to follow the process under the investigation.

To understand how the photo-switching is “turned on” we should also understand how it is “turned off”[1]. Rational design of a non-photoswithcing (non-ps) mutant of Dronpa would provide us with new insight on the photo-switching mechanism. Following previous studies on various GFPs it can be concluded that the flexibility of this protein plays an important role into its' behaviour [2]. A detailed analysis of the flexibility of a set of similar proteins with different properties (ps and non-ps) could reveal the nature of the difference.

To analyze how the flexibility of the GFP beta-barrel influences the photo-switching properties we have conducted an MD study for a set of 6 proteins: EosFp – IrisFP (97%), mAG – Dronpa (73%), EGFP – rsEGFP (96%). Those couples of proteins differ by a minimum set of mutations (sequence similarity is indicated in the brackets), however one of them is photo-switching and the other is not. Primary backbone flexibility analysis was based on a RMSF vs. residue comparison. Partial-least square (PLS) analysis was performed to detect the differences in the ensemble of the non-ps and ps GFPs.

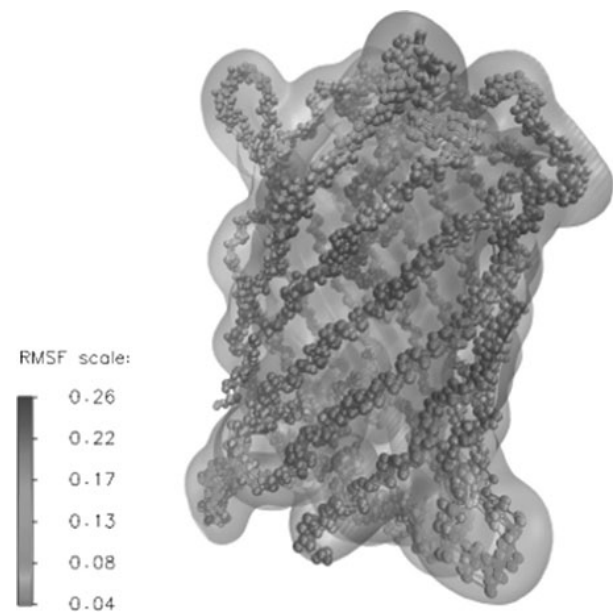

Fig. 1.

The analysis has clearly indicated that the average flexibility of the proteins does not determine the photo-switching properties, while it is the local flexibility which distinguishes the psGFP and non-psGFP (see the picture, which is the superposition of 4x2 proteins. Regions with the lowest RMSF are indicated in red.). The results also indicate the regions as well as the single aminoacids responsible for the photo-switching properties. To test the conclusions mutations were made to psGFP Dronpa which would render it non-ps. The obtained results have confirmed the protocol and can provide insight into the photo-switching mechanism of Dronpa.

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Keywords: fluorescent proteins, Molecular dynamics simulation, protein design.

WED-087

The role of dynamic β-hairpin structures in the catalytic loop of *Mycobacterium tuberculosis* tyrosyl-tRNA synthetase

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Tyrosyl-tRNA synthetase from *M. tuberculosis* (*Mt*TyrRS) is an enzyme that belongs to Class I of aminoacyl-tRNA synthetases, that catalyze the attachment of tyrosine to its cognate $tRNA^{Tyr}$ at the preribosomal protein synthesis step. *Mt*TyrRS is incapable of cross-recognition and aminoacylation of human cytoplasmic $tRNA^{Tyr}$, so this enzyme is a promising target for development of novel selective inhibitors as new antituberculosis drugs. *Mt*TyrRS has the HIGH-like and KFGKS motifs that catalyze the amino acid activation with ATP. We have investigated the dynamic properties of *Mt*TyrRS catalytic loop (-KFGKS-) using molecular dynamics (MD) simulations in solution within a long time interval of 100 ns. In our previous studies we have shown that the catalytic loop can form two dynamic β-strands in its flanking regions. In this study special attention has been given to the role of the β-hairpins in structural dynamics of the catalytic loop.

The crystalline *Mt*TyrRS dimer structure was used as an initial structure (PDB code 2JAN). All-atom MD simulations were performed using the GROMACS 4.5 package with the Amber ff99SB-ILDN force field. The *Mt*TyrRS in solution was simulated for 100 ns at 310 K temperature and 150 mM NaCl salt concentration. To study the influence of the β-sheets on the catalytic loop dynamics and structural-functional state we designed a variant of the *Mt*TyrRS loop with proline substitutions (proline residues effectively break the β-sheets) at sites involved in β-hairpin formation. All MD simulations were calculated using the MolDynGrid virtual laboratory services (http://moldyngrid.org).

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For the wild type TyrRS the catalytic loop has M-like structure, the central part of which points to the active center of the enzyme. We hypothesize that flanking β-hairpins in the catalytic loop keep it in the closed functional M-like state, which directs the catalytic KFGKS motif toward the active center cavity. To verify this, we made a mutant *Mt*TyrRS by substituting residues that are involved in β-sheet formation with proline: T225P, A226P, T230P, K231P, K234P, S235P, and S240P. Remarkably, the catalytic loop starts to move out of the active site cavity from the very beginning of MD simulation. The M-shape of the loop (initial structure) rapidly changes (in a 20 ns time interval) to an "open" ring-like structure (O-structure) that is fully exposed to the solvent. This result elucidates the important role of β-hairpin structures in maintaining the active M-state structure of the catalytic KFGKS loop inside the enzyme active center.

Keywords: Molecular Dynamics simulations, tyrosyl-tRNA synthetase, β-strands.

WED-088

The use of human digestive juices and commercial enzymes preparations for evaluation of biological activities of carp protein hydrolysates

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Fish proteins are considered as an interesting source of peptides with biological activity, including angiotensin converting enzyme (ACE) inhibitory and antioxidant peptides. Carp (*Cyprinus carpio*) is one of the most popular fish in Poland with high digestibility of proteins exceeding 98%, so it may be the source of bioactive peptides in the human diet. The aim of this study was to identify ACE inhibitory, antioxidant peptides and antimicrobial peptides in hydrolysates of carp myofibrillar and sarcoplasmic proteins, obtained *via* different ways of human digestive tract conditions simulation.

The study covered the *in silico* part – with the use of UniProt and BIOPEP databases as well as tools available within, Fragment Ion Calculator and Sequence Specific Retention Calculator application. The next step of analysis was *in vitro* hydrolysis – with the use of commercial enzyme preparations, and *ex vivo* digestion – with the use of human digestive juices isolated from individuals. The method was developed using carp muscle tissue. *In vitro* hydrolysis of protein extracts was carried out using a commercial enzyme preparations such as pepsin and Corolase PP. Bioactive peptides in the obtained hydrolysates were identified using RP-HPLC with MS detector based on the expected retention times.

Peptide sequences, which from the theoretical point of view should be released during the hydrolysis of carp proteins, were selected from the results of the *in silico* part of study. The identification of bioactive peptides in hydrolysates of carp protein isolates was possible *via* the expected retention times for each of the selected sequence calculation. The comparison of theoretical and experimental retention times of searched peptides was the basis for identification and analysis. For example the predicted retention time of peptide FIKK was 19.15 min and the retention time observed during the study was 19.56 min and 19.57 min for *in vitro* and *ex vivo* samples, respectively. ACE inhibitory peptides ALPHA, MNPPK, VKAGF, IVY, GL, HL and antioxidative peptides FIKK, PW, HL were identified in both kinds of hydrolysates but TVY and IW peptides (ACE inhibitors) were presented only in *ex vivo* and *in vitro* hydrolysates respectively. It was proved that during *in vitro* and *ex vivo* digestion different products were released. It was impossible to identify antimicrobial peptides in hydrolysates that were studied. It was concluded that carp proteins can be the source of antihypertensive and antioxidant peptides.

Keywords: bioactive peptides, carp proteins, hydrolysis.

WED-089

Towards to mechanism of uptake bilitranslocase transporter. NMR study of TM2: TM3 assembly and modelling simulations in micellar environment

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Using a combination of genomic and post-genomic approaches is rapidly altering the number of identified human influx carriers. A transmembrane protein bilitranslocase (BTL) is involved in transport of bilirubin and several other organic anions from blood to liver cells. Moreover, the BTL has been identified as a potential target for cellular uptake of several drugs. The drug delivery task requested to knowledge about high-reslution 3D structure and mechanism of functioning of transmembrane channel. Previously, the chemometrics model have been developed in our group on base of the counter-propagation neural network (CPNN). As result of our studies, we possible to predict four transmembrane (TM) regions in BTL protein. To the time being, the 3D structures of TM2 and TM3 BTL fragments in SDS micelle are available [1,2]. According these data, both TM segments – TM2 (^{73}Ser.Leu^{99}) and TM3 (^{220}Ser.Thr^{237}) – are folded as a helix – loop – helix motifs with a kink around the central proline residues (Pro85, Pro231). Very recently, the structure of two TM2 and TM3 transmembrane fragments, dissolved together in SDS lipid media was evaluated on base NMR data [3]. The detail analysis reveal significant alteration of TM2 and TM3 peptides in assebly compare to the previously reported structural data. In addition, the experimental values of ^{15}N R_1, R_2 relaxation rates and ^{1}H-^{15}N *NOE* obtained for ^{15}N-labeled alanines incorporated in both regions reveals existence conformational exchange motions. The efects of such processes more pronounced for alanines located close to central prolines. Finally, taking into account the side chains orientation in hydrophobic and hydrophilic residues we conclude that TM2: TM3 assemble of BTL fragments possible to facilitate formation of a BTL transmembrane anion channel with uptake mechanism based on *cis*/*trans* isomerization.

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Keywords: Bilitranslocase, NMR Spectroscopy, transmembrane protein.

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WED-090
Understanding the mechanism of ASK1 regulation by thioredoxin using biophysical characterization of their complexes

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Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family that activates c-Jun N-terminal kinase and p38 MAP kinase pathways in response to various stress stimuli, including oxidative stress, endoplasmic reticulum stress, and calcium ion influx. The function of ASK1 is associated with the activation of apoptosis in various cells and plays a key role in the pathogenesis of multiple diseases including cancer, neurodegeneration and cardiovascular diseases. The kinase activity of ASK1 is regulated by many factors, including binding of thioredoxin (Trx) and the 14-3-3 protein that both function as inhibitors of ASK1 [1]. However, the mechanisms by which these binding interactions inhibit ASK1 are still unclear.

To better understand the role of Trx binding in the inhibition of ASK1, we prepared the isolated Trx-binding region of ASK1 (ASK1-TBD), performed its structural and biophysical characterization and studied its interaction with Trx under both reducing and oxidative conditions. Data obtained from analytical ultracentrifugation, time-resolved fluorescence anisotropy measurements, circular dichroism and small-angle X-ray scattering suggest that: (1) ASK1-TBD is a compact monomeric and rigid domain that under reducing conditions forms with Trx a stable and well defined complex with 1:1 molar stoichiometry; (2) the structural integrity of the catalytic Trp-Cys-Gly-Pro-Cys motif of Trx is essential for its binding to ASK1-TBD; (3) Trx interacts with the region of ASK1-TBD located in the vicinity of Cys250; and (4) Trx binding does not induce significant structural change of ASK1-TBD. In addition, it seems that the interaction between ASK1-TBD and Trx does not involve the disulfide bond formation, as has been suggested in the literature [2,3].

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Keywords: apoptosis signal-regulating kinase 1, thioredoxin.

WED-091
Urea-unfolded ubiquitin: from NMR to MD simulation

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The delicate equilibrium between the folded and functional structure of a protein and its unfolded state is highly dependent on environmental variables such as the solvent. For example the co-solvent urea is a well-known protein denaturant that displaces the equilibrium towards unstructured and non- functional conformations of proteins. However the molecular mechanism behind its ability remains an enigma and the interpretation of the experimental data is still ambiguous. In this work we present the characterization of the structural, dynamics, and energetics of properties of the urea-denatured state of ubiquitin, a small prototypical soluble protein. By combining molecular dynamics simulations with nuclear magnetic resonance and small-angle X-ray scattering data, we were able to: (*i*) define the unfolded state ensemble, (*ii*) understand the energetics stabilizing unfolded structures in urea, (*iii*) describe the differential nature of the interactions of the fully unfolded proteins with urea and water, and (*iv*) characterize the early stages of protein refolding when chemically denatured proteins are transferred to native conditions. The results provide a new picture of the chemically unfolded state of proteins and contribute to deciphering the mechanisms that stabilize the native state of proteins, as well as those that maintain them unfolded in the presence of urea.

Keywords: None.

WED-092
Utilizing reprogramming technologies to study neurodegeneration: a case study in ALS

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Although many distinct mutations in a variety of genes are known to cause Amyotrophic Lateral Sclerosis (ALS), it remains poorly understood how they selectively impact motor neuron biology and whether they converge on common pathways to cause neural degeneration. We have combined reprogramming and stem cell differentiation approaches with genome engineering and RNA sequencing to define the transcriptional and functional changes that are induced in human motor neurons by mutant SOD1. Mutant SOD1 protein induced a transcriptional signature indicative of increased oxidative stress, reduced mitochondrial function, altered sub-cellular transport, electro-physiological excitability as well as activation of the ER stress and unfolded protein response pathways. Functional studies demonstrated that perturbations in these pathways were indeed the source of altered transcript levels. Importantly, we used a genetic targeting approach to demonstrate that these phenotypes are reversed by genetic correction of the SOD1 mutation.

Utilizing this patient-specific induced pluripotent stem cell (iPSC) approach we next addressed two important, outstanding questions in the field. Why are motor neurons selectively lost in ALS? And are there common molecular pathways between distinct ALS-causing mutations? We found that motor neurons exhibit inherent ER stress that is related to their physiological properties and renders them vulnerable to disease. Finally, inter-

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rogation of stem cell-derived motor neurons produced from ALS patients harboring a repeat expansion in C9orf72 and FUS mutations identified electro-physiological excitability as a major feature of distinct types of ALS. These results provide an insight to the common functional defects that physiological levels of mutant proteins may lead to, in patient motor neurons. More broadly our studies demonstrates that iPSC technology can be used to probe an adult-onset neurological disease such as ALS.

Keywords: neurodegenerative diseases, pluripotent cells, reprogramming.

WED-093
Valorization of agricultural residues for Compactin production by *Aspergillus terreus* MTCC 279 in mixed substrate solid state fermentation

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The present study was aimed to enhance the production of compactin by *Aspergillus terreus* MTCC 279 in mixed substrate solid state fermentation using various solid substrates. Twenty solid substrates including agricultural residues and other solid substrates were tested for the compactin production by *Aspergillus terreus*. Among the twenty substrates tested, the three substrates which gave maximum compactin production was taken for further optimization in mixed substrate solid state fermentation in various combinations using response surface methodology. Green peas, millet and ragi were found to be suitable substrates which produced maximum compactin production in SSF. The combinations of the substrates with 1.5 g of green peas, 1.5 g of millet and 1.5 g of ragi gave maximum production of 389.34 mg/gds compactin. Response surface methodology was used to determine the optimal combination of each substrate. To the best of our knowledge this is the first report on enhancing compactin production in mixed substrate solid state fermentation.

Keywords: Compactin; mixed substrate solid state fermentation; Optimization; Response Surface Methodology; Aspergillus terreus.

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CSIV-05 – The new microbiology

WED-095
A kinetic approach for immobilization of NAD+/NADH on magnetic solid support and its regeneration for enzymatic biosynthesis

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β-nicotinamide adenine dinucleotide (β-NAD) is one of the most important electron carriers in all living cells and used various scientific and technologic fields such as biofuel cell, sensor technology, and hydrogen production. Therefore, the immobilization of β-NAD is of great importance. For this purpose, 3-aminophenyboronic acid (APBA) functionalized Fe_3O_4 nanoparticles were prepared as a magnetic solid support for the purification of β-nicotinamide adenine dinucleotide (β-NAD). The condensation product of 3-aminophenyboronic acid (APBA) was attached on the synthesized magnetite nanoparticles. X-ray diffraction, fourier transform infrared spectroscopy, vibrating sample magnetometer and transmission electron micrograph methods were used to characterize the surface modified magnetic nanoparticles. The reversible immobilization behavior of β-NAD was investigated using the APBA functionalized magnetic particles in batch-fashion. Loading capacity of 3-aminophenyboronic acid (APBA) functionalized Fe_3O_4 nanoparticles for β-NAD adsorption was 13.0 μmol/g. Immobilizaton kinetic and isotherm studies, respectively, showed that the immobilization process followed a pseudo-second-order kinetic model and the Langmuir isotherm model which agrees with experimental data better than the Freundlich isotherm model.

3-aminophenyboronic acid (APBA) functionalized Fe_3O_4 nanoparticles were proposed as alternative support for the β-NAD immobilization. The results elucidated the significance of magnetic separation as a fast, relatively simple and low-cost technique.

Keywords: Magnetic solid support; Immobilization; Elution; Re-usability; cofactor.

WED-096
A lithotrophic microbial fuel cell operated with iron-oxidizing bacteria enriched at the anode

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In this study, we attempted to enrich neutrophilic iron bacteria in a microbial fuel cell (MFC) in order to develop a lithotrophic MFC system that can utilize ferrous iron as an inorganic electron donor and operate at neutral pHs. Electrical currents were steadily generated at an average level of 0.6 mA in MFC reactors initially inoculated with microbial sources and operated with 20 mM Fe^{2+} as the sole electron donor; whereas in an uninoculated control reactor, the average current level only reached 0.2 mA. The MFC inoculated with a natural microbial source (MFC 2) appeared to generate a higher and more stabile current, in comparison with the MFC inoculated with a previously enriched iron-oxidizing culture. Cultivation-based and DGGE analyses both show the dominance of *Pseudomonas* species in the anode communities of the MFC reactors. On the other hand, fluorescent in situ hybridization results revealed significant increases in the quantity of neutrophilic iron-oxidizing bacteria in the anode community of MFC 2. The results, altogether, prove the successful development of a lithotrophic MFC with iron bacteria enriched at its anode and suggest a special metabolic cooperation of the anodic bacteria in such a system. This MFC system might offer unique potential applications.

Keywords: iron bacteria, iron oxidation, lithotrophic microbial fuel cell.

WED-097
A novel mycovirus in the human pathogenic fungus *Aspergillus fumigatus* and its application as a virus-induced gene silencing (VIGS) vector

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A novel mycovirus named *Aspergillus fumigatus* tetramycovirus (AfuTmV-1) was discovered in the human pathogenic fungus, *Aspergillus fumigatus* clinical isolate. AfuTmV-1 is a capsid-less, double-stranded (ds) RNA mycovirus comprised of four genomic segments, ranging in size from 1.1 to 2.4 kbp. Each component (dsRNA1-4) has a high GC content and contains a single open reading frame (ORF) flanked by 5′- and 3′- untranslated regions with strictly conserved termini. The largest component encodes a putative viral RNA-dependent RNA polymerase (RdRP) where the sequence of the most highly conserved motif changes from GDDX to GDNQ. Proteins predicted from the dsRNA 2–4 share similarity with respectively serine proteases, highly basic APC proteins and proline-alanine-serine rich proteins which might be associated with scaffolding functions in cells. The virus causes latent infection with no phenotypic alterations to the fungal host and appears to be a unique dsRNA mycovirus which is still unassigned to a known virus family. The AfuTmV-1 sequence information together with its unique features was exploited to develop a potential tool for silencing genes in *A. fumigatus*. A truncated AfuTmV-1 based vector was successfully constructed and used as a prototype vector for generating a recombinant virus-induced gene silencing (VIGS) vector. Silencing vector was engineered to carry host endogenous gene fragment corresponding to the *ALB1/PKSP* gene responsible for conidia pigmentation and subsequently introduced into the wild type fungal protoplasts. Silencing efficiency and stability of the vector to silence gene in *A. fumigatus* are being investigated using quantitative RT-PCR to investigate the expression of the *ALB1/PKSP* gene. Also, the accumulation of the small interfering (si) RNAs derived from the *ALB1/PKSP* fragment will be determined. We anticipate that the development will provide a powerful reverse genetic tool for functional genomics studies to identify key elements involved in fungal pathogenicity and also provide a medical benefit in exploiting mycovirus as a future therapeutic agent against fungal infections.

Keywords: *Aspergillus fumigatus*, Gene silencing, Mycovirus.

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WED-099

Analysis of lipids of probiotic bacteria

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Lipids are important constituents of bacterial cells, served as structural components of membranes, energy sources, signaling molecules. Characterization of lipidome in bacteria plays a crucial role for taxonomic identification, understanding of metabolic processes, selection of valuable strains for biotechnology.

The lipidomes of three industrial probiotic bacteria *Bifidobacterium longum*, *Lactobacillus paracasei*, *Enterococcus faecium* were studied by means of thin layer chromatography (TLC) and matrix assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis of the total lipid extracts. Analysis of lipid extracts of probiotic bacteria by one- and two-dimensional TLC demonstrated the presence of major phospholipids (PLs), glycolipids (GLs) and lipids with free aminogroups (ALs) with different chromatographic mobility (R_f). The major lipids of *B. longum* were PLs (R_f=0.25, R_f=0.35, R_f=0.40, R_f=0.45), GLs (R_f=0.14, R_f=0.22, R_f=0.25, R_f=0.35, R_f=0.40, R_f=0.8), AL (R_f=0.40); *L. paracasei* – PL (R_f=0.45), GLs (R_f=0.15, R_f=0.31, R_f=0.36, R_f=0.59, R_f=0.73), ALs (R_f=0.24, R_f=0.49); *E. faecium* – PL (R_f=0.45) and GLs (R_f=0.25, R_f=0.72). Comparative analysis of lipid extracts and PL standards – diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidic acid (PA) revieled that PG (R_f=0.45) is major PL in *L. paracasei, E. faecium*, and one of the dominat PLs in *B. longum*. Other PLs of *B. longum* were not similar to PL standards in their chromatograthic mobility. Analysis of lipid extracts of probiotic bacteria by MALDI-TOF MS showed characteristic peaks with *m/z* 478.7, 780.6, 1459.5, 1573.4, 1601.3, 1613.9, 1628.6, 1669.9 for lipid components of *B. longum*, *m/z* 478.7, 808.4, 850.5, 914.7, 982.7, 1118.9 – of *L. paracasei*; *m/z* 478.7, 780.3, 834.4, 954.6, 1110.8, 1196.9, 1238.9, 1325.1, 1425.2 – of *E. faecium*. Significant differences were observed among MALDI-TOF mass spectra of lipid extracts of probiotic bacteria, and they can be easily distinguished by lipid mass peak profiles. The fatty acid (FA) composition of lipids of probiotic bacteria was determined using gas-liquid chromatography/mass spectrometry. Normal saturated, unsaturated and cyclopropane FA were detected in analized bacteria. Major FA of *B. longum* were $C_{14:0}$ (14%), $C_{16:0}$ (50%), $C_{18:0}$ (9%), $C_{16:1}$ (5%), $C_{18:1}$ (8%), *cyc*-C_{19} (10%) acids, *L. paracasei* – $C_{16:0}$ (19%), $C_{18:0}$ (13%), $C_{16:1}$ (6%), $C_{18:1}$ (35%), *cyc*-C_{19} (20%) acids, *E. faecium* – $C_{14:0}$ (3%), $C_{16:0}$ (17%), $C_{18:0}$ (12%), $C_{16:1}$ (10%), $C_{18:1}$ (14%) and *cyc*-C_{19} (30%) acids. Further studies of the lipid extracts of probiotic bacteria will be performed to determine their biological activity.

Keywords: None.

WED-100

Analysis of structure, purity and activity of recombinant human growth hormone produced in *Escherichia coli*

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Human growth hormone (hGH) was one of the first recombinant proteins approved for the treatment of human growth disorders. Its small size (191 amino acids), possession of only 2 disulphide bonds and absence of posttranslational modifications make *Escherichia coli* the host of choice for its production on any scale. Previously, we have presented s novel *E. coli* based expression system capable of producing high quantities of soluble recombinant hGH. In this work, we have analyzed amino acid sequence of hGH using the in-source decay and peptide mapping mass spectrometry. Further, we have determined purity using reverse-phased HPLC, size-exclusion HPLC and endotoxin concentration assay (LAL assay). Secondary structure has been determined by the circular dichroism measurements and the activity of the recombinant hGH has been tested by Nb2 cell line proliferation assay. Results show that recombinant hGH produced in *E. coli* has the structure and activity indistinguishable from the native product, while the purity after three steps of chromatography purification reaches level of more than 99%. This data supports the claim that our system for the production of high levels of soluble recombinant hGH represents a viable production process adaptable to large scale.

This contribution is the result of the projects implementation: "Centre of Competence of Comenius University in Bratislava for R&D in molecular medicine" (ITMS 26240220071) and "University Science Park of Comenius University in Bratislava" (ITMS 26240220086) supported by the Research & Development operation Programme funded by ERDF
Keywords: *E.coli*, hGH, purification.

WED-101

Antibacterial mechanism for the apoptosis-like response in *Escherichia coli* induced by silver nanoparticles

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Silver nanoparticles are known to have antimicrobial properties and have been used extensively in medicine, although the mechanism(s) of action have not yet been clearly established. In the present study, the findings suggest a novel mechanism for the antibacterial effect of silver nanoparticles on *Escherichia coli*, namely, the induction of a bacterial apoptosis-like response. We propose a possible mechanism for the bacterial apoptosis-like response that includes the following: accumulation of reactive oxygen species (ROS), in particular hydroxyl radicals (·OH) (detected with HPF staining), increased intracellular calcium levels (detected with Fura-2 AM), phosphatidylserine exposure in the outer membrane (detected with Annexin V) which is the hallmarks of early apoptosis, disruption of the membrane potential (detected with $DiBAC_4(3)$), activation of a bacterial caspase-like protein (detected by FITC-VAD-FMK staining) and DNA degradation (detected with TUNEL assay) which is the hallmarks of late apoptosis in bacterial cells treated with silver nanoparticles. We also performed RecA expression assay with western blotting and observed activation of SOS response to repair the damaged DNA. To summarize, silver nanoparticles are involved in the apoptosis-like response in *E. coli* and the novel mechanisms which were identified in this study, suggest that silver nanoparticles may be an effective antimicrobial agent with far lower propensity for inducing microbial resistance than antibiotics.

Keywords: Antibacterial effect, Apoptosis-like response, Silver nanoparticle.

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WED-102
Antifungal activity of lactic acid bacteria isolated from Armenian dairy products

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Food and feed spoiled by moulds and yeasts cause great economic losses worldwide. Furthermore, the presence of moulds with the concomitant production of allergenic spores and possibly mycotoxins makes them serious potential health hazards. The reduction of mould and yeast growth in food and feed production and storage is primarily important. The development of efficient and safe strategies for solution of this purpose attaches attention. In this context, the application of bio-preservation, i.e. control of one organism by another, has got much interest in recent years. The natural origin and good study of producers, isolated from wild nature, are the primary demands. The best candidates suitable for these demands are lactic acid bacteria (LAB)

The aim of this study was to reveal the antifungal activity of lactic acid bacteria isolated from dairy products of Armenia different regions. The temperature and pH stability, as well as the effects of elicitation on synthesis of antifungal components were studied too.

Primary screening of LAB revealed the antifungal activity of *Lactobacillus delbrueckii* subsp. bulgaricus RIN-2003-Ls, *L. delbrueckii* subsp. bulgaricus INRA-2010-4.2, *L. rhamnosus* R-2002, *L. cryspatus* INRA-2010-5.2 against *Fusarium oxysporum* CB1853, *Cladosporium herbarium, Mucor plumbeus, Penicillum aurantio-violaceum, Debaromyces hansenii.* The ability of LAB inhibited the growth of 10^4 spores of moulds was used as the minimal inhibitory activity. The synthesis of antifungal agents started only after 48 h of LAB cultivation. The effects of different temperature on the antifungal activity revealed a high sensibility of components. The treatment at 45°C leaded to inhibition of antifungal activity of *L. rhamnosus* R-2002 against *M. plumbeus,* while treatment at 55°C inhibited activity against *Penicillum aurantio-violaceum*. The effects of different pH on the antifungal activity determined a high stability of antifungal substances. *L. rhamnosus* R-2002 showed the antifungal activity in broad spectra of pH from 2 to 10. The cell free supernatant (without concentration, as well as 10 or 22.5× concentrated) did not show antifungal activity. Moulds' autolysates which were used as elicitors during the cultivation of LAB did not increase the antifungal activity. The association of antifungal components with cell wall was determined.

All studied strains are suggested to be able to be used as starters for production of functional food, as well as preserving strain–producers.

The work was supported by grant from the Armenian National Science and Education Fund (ANSEF) based in New York, USA.
Keywords: antifungal activity, lactic acid bacteria.

WED-103
Antimicrobial activity of samarium doped hydroxyapaptite prepared by co-precipitation method

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This research focuses on understanding the antimicrobial activity of samarium doped hydroxyapatite (Sm:HAp). Samarium doped hydroxyapatite, $Ca_{10-x}Sm_x(PO_4)_6(OH)_2$ with $x_{Sm} = 0.7$ were developed by coprecipitation method. The bactericidal effect against common Gram-positive bacteria and fungus has been also investigated. Elemental maps for the samples prepared with $x_{Sm} = 0.7$ are also shown. The spectrum and images confirmed the presence of samarium in the samples. The EDAX spectrum of Sm:HAp confirms the presence of calcium (Ca), phosphor (P), oxygen (O), and samarium (Sm). They show that a substantial Sm content has been enrobed in the hydroxyapatite. The XPS spectra revealed the presence of a material composed mainly of phosphate, calcium, oxygen, hydrogen and samarium.

This study showed that Sm:Hap with $x_{Sm} = 0.7$ presented a good antimicrobial activity against *Bacillus Subtilis* and *Candida Albincas*. The antimicrobial activity of Sm:HAp was tested using the standard microdilution method. *Bacillus Subtilis* and *Candida Albincas* were grown on LB agar broth with Sm:HAp ($x_{Sm} = 0.7$). Yeast extract agar plates were incubated for 24 h at 37°C and the obtained colony forming units (CFU) were visually counted. The Sm:HAp ($x_{Sm} = 0.7$) show strong antibacterial activity.

The results of this study clearly showed that the Sm:HAp with $x_{Sm} = 0.7$ inhibited the growth and multiplication of the *Bacillus Subtilis* (gram-positive) and *Candida Albicans*. These data suggest that development of novel Sm:HAp is a promising material with the antimicrobial properties that may be used for covering the various surfaces of ambulatory and other medical devices.

Keywords: samarium, hydroxyapatite, antimicrobial activity.

WED-104
Anti-microbial compounds targeting the alternative thymidylate synthase ThyX

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The growing problem of antibiotic-resistant bacteria in clinical settings points to a need for new anti-infective therapies to address global health problems. However, the rate of new antimicrobial compounds to be developed by the pharmaceutical industry alone will not be sufficient to meet the expected need for the foreseeable future.

We have exploited the alternative thymidylate synthase ThyX proteins discovered in the laboratory as a new target for antimicrobial compounds. This is highly justified as ThyX enzymes are essential, present in many pathogenic bacteria (e.g. *Helicobacter*, *Mycobacteria*, *Chlamydia*, *Rickettsia* and *Clostridium* species, 30% of completed bacterial genomes carry *thyX*), but absent in humans. The active site configurations and catalytic reaction mechanisms differ drastically between human thymidylate synthase and ThyX proteins, thus further facilitating conception of ThyX-specific inhibitors. Moreover, recent genome-wide sequencing studies have established ThyX proteins as a virulence factor in *Leptospira* and revealed that overexpression of *thyX* is associated with resistance to the first- and second-line tuberculosis drugs.

We describe how the use of medium-throughput robotized tests for detecting ThyX activity led to the discovery of specific ThyX inhibitors that do not act on human thymidylate synthase. The most interesting ThyX inhibitors inhibit ThyX in genetically modified bacterial strains and show bactericidal activity in the micromolar range against laboratory and clinical strains of *Helicobacter* and *Mycobacteria* species. These inhibitors bind in the vicinity of the redox active co-factor flavin adenine nucleotide, in unexpected configuration.

Our molecules are the first non-substrate based ThyX inhibitors with activity against whole cells. Our biochemical, biophysical and structural studies provide means for development of more potent ThyX inhibitors in order to the fight the problem of antibiotics resistance.

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Keywords: antibacterial, microbiology, Thymidylate biosynthesis cycle.

WED-106
Changes of catalase, superoxide dismutase and peroxidases activities after UV-C exposure of *Pseudomonas aeruginosa*

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Ultraviolet (UV) light is the most commonly technology used in wastewater disinfection. However, bacteria have evolved several strategies to counteract oxidative stress generated by UV-C rays. As a first line of defense against potentially toxic levels of endogenous superoxide, *Pseudomonas aeruginosa* possesses superoxide dismutase (SOD), catalase (CAT) and peroxidases (POX) enzymes that are involved in the detoxification of O^{-}_2 and H_2O_2.

In the present study, we investigate the expression of CAT, POX and SOD enzymes of *P. aeruginosa* after exposure to UV-C radiations. Furthermore, different SOD isozymes were separated by a non-denaturating polyacrylamide gel and MDA content was determined for the same UV-C exposure times.

Our results showed that CAT and POX activities exhibited a gradual increase after 5 and 15 min. These variations were concomitant with SOD activity peak at 15 min. For shorter exposure time, SOD activity was inactivated by MDA products, highly generated under UV-C short exposure time. Furthermore, gel activity staining assay detected the presence of three differentially regulated SOD isozymes with an important role of iron-cofactored isoform in response to UV-C stress.

In conclusion, the results of these studies suggest that antioxidant enzymes act in a coordinated manner to counteract stress generated by UV-C. Moreover, we suppose that CAT and POX enzymes have the same importance in scavenging H_2O_2 at shorter and longer exposure times and this may confer additional protection to SOD from damage that is mediated by MDA products.

Keywords: antioxidant enzymes, P. aeruginosa, UV-C.

WED-107
Characterization of DnaB-like proteins in *Acetobacter pasteurianus*

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The DnaB protein is an essential replication protein in *Escherichia coli* which is involved in both the initiation and elongation stages of DNA replication. The protein is the *E. coli* primary replicative helicase, the factor responsible for unwinding the duplex DNA in front of the replication fork. Studies *in vitro* have shown that the DnaB protein elicits multiple activities: 1) binding of rNTPs and dNTPs; 2) ATPase and ribonucleotide triphosphatase activities; 3) binding to single- and double-stranded DNAs; 4) interactions with DnaC protein and primase (DnaG protein); and 5) helicase activity. These multiple activities reflect the essential role of the DnaB protein in *E. coli* cell's DNA metabolism and involvement in crucial interactions in the primosome as well as in the protein-nucleic acid complexes formed at the origins of DNA replication. The protein has been studied intensively in *E. coli* strains. In this presented work we focused on the study of the DnaB-like proteins which are encoded by the chromosome of *Acetobacer pasteurianus*. Based on the nucleotide sequence of the genome of *A. pasteurianus* IFO 3281-01, we designed PCR primers to amplify *dnaB-like* genes from chromosome of the bacterial strains *A. pasteurianus* LMG 1513 and *A. pasteurianus* CCM 3610. We cloned the PCR products into cloning vector pGEM^{R}-T Easy and we used these prepared constructs for sequencig analysis. We compared the primary nucleotide and amino acid sequences of the *dnaB-like* genes to each other, as well as to the sequences of *dnaB* gene from *E. coli* strains in BLAST database. Bioinformatic analysis showed sequence similarity between both *Acetobacter* strains but low sequence similarity between the compared *Acetobacter* and *E. coli* strains. Based on the primary amino acid sequences and by using the PSIPRED program we predicted the secondary structure of both DnaB-like proteins from *Acetobacter* strains. We recloned the targeted *dnaB-like* genes from vektor pGEM^{R}-T Easy into expression vector pET30a+. We transformed the competent cells *E. coli* BL21 (DE3) with this newly prepared constructs that carried *dnaB-like* genes. We optimalized the overexpression of our targeted proteins that were in fusion with a hexahistidine sequence and then we purified them by afinity chromatography. We measured the ATPase and helicase activity of the purified proteins and compared them with enzymatic activity of DnaB protein from *E. coli* K12.

Keywords: Acetobacter, DnaB protein, enzymatic activities.

WED-108
Characterization of the genomic island harboring the gene cluster for production of the antibacterial peptide microcin E492

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Microcin E492 (MccE492) is a peptide produced and secreted by *Klebsiella pneumoniae* RYC492 (*Kp*RYC492), which has antibacterial effects against *Enterobacteriaciae*, antitumoral effects over some human cell lines, and forms amyloid fibers modulating their activity. Genetic determinants for the MccE492 production are encoded in a gene cluster that has been cloned and studied in *E. coli*. However, genomic context of the cluster in the former strain has not been explored. Analysis of *Kp*RYC492 genome revealed a sequence context suggesting that MccE492 cluster is allocated in a putative ~23 kbp genomic island named *Kp*GI-E492. In this work we show that *Kp*GI-E492 is an unstable mobile element, unveiling their structural features, and evaluating factors influencing their excision from their host chromosome.

Keywords: antibacterial peptide, Genomic Island, microcin e492.

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WED-109
Chemical composition and antimicrobial activity of *Artemisia annua* essential oil

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Artemisia annua, a native species of East Asia, due to its high degree of morphological and reproductive plasticity and massive seed production become widespread in temperate regions, including Europe. In Romania this species is included in the invasive plants category.

Considering the fact that the eradicating methods are extremely costly and furthermore not ecological, therefore, in the desire to offer a viable alternative for the approach of this phenomenon, our objective was to study the chemical composition and the antimicrobial properties of the *A. annua* essential oil extracted from leaves.

The volatile constituents of *A. annua* L. have been analyzed using GC/MS. The qualitative screening of *Staphylococcus aureus*, *Bacillus subtilis, Enterococcus fecalis, Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumanii* and *Candida* sp. (clinical and reference strains) susceptibility to the *A. annua* essential oil was performed using Kirby-Bauer diffusion method. The quantitative analysis of the antimicrobial activity was performed by using binary serial microdilution method in liquid medium. Positive and negative controls for microbial growth, as well as for the antimicrobial activity of the solvent were used. The influence of the obtained plant extracts on the microbial adherence capacity on the inert substratum was studied using the biofilm microtiter method.

The extract yields of essential oil were 0.783%. A total of compounds were identified by GC/MS was 91.7%, the main components were α-pinene, camphene and eucalyptol. The tested microbial strains proved to be susceptible to the essential oil of *A. annua*, including those clinical strains resistant to antibiotics. The minimum inhibitory concentrations of stock solutions from *A. annua* essential oil solubilized in DMSO (1:2) ranged from 0.51 to 16.33 mg/ml. The tested essential oil showed good antibiofilm activity, inhibiting the initial stage of formation of the microbial cells adhesion to the inert substratum, but was also active against the mature, pre-formed bilofilms.

Our results demonstrated that among other biological activities, the essential oil of *A. annua* contains antimicrobial active compounds with selective activity on Gram-positive, Gram- negative bacterial and yeasts species and interfere with the microbial adhesion and biofilm development.

Keywords: antimicrobial activity, essential oil, GC-MS.

WED-110
Chitinolytic enzymes from *Clostridium paraputrificum* for biomedical applications

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Gastrointestinal tract with its microflora remains an interesting target for search for new biomedical applications. Presence of chitin degrading enzymes in human colon can play an important role in defense against fungal invasions. We have isolated and characterized bacterium *Clostridium paraputrificum* strain J4 (CpJ4) that is able to degrade chitin using wide range of extracellularly produced chitinolytic enzymes.

We have characterized the expression spectrum of CpJ4 and cultivated sufficient amount of CpJ4 for DNA isolation and consequential whole genome sequencing. We have developed several procedures to isolate three major chitinases naturally produced by CpJ4 grown in colloidal chitin containing medium. Furthermore, we have identified the three chitinases in the genome of CpJ4 and cloned them for production in *E. coli*. Two of the chitinases (chitinase B and chitinase Chit62J4) have been already recombinantly produced. We have performed comparative analysis of natural and recombinant proteins with focus on their activity towards chitin-derived substrates. We have observed remarkable differences between the proteins. *E.g.* in the case of chitinase Chit62J4, both forms exhibit almost identical characteristics in activity studies. Surprisingly, differences in potency to inhibit or enhance the activity of natural and recombinant protein have been observed for several compounds used in our analysis.

Our results show that there are significant differences between the natural and the recombinant proteins as for their activity profiles. Design of purification techniques for chitinolytic enzymes produced in natural source organism remains important for intended biomedical applications.

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Keywords: None.

WED-111
Combined use of genetics and metabolomics to study the biosynthesis and function of small antioxidative metabolites in *S. pombe*

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Our laboratory studies basic mechanisms that control the transition between cell division and quiescence in response to nutritional conditions. We use fission yeast *Schizosaccharomyces pombe* as a model. Since our recent results suggest that oxidative stress plays a significant role in the regulation of cell division and quiescence, we are interested in the biosynthetic pathways of small antioxidative metabolites. We employ genetic and metabolomic approaches to obtain detailed, quantitative data on the function of the biosynthetic enzymes. Recently, we characterized the two-step (Egt1 and Egt2) biosynthetic pathway of a proposed antioxidant ergothioneine, and its selenium-containing derivative, selenoneine (Pluskal et al, 2014). Currently, we focus on the biosynthetic pathway of a major antioxidant, glutathione, consisting of Gcs1 (glutamate cysteine ligase) and Gsa1 (glutathione synthetase) enzymes in *S. pombe*. The same pathway is reportedly used to synthesize ophthalmic acid, the function of which is completely unknown. In addition, glutathione can be polymerized by Pcs2 enzyme into phytochelatin, a compound required for protection from heavy metal stress. We found that deletion mutant Δ*gsa1* fails to grow in a synthetic minimal medium (EMM2). This defect can be rescued by supplementation with a tiny amount (5 μg/ml) of glutathione, but not by other antioxidants such as

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N-acetylcysteine or ascorbate. Interestingly, deletion mutants of other oxidative-stress related genes, such as *sod1*$^{+}$ (superoxide dismutase) or *trx1*$^{+}$ (thioredoxin), also show growth defects in the synthetic medium, suggesting a common underlying mechanism. In spot test experiments, the Δ*gsa1* mutant was highly sensitive to copper and cadmium, presumably due to the lack of phytochelatin. Metabolomic analysis of the Δ*gsa1* mutant has shown a complete absence of glutathione and increase in γ-glutamylcysteine, its precursor. In addition, we found an accumulation of thiamine disulfide, suggesting that thiamine might complement the antioxidative function of glutathione in Δ*gsa1* mutant. As ophthalmic acid was also absent in this mutant, we are interested to study the function of this poorly understood compound using the combined genetic/metabolomic system.

Reference

Pluskal, T., Ueno, M. and Yanagida, M. (2014) Genetic and Metabolomic Dissection of the Ergothioneine and Selenoneine Biosynthetic Pathway in the Fission Yeast, *S. pombe*, and Construction of an Overproduction System. *PLOS ONE* **9(5)**: e97774

Keywords: Antioxidants, Glutathione, metabolomics.

WED-112
Comparative assessment of poly-β-hydroxybutyrates synthesis in bacteria and yeasts

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Poly-β-hydroxybutyrates are biodegradable polymers which accumulate intracellularly as storage granules in bacteria. They can be obtain from renewable sources and have important medical and environmental advantages.

Colony PCR techniques were used for screening poly-β-hydroxybutyrates (PHB) producers isolated from different soils from Transylvania, Romania (soils from alpine meadow, karstic zone with limestone meadow, hill zone with xeric meadow, flood plain). The primers used in colony PCR were used in order to detect *phbC* synthase gene. *Azotobacter vinelandii* 720 DSMZ, Germany, was used as control strain.

A recombinant plasmid was constructed using *phb* gene (1704 pb, NC_012560.1) and *pJET1.2* vector, which codifies *phbC* synthase gene from *Azotobacter vinelandii.* This construct was chemically transformed and introduced in competent cells of *Escherichia coli* strain XL1 Blue. Then it was trasfered in *Saccharomyces cerevisiae* by lithium acetate transformation. The transformed groups were recovered on leucine deficient medium.

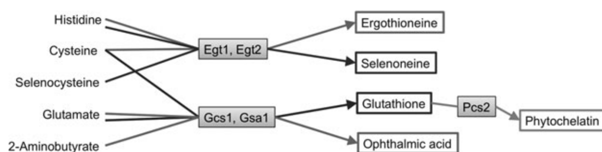

Fig. 1.

Comparative assessing of PHB synthesis in bacteria and yeasts in different experimental conditions led to the conclusion that the synthesis reached the maximum to the *Azotobacter* strains on YE medium with mannitol and tryptone. The highest PHB contents were found to the standard strain *Azotobacter vinelandii 720* (3.65 μgPHB/mg pellet) and to the *Azotobacter* strain from alpine meadow (1.96 μgPHB/mg pellet).

Keywords: bacteria, poly-β-hydroxybutyrates, yeast.

WED-113
Comparison of activity of immobilized and free recombinant formate dehydrogenase expressed in *Escherichia coli*

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The enzymatic bioconversion of aromatic compounds in food and cosmetic industry is often accompanied by the limitation in terms of co-factor depletion or its insufficient regeneration. To address this issue, we have constructed an efficient *E coli* based expression systems capable of producing functional yeast formate dehydrogenase (FDH) and alcohol dehydrogenase (ADH), enzymes used in conversion of trans-2-hexenal to trans-2-hexenol. To further improve this reaction, we have immobilized FDH onto magnetic Fe_3O_4 nano-particles and tested the ability of produced enzyme to improve activity of ADH by NAD+/NADH conversion. The activity of FDH has been tested by applying various physical and chemical condition changes, such as buffer pH and reaction temperature. The results show that although the activity of the immobilized FDH decreased compared to the one of free enzyme, the stability increased significantly allowing prolonged incubations or repeated use of a single batch.

This work was supported by the Slovak Research and Development Agency grant APVV-0061-11 and is also result of the "Competence centre for R&D in molecular medicine" (ITMS 26240220071) project implementation supported by the Research and Development Operational Program funded by the ERDF.
Keywords: formate dehydrogenase, immobilization.

WED-115
Copper (II) complexes with Schiff base from methionine and o-vaniline as antimicrobials – synthesis and characterization

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In the last decade, interest in copper (II) complexes has been increasing because of their potential use as antiviral, anti-inflammatory, antipyretic or enzyme inhibitors.

A new Schiff base derived from methionine and o-vaniline has been synthesized. The structures assigned to Schiff base are supported by elemental analysis, IR, H^1-NMR, C^{13}-NMR and UV-Vis spectra.

A number of six complexes with this Schiff base and copper salts (chloride, bromide, sulphate, nitrate, perchlorate and acetate) have been also synthesized through the reaction of the Schiff base with copper (II) salt. The structures assigned to complexes are supported by elemental analysis, thermal studies, IR, UV-Vis and RPE spectra.

All compounds have been assayed to demonstrate the changes in biological activity due to the coordination of Schiff base to Cu (II) comparing with the Schiff base and for complexes on copper salt changing.

The antimicrobial activity was tested against reference and clinically isolated resistant strains of Gram-positive bacteria (*B. subtilis*, *S. aureus*), Gram-negative bacteria (*E. coli*, *P. aeruginosa*) and fungal strains (*C. albicans*) using the screening disk dif-

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fusion method and the quantitative, serial microdilution method for establishing the minimal inhibitory concentration values (MIC) for each compound.

The antimicrobial activity assayed by *in vitro* qualitative and quantitative methods showed that the complexes exhibited an improved antimicrobial activity as compared to non-coordinating Schiff base. The best activity was obtained for the complexes with copper chloride, sulphate and nitrate on *B. subtillis* and for the one with copper sulphate on *C. albicans*.

The compounds that exhibited the best antimicrobial activity were tested for the influence on the bacterial adhesion to HT-29 eukaryotic cell (Human colon adenocarcinoma grade II) and HEp-2 cell (human epithelial carcinoma) lines, using Cravioto method, and for the biofilm development on inert substratum.

Through their microbicidal, anti-biofilm and anti-pathogenic features, the obtained complexes open new perspectives for the development of the novel anti-infective strategies.

Keywords: antimicrobial activity, copper complexes, Schiff base.

WED-116
De novo evolution of kin discrimination in a social bacterium

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The capacity to distinguish self from non-self, or kin from non-kin, critically determines if and how cooperative behavior evolves across the biological spectrum, from complex vertebrates to microbes. Kin discrimination favors high genetic relatedness amongst interacting individuals and can hinder the spread of "cheaters", or non-cooperative organisms that exploit cooperative individuals and take advantage of public goods. Various forms of microbial kin discrimination have been reported, including colony-merger incompatibilities in the model cooperative bacterium *Myxococcus xanthus,* in which swarming groups of distinct genotypes fail to merge during group swarming on agar medium. Most natural isolates of *M. xanthus* cooperate well with clonemates but display a high degree of social incompatibility with distinct natural genotypes. Such incompatibilities during swarming appear to be relevant for understanding the social structure of natural populations, as they were found to limit co-aggregation during multicellular fruiting body development among very similar natural strains that had diverged recently in sympatry.

We examined experimentally evolved populations of *M. xanthus* to investigate the evolutionary and molecular origins of microbial kin discrimination. More than 120 populations that evolved independently across a variety of selective environments were screened and swarming colonies from ~60% of these populations failed to merge freely with colonies of their ancestor on agar medium, whereas control swarms of the same genotype always merged. A similar proportion of evolved populations failed to merge with other independently evolved populations. Thus, kin discrimination – broadly defined as reduced cooperation toward less related genotypes – was found to have pervasively evolved as a by-product of adaptation to a variety of selective conditions.

Kin discrimination phenotypes appeared either abruptly as single-step phenotypic jumps to the full incompatibility phenotype or gradually as continuous increases over evolutionary time, with roughly equal numbers of populations showing each pattern. Whole-genome sequencing (WGS) of evolved lineages indeed revealed that each temporal pattern of phenotypic change correlates with point mutations in two different genes. Additionally, WGS revealed strong links between the origin of kin discrimination and CRISPR immunity pathways.

Keywords: evolution of cooperation, kin discrimination.

WED-118
Development of genetic engineering systems in *Actinomyces* spp.

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Background: Recent studies have revealed that oral *Actinomyces* spp. plays an important role as an initial colonizer in dental plaque formation. However, only a few systems for genetic engineering is available for this species. Here, we constructed new pJRD215-based plasmids with the high efficiency for cloning We also established an inducible expression system using a P_{BAD}/*araC* system.OBSERVATIONS: pJRD215 is the only plasmid that can replicate in some *Actinomyces* spp. We determined the whole sequences to elucidate the genetic background of this plasmid. Then we reconstructed smaller plasmids, pCMDk (KmR) and pCMDs (SmR), by eliminating non-essential regions, and evaluated their transformation efficiency in *A. oris* strain MG-1. Both plasmids resulted in a 100-fold increase in the transformation efficiencies compared to those of pJRD215. Furthermore, to demonstrate the utility of *Actinomyces* b -galactosidase gene (gene locus: ANA1492) as reporter, we made an in-frame deletion mutant of ANA1492 (*A.oris* DANA1492) by a two-steps allelic exchange method using *galK* (coding for galactokinase) as a counter-selectable marker. We also constructed the plasmid expressing ANA1492 for complementation (pCMDk::ANA1492). We transformed DANA1492 with pCMDk::ANA1492, then detected that transformant has a strong b-galactosidase activity compared to *A.oris* DANA1492 on HIA plate containing X-gal chromogenically. Finally, we constructed a new expression vector containing the P_{BAD}/*araC* system by subcloning the relevant portion of pBAD/HisA (Invitrogen) into a pCMDk, resulting in pCMiex1. The Reporter gene constructed in pCMiex1 was successfully used to provide b -galactosidase (ANA1492) in *E.coli*.

Conclusions: We constructed pJRD215-based new plasmids with higher transformation efficiencies (pCMDk and pCMDs), and confirmed pCMiex1 as an inducible expression vector.

Keywords: oral bacteria Actinomyces pJRD215.

WED-119
Differentiation of bacilli on genera level based on tRNA modification profiles

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Transfer RNAs (tRNAs) are widely speculated to be among the most ancient RNA molecules, present at the dawn of life in the last universal common ancestor. Recently by using a parallel systems-type approach it was shown that the collective set of modified bases is highly species-specific and linked to phylogeny.

The aim of this study was to characterize of aerobic bacilli isolated from Armenian geothermal springs based on tRNA modification profiles. The objects for investigation were 14 aerobic bacilli strains including thermophilic (*Anoxybacillus, Geobacillus*) and mesophilic (*Bacillus, Brevibacillus, Ureibacillus*, *Paenibacillus*) representatives isolated from Armenian geothermal mineral springs. LC-MS was applied for the quantification of modified nucleosides by using isotopically labeled standards. 16 tRNA modifications (m^1A, m^6A, Am, t^6A, i^6A, ms^2i^6A, Q, m^1G, $m^6{}_2A$, m^2G, $m^2{}_2G$, Gm, m^2A, m^7G, m^5C, Cm) in both their natural and isotopically labeled forms were synthesized for the parallel quantification.

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Results obtained were confirmed presence of m^6A, i^6A, $m^6{}_2A$ and Cm modifications at low levels in all the species studied. In contrast m^2A and m^7G modifications were present at high levels in all species indicating their oldest origin, which likely contributed to the very early development of bacilli. Relatively high level of i^6A modification was observed in *Paenibacillus*. The lowest level of Cm modification was found in *Bacillus*. The highest level of m^5C modification was presented in *Ureibacillus*. The ms^2i^6A modification was found in all bacteria except *Ureibacillus*. On the other hand, only in *Ureibacillus* was observed modifications Am and $m^2{}_2G$. In *Brevibacillus* was absent m^1G modification. While both thermophilic and mesophilic species contains Gm, m^1G and ms^2i^6A modifications, large quantities of them (especially ms^2i^6A and Gm modification) were detected only in thermophilic ones (representatives of genera *Geobacillus* and *Anoxybacillus*). The high resolution of the analysis indicated that quantification of tRNA modifications could be applicable for use in reliably distinguishing thermophilic bacilli from related mesophilic species. The presented results offer a deeper insight into the evolution of tRNA modifications, and shows that they characterize species at a very fine level and are linked to phylogenetic variation of endospore-forming bacteria.

The work was supported by the DAAD award A/13/03781.
Keywords: bacilli, thermophiles, tRNA modification.

WED-120
Diversity of cultivable bacteria and analysis of bacterial secreted proteases from a tropical composting operation

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Composting operation systems are a wide source of microorganisms and enzymes produced by them. Among these enzymes, proteases, besides their role during the composting process as appropriate indicators of organic matter decomposition, are also important in industry, accounting for about 35% of microbial enzymes. In this study, we assessed the diversity of cultivable bacteria and extracellular proteases produced by some selected species from the facility inside the São Paulo Zoo Park (SPZPF) in Brazil, the biggest zoo in Latin America. Three hundred bacterial isolates were obtained and identified through 16S rRNA sequencing as belonging to 13 different genera. The predominant genus prospected was *Bacillus* (67%), which encompasses potential new species. Some of these strains showed high proteolytic activity. We biochemically characterized the secreted proteases of the potential new species of *Bacillus sp* through different techniques such as Fluorescence resonance energy transfer (FRET) peptides and gelatin zymography and found a repertoire of serine and metallo proteases with different molecular weight; among them, we are interested in some low molecular weight proteases (<15 kDa) that are related with sporulation. This general screening can be geared toward prospection of proteases with distinct properties.

Funding: São Paulo Research Foundation
Keywords: Bacillus, proteases.

WED-121
Down-stream processing for bioethanol production and high-added value products

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Wine industries produce a vast amount of wastes such as grape pomace, lees and tartar. Their organic content consists of dissolved sugars, alcohol, acids, polyphenols, tannins and lignins. The need for specific winery waste management plans for production of added value products has been widely understood. Red and white grape pomaces from Gerovassiliou wine industry (Thessaloniki, Greece) were used in this study. Extraction of main constituents, polyphenols and sugars, was performed using chromatographic or/and selective biosorption techniques. Fractions enriched in sugars after removing the majority polyphenols, were fermented for ethanol production. Pretreatment of samples with cellulases (in suspension or immobilized in calcium alginate beads) was also performed prior to fermentations. *Saccharomyces cerevisiae* and a mutant strain of *Zymomonas mobilis* CP4, tolerant to sucrose up to 40% (w/v), were used to produce ethanol from the wine wastes supplemented with different salts. Results revealed that ethanol production from wine wastes fractions was promising with possible commercial interest.

Keywords: bioethanol, down-stream processing.

WED-122
Dynamic processes in the heme-based transcription factors CooA and DNR

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A group of transcription factors is specifically activated by the binding of diatomic signaling molecules like carbon monoxide (CO), nitric oxide (NO) or dioxygen (O_2). These small gaseous ligands are selectively bound to a single heme-cofactor in a protein sensor domain. The presence or absence of the effector gas triggers a conformational change by which the protein passes from an inactive to an active state, ultimately leading to binding to target DNA and regulatory events. The bacterial transcription factors CooA[1] and DNR[2] are members of the CRP/FNR family. Both proteins contain a six-coordinate heme and are thought to undergo large and delocalized conformational changes upon binding of their physiological ligand. Purple bacterial CooA initiates the CO-dependent transcription of genes encoding the CO oxidation system, whereas the dissimilative nitrate respiration regulator DNR mediates NO-dependent induction of denitrification in the opportunistic pathogen *Pseudomonas aeruginosa*. We used biochemical approaches and advanced optical spectroscopy to investigate the mechanism and molecular pathways of the activation and deactivation of these gas-sensing transcription factors. Binding of CooA and DNR to their respective target DNA was studied by fluorescence anisotropy measurements and demonstrated that activation is highly specific for the physiological ligand. FRET measurements allowed direct determination of the distance between target DNA and the native heme, opening up the perspective of mapping out global protein conformational changes using time-resolved FRET techniques. Primary mechanistic processes in heme-based sensor proteins are initialized by ligand binding and dissociation from the heme[3]. Our ultrafast studies of ligand dynamics in the heme environment of wild type and mutant CooA proteins, combined with molecular modeling

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approaches, suggest a large conformational change of the protein backbone upon CO binding, involving a dramatic move of one heme-ligand. In DNR, NO recombination is very fast and nearly complete, revealing “NO-trapping” properties similar to what has been observed in other heme-based NO sensors and possibly providing means to control the effects of fast environmental fluctuations. The energetic barriers involved in CO migration have been determined in both transcription factors by temperature-dependence studies and compared to the five-coordinate heme sensor protein DosT and the electron transfer protein cytochrome *c*. The results will be discussed in the light of a general mechanism for ligand dynamics in heme-based sensor proteins.

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Keywords
codon variation, computer simulation, INFLUENZA VIRUS.
Keywords: Dynamic events, Gas sensor proteins, Heme-based transcription factors.

WED-124
Enzymatic dipeptide synthesis

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We previously reported an acyl-CoA synthetase, AcsA, which plays an essential role in acid utilization in the nitrile-degrative pathway of Pseudomonas chlororaphis B23 [1]. Acyl-CoA synthetase, which is one of the acid-thiol ligases, catalyzes the ligation of an acid with CoA in the presence of ATP and Mg^{2+}. The reaction mechanism and structures of acyl-CoA synthetase have been extensively investigated. During studies on the characterization of AcsA, however, we discovered a new reaction. When L-cysteine was used as the substrate instead of CoA, *N*-isobutyryl-L-cysteine was surprisingly detected as the reaction product [2]. This finding demonstrated that the enzyme formed a carbon-nitrogen bond involving the carboxyl group of the acid and the amino group of the L-cysteine, although the enzyme essentially catalyzes the formation of a carbon-sulfur bond. Furthermore, this unexpected enzyme activity was also observed for acetyl-CoA synthetase and firefly luciferase, both of which belong to the same superfamily of adenylate-forming enzymes. Therefore, all enzymes in this superfamily of adenylate-forming enzymes are likely to synthesize *N*-acyl-L-cysteine when L-cysteine is used as one of the substrates.

An adenylation domain of nonribosomal peptide synthetase (NRPS) also belongs to this superfamily of adenylate-forming enzymes. This domain is known to be responsible for the selective substrate recognition and formation of acyl-AMP as an intermediate under ATP consumption. Here, we found that N-aminoacyl-L-cysteine (dipeptide) was synthesized by a bacterial enzyme, which is homologous to the adenylation domain of NRPS. When the reaction mixture including the enzyme, ATP, an amino acid, and L-cysteine were analyzed by HPLC, a significant increase in AMP was observed. The increase in the amount of AMP with consumption of the amount of ATP indicates that the reaction proceeds. In addition, a new product peak other than that of AMP was detected by HPLC analysis. No other new peaks were detected. Without the enzyme, the peaks due to the new product and AMP were not detected. So, we purified and identified the product by LC-MS/MS and NMR analyses. The results of these analyses showed that the new product is not *S*-aminoacyl-L-cysteine but *N*-aminoacyl-L-cysteine (namely, dipeptide).

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Keywords: None.

WED-126
Expression and purification of proteoglycan-like domain of carbonic anhydrase IX in *Escherichia coli*

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Carbonic anhydrase IX (CAIX) is a transmembrane enzyme expressed in some types of tumors. It is commonly used as a marker or a target for potential therapy using monoclonal antibodies, generated using intact enzyme, but recognizing the epitope located in the extracellular proteoglycan-like (PG) domain. Therefore, isolation of only this part of the enzyme could potentially increase the efficiency of production and improve properties of monoclonal antibodies against CAIX. In this work, we present the construction of an efficient *E.coli* based expression system capable of producing high levels of PG-domain fused to thioredoxin (Trx-PG) and its purification using affinity chromatography. The product was expressed in soluble form at levels reaching 25% of total cell proteins. Using two steps of affinity chromatography and enterokinase cleavage we have gained recombinant PG domain with sufficient purity for generation of monoclonal antibodies against CAIX.

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Keywords: CAIX, E.coli expression, PG domain.

WED-127
Expression, localization and killing mode of yeast K2 toxin

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Budding yeast produces four types of dsRNA virus-encoded killer toxins (K1, K2, K28, and Klus) capable of killing non-killer yeast as well as killer yeast of the different types. Despite some similarities in their mode of production, killer toxins have different biochemical properties and profoundly distinct modes of action. While K28 toxin primarily acts in the nucleus of the host cell interfering with gene expression, K1 and K2 are active at the cell wall where they ultimately disrupt the structural and/or functional integrity of the plasma membrane. Mode of action of the Klus toxin is not completely understood.

In this work we demonstrated that K2 killer toxin, when expressed either from dsRNA or cDNA, is found in secretion vesicles of yeast cells and in extracellular medium. We have shown that K2 cDNA containing yeast exhibited lower toxin expression level even under inducing conditions comparing to the

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dsRNA-conditioned synthesis. Based on *in vivo* analysis of toxin activity and detection of the K2 protein by the Western blot, we discovered that killer protein is secreted when the yeast were grown at different pH values, even non-permissive for toxin activity. By modifying the pH of the growth medium, we showed possibility to revert the compromised activity. In order to explore the killing mode of K2 toxin, we investigated the dynamics of K2 protein binding to the cell wall-localized receptors, evaluated the dependence of K2 binding and killing activities on the presence of different glucans *in vitro* and *in vivo*. We established that cytotoxic effect of the K2 toxin on the *S. cerevisiae* cells assert very quickly, when fast decline of living cell population occurs due to the toxin-induced damages.

Keywords: yeast toxin, expression

WED-128
Extracellular vesicles and development of resistance to fluoroquinolones in *Acholeplasma laidlawii*

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Acholeplasma laidlawii (class Mollicutes) is an ubiquitous mycoplasma found in the tissues of humans, animals and plants, the causative agent of phytomycoplasmoses and the major contaminant of cell cultures. Mycoplasmas quickly become resistant to antibiotics. Despite its low efficiency, antibiotic therapy remains the primary tool used for the treatment of mycoplasma infections and decontamination of cell cultures. The elucidation of the mechanisms of the rapid development of antibiotic resistance and solving the problem of control of mycoplasma infections are connected with the investigation of molecular-genetic mechanisms of mycoplasma adaptation to stress. In our studies the stress-reactive proteins and genes of *A. laidlawii* PG8 were first identified, and it was shown that adaptation of the mycoplasma to environment was associated with the secretion of extracellular vesicles. Recent studies suggest the possible involvement of vesicles in the development of resistance to antibiotics in bacteria, however, similar studies in mycoplasmas have not been conducted yet. Characterization of extracellular vesicles and elucidation of the possibility for its participation in the development of resistance to fluoroquinolones (ciprofloxacin) in *A. laidlawii* was the purpose of our work.

In our studies it was shown that the level of secretion of vesicles in strain *A. laidlawii* with higher level of resistance to ciprofloxacin was found to be significantly more than that in the original parent strain. The cells and vesicles of this strain contained mutant nucleotide sequences of the target gene of ciprofloxacin (*parC*), exported the antibiotic from cells and displayed bacteriostatic effect toward the ciprofloxacin-sensitive bacteria strain. As a result of a global proteome profiling of *A. laidlawii*-derived vesicles it was revealed that vesicles from the mycoplasma were enriched in virulence factors. Thus our study allow to conclude that development of resistance to ciprofloxacin in mycoplasmas turns out to be related to mutation in gene of DNA-gyrase, the secretion of extracellular vesicles which mediate export of DNA sequences coding the protein-target for antibiotics and the traffic of ciprofloxacin. The vesicle mediated export of DNA sequences coding the proteins-targets for ciprofloxacin may promote the distribution (in bacterial populations) mutant versions of the respective genes via lateral transfer. The obtained data may facilitate the development of effective approaches to control mycoplasma infections and the contamination of cell cultures.

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Keywords: antibiotics, extracellular vesicles, mycoplasma.

WED-129
Formation of chromosome in bacteria

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The genome of bacteria is classically separated into the essential, stable and slow evolving chromosomes and the accessory, mobile and rapidly evolving plasmids. This paradigm is being questioned since the discovery of extra-chromosomal essential replicons (EER), be they called "megaplasmids", "secondary chromosomes" or "chromids". These genomic elements are thought to be indispensable based on their having a GC content identical to that of the chromosome and harboring essential genes, but plasmidic in origin because of the structure of their replication origin. However, none of these criteria are universally applicable and the true nature of these replicons is yet to be formally determined. Here, we explore the relationships of chromosomes and plasmids with reference to their genetic information inheritance systems (GIIS), under the assumption that the inheritance of EERs is integrated to the cell cycle and highly constrained in contrast to that of standard plasmids.

We performed a global comparative genomic analysis including all bacterial complete genome sequences available in NCBI RefSeq. Using ACLAME and KEGG GIIS-related proteins as input, we first identified GIIS functional homologs from all bacterial genomes. These homologs were then clustered according to function and sequences homology. The relationships between all bacterial replicons were then investigated using the GIIS clusters as parameters and unsupervised analyses (projections and graphs, and clustering. Finally, identification of putative EERs and of trends in GIIS usage were performed using supervised analyses (classification, regression).

This strategy clearly discriminated between chromosomes and plasmids with respect to their GIIS usage, and revealed another class of genomic elements that corresponds to EER-annotated replicons. They are characterized by a specific GIIS usage that testifies of the continuity of the genomic material in bacteria. Furthermore, whereas some are plasmidic in origin, others derive from the cleavage of an ancestral chromosome. Our study provides insights into the formation of "neo-chromosomes" and the

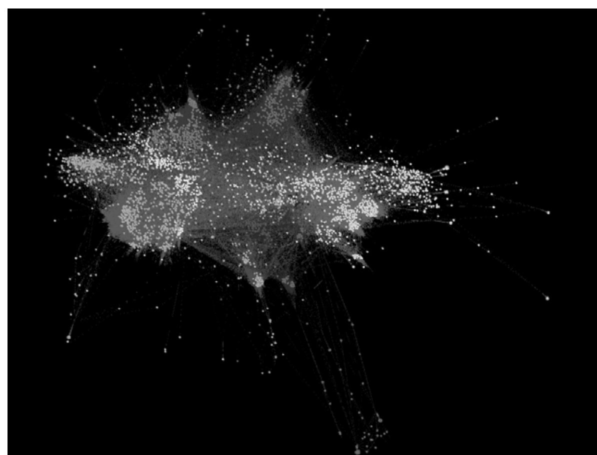

Fig. 1.

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emergence of multipartite genomes in bacteria, as well as clues about the forces shaping the genome.

Keywords: chromosome, large-scale analysis of genomes, Plasmids, replication proteins, evolution.

WED-130
Functional characterization of *Zymomonas mobilis* polyphosphate kinase 2 (PPK2); implications for polyphosphate and nucleotide metabolism

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Inorganic polyphosphate (poly-P) is a linear biopolymer comprised of tens to hundreds of phosphate monomers. Poly-P plays a variety of important physiological roles in all living organisms; for example, helping microbial cells survive and adapt to external stresses. Type 2 polyphosphate kinase (PPK2) enzymes are encoded in a wide variety of bacterial organisms. General consensus is the primary function of PPK2 proteins is to catalyze the phosphorylation of nucleotide monophosphate (NMP) or nucleotide diphosphate (NDP) substrates using polyphosphate as the phosphate donor. However, PPK2 proteins are diverse, and their full range of functions remains to be determined. Here, we report the biochemical characterization of the PPK2 homologue encoded by the ethanol-producing bacterium *Zymomonas mobilis* (ZM-PPK2). The ZZ6_0566 gene from *Zymomonas mobilis* subsp. *mobilis* ATCC 29191, which encodes a putative one-domain PPK2 protein of 261 amino acids, was cloned and overexpressed in *Escherichia coli*. Size exclusion chromatography indicated that the purified recombinant ZM-PPK2 protein adopts a stable tetrameric arrangement in solution. Its substrate range and biochemical activities were determined by analyzing reaction mixtures containing different nucleotide substrates using chromatography and gel-based approaches; in conjunction with fluorometric and spectrophotometric assays. Results indicated that purine 5′-monophosphates, e.g. AMP and GMP were efficiently phosphorylated to the corresponding 5′-diphosphates using poly-P as the phosphate donor. Pyrimidine 5′-monophosphates e.g. UMP were not utilized. To a lesser extent, ZM-PPK2 also catalyzed the transfer of phosphate units from poly-P to GDP to form GTP; but did not utilize ADP. Medium and long chain length poly-P substrates were utilized more effectively than short chain lengths, and the reverse reactions (i.e. polyphosphate synthesis) were not catalyzed to notable levels. Mg(II) or Mn(II) ions were essentially required and the activity of ZM-PPK2 displayed a broad pH optimum (pH 6.8 to 8.8). Studies are ongoing to establish its biological functions. In conclusion, our preliminary results suggest that the primary function of ZM-PPK2 is to utilize medium to long chain polyphosphate for the synthesis of GDP and ADP nucleotides.

Keywords: Bacteria, Enzyme, Polyphosphate metabolism.

WED-131
Functional gene responses in a perfect world – can we trust quantification of transcripts in soil in response to manmade chemicals?

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Analyzing environmental relevant microbial processes in situ is extremely challenging. Nucleic acid based studies are becoming more and more popular, but pitfalls exist and should be taken into account. In a perfect world we would know the genes involved, we would know the expression pattern, we would know the mRNA degradation rate, we would know the nucleic acid extraction biases, we would trust our DNase treatment, reverse transcriptase reaction and the quantitative PCR. Does the perfect world exist?

In a less perfect world the functional genes might not be known – or at least only some of them are known. Such a scenario might be when searching for *tfd*A degradation genes for modern herbicides in a soil originating from below a burial mound that has been isolated from the surrounding environment for more than 5000 year.

Another less perfect world would be RNA/DNA extraction from inorganic clay sediments – in this scenario the problem is that when cells are lysed in the nucleic acid extraction procedure the nucleic acids sticks to the clay due to the phosphate backbone. However, using an optimized RNA/DNA extraction protocol including the patented G2 blocking solution, we were able to obtain high-resolution expression profiles of the functional reductive dehalogenase genes *bvcA* and *vcrA* during two consecutive dechlorination events of trichlorethene (TCE), *cis*-dichlorethene (*cis*-DCE) and vinyl chloride (VC) in a clay subsurface environment. Up-regulation of the *bvcA* (for the biostimulated microcosms) and *vcrA* (for the bioaugmented microcosms) gene expression fitted well with high rates of dechlorination of VC, while no known transcripts could be measured during TCE and *cis*-DCE dechlorination. But is this trustable?

What is needed to further establish quantitative transcript based analysis of functional genes in environmental samples? – will we be able to adapt rules for gene expression as used in mammalian cells? Should a housekeeping gene be used for validation? – if so which?

Keywords: microbiome; soil; mRNA.

WED-132
Gene analysis of all two-component systems of *Thermus thermophilus* HB8 and their molecular cross-talk

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Bacterial proliferation and adaptation are governed by sophisticated signal transduction networks, including the versatile two-component systems (TCSs) that comprise sensor histidine kinases and response regulators and rely on phosphotransfer to exert their molecular function. Based on the comparative study of several databases involving genome and protein family domain annotations and that the complete genome of *Thermus thermophilus* HB8 has been sequenced, 11 HKs and 14 RRs were identified. The genes of all TCSs are located on the bacterial chromosome except for a cognate pair that is found on the pTT27 megaplasmid. For the functional characterization of all TCSs *in vitro* and the related phosphatases, the full-length coding sequences of the 14 RRs, 11 HKs and 2 protein phosphatases near to the TCSs, were amplified. Each amplified sequence was cloned into pET-29c vector and the overexpressed proteins were purified by affinity metal chromatography. Results from *in vitro* phosphorylation assays revealed the communication of the cognate pairs, as well as other intense interactions indicating the cross-talk between the non-cognate components. The biochemical and molecular exploration or new cellular functions in *T. thermophilus,* in which the TCSs participate to the bacterial signaling or to other mechanisms, remains to be elucidated.

Keywords: cell signaling, thermophiles,, two component systems.

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WED-133
Genetic variability of *Listeria monocytogenes* strains persisting in food processing plants

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Listeria monocytogenes is opportunistic foodborne bacterial pathogen that represents an important hazard to human health because it is capable of causing listeriosis mainly in newborns, elderly, immuno-compromised individuals, and pregnant women. Contamination of food products could be the be the result of *L. monocytogenes* persistence in the food processing plant.

In the present study, genetic variability of *L. monocytogenes* strains from meat processing plant were studied. Total amount of 33 *L. monocytogenes* strains were isolated from different places of food production plant, from food contact and non-food contact surfaces as well as raw materials, semiproducts and final products, during half-year sampling in years 2011–2014.

Identity of isolates was analyzed by PFGE, PCR-serotyping and MLST. Seven different PFGE profiles and three different serovars were distinguished, with preference contribution to PFGE profile 2 (21 %) and profile 9 (21 %) and serovar 1/2a (34 %), followed by serovar 1/2c (21 %) and 4b (21 %). While *L. monocytogenes* PFGE profile 2 strains were obtained regularly during whole sampling period, strains with profile 9 were isolated only since 2013 and repeatedly from three subsequent samplings. Our results emphasize the importance of environmental monitoring to identify potential contamination sources and transmission routes, particularly of *L. monocytogenes* persistent strains in food production chain.

Keywords: bacteria, Listeria, pathogen

WED-134
Genomic footprints of domestication in cheese-making fungi

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Domestication is an evolutionary process studied since Darwin times as a model of rapid selection and diversification. Much insight has been gained from the study of domesticated plants and animals, both on the type of genes under selection during phenotypic and molecular evolution, and on the genomic architecture of adaptation. The process of domestication has been much less studied in eukaryote microorganisms than in plants or animals. Domesticated fungi, such as the ones used for cheese production, represent ideal eukaryote models for studying the evolutionary processes involved in domestication. The bacteria and fungi used for producing cheese are amongst the earliest human domesticated micro-organisms, their evolutionary histories have been shaped by the strong selection imposed by growing in a nutrient-rich media. This selection implies some adaptation of cheese-making fungi to a novel, human-made, environment with many potential competitors, thus providing a good model for studying several aspects of rapid eukaryotic evolution. In this study, we compared the whole genome sequences of two fungal species widely used for the production of mold-ripened cheeses (*Penicillium roqueforti* and *P. camemberti*), with those of other three publicly available (*P. chrysogenum*, *P. rubens* and *P. digitatum*) plus five newly sequenced non cheese-making species from the same genus (*P. nalgiovense*, *P. biforme*, *P. fuscoglaucum*, *P. paneum* and *P. carneum*). Seven of these species are known to occur in the cheese environment, either as desired or contaminant species. We compared the whole genomes of the ten species in order to elucidate the genomic processes involved in the domestication of the cheese-making Penicillium and in the adaptation to the cheese substrate. For this goal, we looked for gene gains and gene losses, gene family expansions and genes showing cheese-making specific selection regimes. We also investigated the content and insertion sites of Transposable Elements (TE). In the end, we revealed that TE-mediated genomic events and gene family expansions have been crucial processes of adaptation as mechanisms allowing rapid acquisitions of novel functions while fine tuning of preexisting functions also occurred via amino-acid substitutions.

Acknowledgements: Partially supported by ERC GENOMFUN, ANR FOODMICRIBIOME, CNRS and UPSUD grants to TG and ANR FROMAGEN to AB. RCRV work received founding from the European Union Seventh Framework Program (FP7/2007-2013) under grant agreement COFUND PRESSUD No. 246556.
Keywords: Comparative genomics, Domestication, Fungi.

WED-135
Growth inhibition of Cronobacter in reconstituted infant milk formula using cocktail of T4-like and T7-like bacteriophages and antimicrobial human cathelicidin LL-37

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Cronobacter is a foodborne pathogen responsible for infections in neonates. Powered infant milk formula has been implicated as the vehicle of transmission. Bacteriophages can be used as perspective biocontrol agents against food contamination by these pathogens. The human antimicrobial peptide LL-37 of the cathelicidin family is a host defense molecule and a potent killer of different microorganisms. The aim of the present study was to determine efficiency of *Cronobacter* inactivation in reconstituted infant milk formula by new isolated bacteriophages and to observe synergism between bacteriophages and the antimicrobial peptide. Phage and cathelicidin inhibition of bacterial growth was measured in LB medium and in reconstituted infant milk formula. Different strains belonging to *C. sakazakii, C. turicensis* and *C. dublinensis* were used. Bacterial lysis in LB was measured by spectrophotometry, growth of bacteria in reconstituted milk was determined by colony counting. Bacteriophages used in experiments were isolated from waste water. According to the genome sequences they belonged to T4-like group of *Myoviridae* (Dev-CS-701 and Pet1) and to T7-like and KMV-like groups of *Autographivirinae* (Dev2, Dev-CD-23823 and Dev-CT-57). We observed that application of individual phages decreased numbers of *Cronobacter* cells in reconstituted infant formula and the highest reduction was obtained with Dev-CS-701 and Dev-CT-57 phages. By using high initial phage concentrations (10^8 PFU/ml) numbers of *Cronobacter* strains decreased under detection limit after 4–6 hours of cultivation. However, lowering phage concentration to 10^4 PFU/ml caused overgrowth of resistant bacteria in later periods of time. Application of mixture consisting of all phages showed the highest efficiency as it eliminated bacteria in both high and low phage concentrations. In further experiments the recombinant cathelicidin LL-37 expressed in *E. coli* system was employed to enhance the inhibition effect of phages. Cathelicidin LL-37 alone caused inhibition on bacterial growth in first two hours, but regrowth of the culture occurred after prolonged cultivation. Addition of LL-37 to phage treatment further decreased bacterial numbers in the range 1–2 log comparing to

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the phage alone experiment. Synergic effect of phages and antimicrobial peptides could be therefore used in food protection measurements.

Keywords: bacteriophage, food safety

WED-136
Gut microbiome in inflammatory bowel disease patients with and without arthropathy

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Introduction: Inflammatory bowel disease (IBD) is considered to result from an abnormal innate immune response elicited by components of the gut microbiota in genetically predisposed individuals. The gut microbiota shows alterations (dysbiosis) in IBD. Joint disease or arthropathy occurs in IBD. We hypothesized that the gut microbial alterations in IBD patients would be different in those with arthropathy compared to those without arthropathy.
Objective: To compare gut microbial phylotypes in IBD patients with and without arthropathy.
Methods: IBD patients were recruited from outpatient departments of Christian Medical College from January 2007 to March 2009. Clinical details, laboratory results, and severity of joint involvement (by rheumatologist, RG & DD) were entered into structured forms. Patients who had received antibiotics in the 60 days prior to study were excluded. This study was approved by Institutional research board. Fresh samples of stool were collected, DNA extracted and DNA libraries prepared using primers targeting hypervariable regions (HVR) 3 and 4 of the 16S rRNA gene using multiplex identifier sequence tags. The DNA libraries were sequenced in a 454 sequencing platform. The metagenomic diversity and phylogenetic analysis was assessed using the MG-RAST pipeline. Taxonomic comparison of bacteria between the groups was performed using Kruskal-Wallis test and pairwise Wilcoxon test in linear discriminate analysis effect size (LEfSe) program.

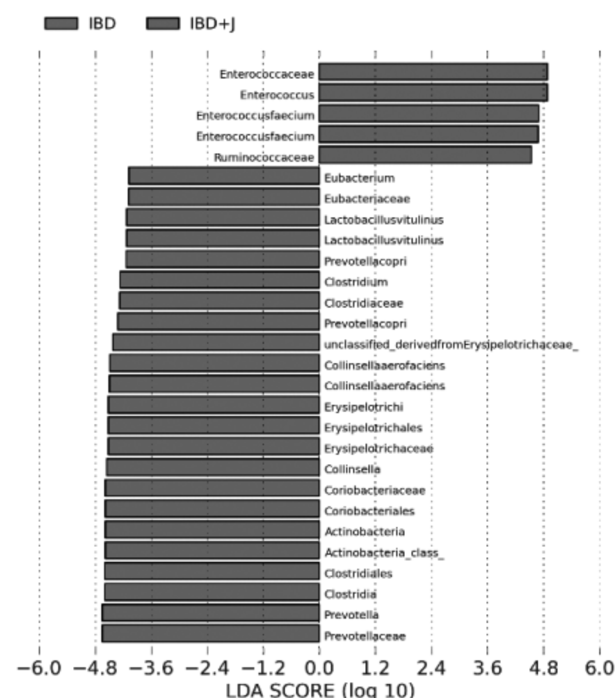

Fig. 1.

Results: Twenty four IBD patients (12 with and 12 without arthropathy) were recruited for study. Arthropathy patients included five each with isolated axial and peripheral and 2 with mixed type of joint involvement. A total of 800,968 reads were generated for the current study. The median read count of the samples was 24,884 (range 17,774–48,477). Alpha (Shannon) diversity index was significantly different between the groups (21.7 ± 4.4 Vs 38.41 ± 4.1; $p < 0.05$) with significantly higher diversity in IBD with arthropathy. The taxonomic comparison between the groups revealed that statistically significant differences in the microbial phylotypes were noted from Class to Strain levels. The important observation noted in the study that *Enterococcaceae, Enterococcus* and *Enterococcus faecium* were increased in IBD with arthropathy compared to IBD without arthropathy (Figure).
Conclusion: An increase in the abundance of *Enterococcus* and its species in the faeces differentiated IBD patients with arthropathy from those without arthropathy. Enterococcus may be relevant to the pathogenesis of arthropathy in IBD.

Keywords: 16s rRNA Gene, Microbiota, Pyrosequencing.

WED-137
HicA3-HicB3, a novel toxin-antitoxin system of *Yersinia pestis*

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Toxin-antitoxin systems (TASs) are widespread in bacteria. They are encoded by bicistronic operons born on plasmids or on the chromosome. In type II TASs, the toxin and antitoxin are both small cytoplasmic proteins. The toxin is usually stable and targets essential cellular processes such as replication, translation and cell division. The antitoxin is unstable and has a double function: it represses the TA operon promoter and neutralizes the toxin by direct binding. Under normal growth conditions, the level of expression of a TA operon is low due to autorepression by the antitoxin or the TA complex. Under stress conditions, the antitoxin is degraded by cellular proteases, the TA promoter is derepressed, and the toxin is then free to poison the cell. TASs have multiple roles including stabilization of mobile elements, biofilm formation, bacterial persistence or virulence.

In previous research, we found that a *Yersinia pestis* mutant lacking the HicB3 protein was attenuated for virulence in a murine model of bubonic plague. It had been suggested that HicB3 was an orphan antitoxin. Here, using biochemical and mutagenesis-based approaches, we identified the partner of the HicB3 antitoxin that we called HicA3. We demonstrated that the HicA3 toxin is an RNase with a catalytic histidine residue and that HicB3 neutralizes HicA3. In addition, HicB3 binds to two operators in the *hicA3B3* promoter region to repress the transcription of the *hicA3B3* operon. Thus, HicA3-HicB3 constitutes a new functional TAS of *Y. pestis*. We solved the X-ray structures of the HicB3 antitoxin and of the HicA3-HicB3 complex. HicB3 forms tetramers which can bind two HicA3 toxin molecules. The HicA3 RNase is monomeric and folds as a double-stranded RNA-binding domain. HicB3 occludes the HicA3 active site via its N-terminal domain, while its C-terminal domain folds as a Ribbon-Helix-Helix (RHH) DNA binding motif.

Keywords: Bacterial gene regulation, Crystal structure, Protein interactions.

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WED-138

Identification and characterization of plant cell wall degrading enzymes from analyses of genomic libraries of *Bjerkandera* adusta

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The use of lignocellulosic biomass as raw material in various industrial processes, has received much interest. Among the enzymes that degrade biomass, which are produced by different organisms, carbohydrate esterases (CE) and xilanases (Xil) are required for complete degradation of plant material. Hemicellulose shows modifications (presence of acetyl groups) that may be eliminated by the CE. In addition, Xil catalyse the depolymerisation of hemicellulose, being this an essential step for the conversion of lignocellulosic biomass. *Bjerkandera* is a genus that groups several Bacidiomycetes causing white rot of wood, and their enzymes have great potential for biotechnology applications. Performing an analysis of sequences from a cDNA library of *Bjerkandera* grown in the presence of crude oil, sequences with similarity to CE and Xil were found. Knowing the roles of these proteins, it will be interesting to characterize and express these genes in a heterologous system, allowing us to characterize biochemically. To predict the size of the transcript, Northern blot was performed, and full genomic and cDNA sequences were obtained using G.Walker and RLM-RACE kit, respectively. The cDNA was cloned into the pPICZ-αA vector, for expression in *P. pastoris* and the transformant strains was analysed. The complete gene for CE has an ORF of 470 aa and high identity with family 4 CE. The protein was partially characterized and shows a temperature and a pH optimum of 28°C and 6.0 respectively, and the substrate specificity was better for the 2-naphthyl acetate and 4-ethyl-p-Nitrophenol as compared to other substrates tested. Acetic acid release was measured from natural substrates, confirming the activity of deacetylase. These results correlate with the possible function of the protein predicted by in silico analysis. On the other hand, characterization of a Xyl gene demonstrated that this sequence was incomplete. Genome Walker and 5′RACE allowed progress in sequence from genomic DNA and cDNA. The sequence of the gene from genomic DNA is comprised of 1500 bp. Blastx with this sequence showed homology with fungal glycosyl hydrolases. Currently, we are working on the cloning and expression of this gene in *P. pastoris*, for the subsequent functional characterization of the enzyme. This study demonstrates that the application of genomic tools for characterization of new genes is a novel approach to study the physiology of poorly characterized organisms.

Keywords: Bjerkandera adusta, Carbohydrate esterase, xilanase.

WED-139

Improved baculovirus vector as high-productive expression system for developing a subunit vaccine against influenza

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Influeza virus causes morbility and mortality every year due to its high rate of mutation capacity. Production systems currently used for egg-based vaccines are time-consuming and expensive; thus, they would not be the best option neither in a possible pandemic situation nor in a seasonal-repetitive campaign of vaccination. So that, developing a fast, reliable and low-cost production system for facing this economical important disease has a high relevance. Recombinant vaccines are good candidates, moreover when these are producing using cost-cutting technologies such as baculovirus expression system (BEVS). Lately, this system has risen up as a trustable and quick approach to produce high yield of recombinant proteins. However, the lytic nature of this system implies baculoviral-infected cells undergoing to a severe cytopathic effect that usually ends in degradation of the expressed protein, interfering in its quantity as well as its quality. A recently described improved baculovirus expression vector (TOPBAC®) is characterized in this work as an alternative to overcome this bottleneck to produce the globular domains (aa 18-529) of hemagglutinin (HA) protein from mouse-adapted H1N1/PR/8/34/Pto Rico influenza virus. This vector has been previously described in our laboratory as a new vector that is able to minimize cytopathic effect and improve recombinant protein production rates. In the present research, we have compared the expression levels of this protein using the TOPBAC ® or a conventional expression vector in *Sf*9-suspension culture assays. Moreover, we have used two approaches for the improvement of the protein's folding pattern: the use of the signal peptide melittin (Mel), which facilitated an efficient secretory pathway in insects, or the endoplasmic reticulum retention signal (KDEL), that had been used before in other productive models to increase recombinant protein production. The results have shown a reduced cytopathic effect in TOPBAC® infected suspension cultures compared to those infected with the conventional vector and an improvement of the secreted protein yield: considering the best production conditions, increments of 1.2 times (in the case of the melittin version) and up to 4.5 times (in the case of KDEL option) have been achieved in supernatant fractions when TOPBAC® vector was used. Also, increments of around 2 times were observed in total protein production (supernatant plus intracellular fractions yields) in the case of KDEL signaling. In conclusion, this alternative system would allow a fast production of more vaccine doses at lower costs, giving an attractive and feasible technology to fight seasonal and, even, a pandemic, influenza attack.

Keywords: Baculovirus expression system, INFLUENZA VIRUS, Vaccine.

WED-140

Lactococcus lactis, a new bacterial factory for functional expression of membrane proteins

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In spite of the functional and biotechnological importance of membrane proteins, their study remains difficult due to their hydrophobicity and relatively low abundance in the cells. Moreover, in the well-known heterologous systems, these proteins are often produced at very low rates, often toxic and/or not correctly folded. In the last decade, *Lactococcus lactis*, a Gram-positive lactic bacterium, proved to be a valuable alternative system for functional expression of MPs. This bacterium, traditionally used in food fermentation, is now largely used in biotechnology for large-scale production of functional prokaryotic and eukaryotic MPs from diverse origins and functions. Initially, several yeast mitochondrial carriers were successfully expressed in a functional

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state. Then, both prokaryotic and eukaryotic ABC transporters could be expressed and characterized in their structural and functional manner. More recently, four chloroplast MPs of *Arabidopsis thaliana* tested could be produced at levels compatible with further biochemical analyses (1,2). Processes of solubilization and purification could also be developed and several proteins were active (1,3). Interestingly, few human proteins were expressed in active forms in this system (4, unpublished data). These recent data suggest that *L. lactis* can be an attractive system for effective and functional production of difficult membrane proteins.

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Keywords
lactic acid bacteria, membrane proteins, NICE system.

WED-141
LC-MS analysis of antimicrobial peptides from *Lactobacillus plantarum* 8PA-3

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Fighting against pathogenic microorganisms is very essential nowadays. Bacreriocins – bacterial antimicrobial proteins and peptides can be considered as potential antimicrobial and antifungal agents which regulate bacterial population growth and provide colonization stability of human body. Sequencing of genome of commercial probiotic strain *Lactobacillus plantarum* 8PA-3 found a locus that can be responsible for synthesis of two bacteriocins EF and NC8.

The mostly potent against standard test-cultures strain L. plantarum 8PA-3 was chosen for purification and characterization of antimicrobial peptides. Peptides were extracted from culture using 5% acetic acid and afterwards were ultrafiltrated through 10 kDa and 1 kDa membranes. Fractionation of peptides were performed using cation-exchnge chromatography on CM-sephadex G25 column and RP-HPLC on Discovery HS C18 column on ÄKTAexplorer 10S instrument. For testing antimicrobial activity of peptide fractions were used radial diffusion and overlay methods. Molecular weights of fraction's components were calculated using LC-Q-TOF instrument Agilent Tehnologies (LC-1260, MS-6538 UHD Accurate-Mass Q-TOF).

During LC-MS analysis of mostly active fraction of peptides (molecular weights 1 - 10 kDa) were found a lot of single-charged components of culture medium MRS (m/z 300n - 750) and some peptides with MW: 2946,7 Da; 3784,2 Da; 3883,9 Da; 3896,2 Da; 3900,0 Da; 4611,1 Da; 5454,9 Da and 6280,4 Da.

Despite of strong antimicrobial activity of low molecular weight peptides fraction from Lactobacillus plantarum 8PA-3 MW of characterized using LS-MS peptides don't mach to MW of predicted bacteriocins.

Work was made within the terms of SRW (scientific research work) SPbSU Ф-№0.37.123.2011 "Morphophysiological and biochemical aspects of bacteriocins antimicrobial action".

Keywords: bacteriocins, Lactobacillus plantarum, antimicrobial peptides.

WED-142
Molecular analysis of the acid phosphatase from *Trichoderma harzianum* ALL 42

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Acid phosphatase (ACP) is a hydrolase that converts organic phosphate into a soluble inorganic form by monoester phosphate hydrolysis and transphosphorylation reactions. It has been found in seeds and roots of the plants; kidneys, prostate, bones; in mycelia and secretions of microorganisms, presenting different physiological activities. These enzymes have been applied in clinical diagnostic and remediation processes recycling organic phosphate. *Trichoderma harzianum* is a saprophytic fungus worldwide known as a biological control agent, able to produce hydrolytic enzymes of economic interest. In this work we purified, sequenced and identified a new acid phosphatase from *T. harzianum*. The PCR and RT-PCR assays was done and the genomic DNA was analyzed (gene and Open Reading Frame) by Sanger sequencing. The crude extract was obtained from culture of spores previously inoculated in minimum medium 1.5% glucose and 20-fold concentrated by ultrafiltration. The protein was purified by molecular exclusion chromatography on a Superdex 200 column using FPLC facility (GE Healthcare) in 0.15 M sodium acetate pH 4.8. The identification of the enzyme was carried out based on amino acid sequence of seven peptides, obtained from the protein hydrolyzed with trypsin, using MALDI-TOF/TOF spectrometry and compared to those sequences from GenBank. Enzyme production was 11.33 ± 0.10 U/mg and molecular weight of 95 kDa. A broad substrate specificity was observed for this enzyme, specifically glucose 1-phosphate, phenylphosphate and phytic acid. The optimum pH and temperature were estimated in 3.8 and 65°C, respectively, using pNP-P substrate. The peptides sequences are similar to phytase of *T. harzianum*, histidine acid phytase of *T. pleuroticola* and a protein of *T. virens*. The RT-PCR assays revealed high expression in medium containing only glucose as a carbon source. Semi-quantitative PCR fragments were amplified containing gene with five introns and ORF with signal peptide of 21 amino acids. Genomic DNA was sequenced and compared with those described in JGI. It presented some differences considering the *T. harzianum* strain. Taken together, the sequence analysis of peptides and biochemical features and sequencing genes allow to conclude that the purified protein is a bifunctional enzyme belonging to the acid phosphatase and phytase families.

Supporting: UNB, CNPq, CAPES, FAP-DF, UFG.

Keywords: Acid phosphatase, Phytase, Trichoderma harzianum.

WED-143
Mycobacterial cell wall components – oligosaccharide-oligopeptides – are resuscitation inducers of the dormant mycobacteria

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Since the R. Koch's discovery of the causative of tuberculosis - *Mycobacterium tuberculosis* in 1882, the world tuberculosis pan-

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demic is still a crucial problem of the society. In spite of the fact, that chemotherapeutic approaches have already been known for 50 years, one third of population carries tuberculosis infection in a latent state, being in a constant risk of transformation of the latent disease into the active form. Few years ago in our lab a protein Rpf (Resuscitation promoting factor), that stimulates reactivation of the "non-culturable" mycobacteria (incl. *M. tuberculosis*) into the active state was discovered. It had been proven, that Rpf possess enzymatic activity, namely – peptidoglycanhydrolase. But then the question arises how both activities - resuscitation and enzymatic are bound together. We supposed first, that low-molecular weight metabolites oligosaccharide-oligopeptides might be released in milieu as a result of peptidoglycan Rpf hydrolysis. These products presumably could serve as a signal directly toward the cell and the other surrounding cells. Though, it is still unclear, which compounds are being released under peptidoglycan Rpf treatment and which of them directly induce reactivation. In the current study we demonstrated the ability of Rpf to hydrolyze mycobacterial cell wall peptidoglycan, and it has been shown that, as low molecular weight as well as high molecular weight products were able to stimulate resuscitation of the dormant mycobacteria. High molecular weight products, as a matter of fact, served as a substrate for Rpf, with subsequent release of the products with the lower molecular mass. We identified, that the end product of mycobacterial PG joint degradation by Rpf with endopeptidase RipA (resuscitation promoting factor interacting protein) is N-acetylglucosaminyl – (ß1→4)-N-glycolyl-1,6-anhydromuramyl-L-alanyl-D-glutamin. Comparison of the resuscitation activity of some synthetic muropeptide-derivates allowed us to suggest, that disaccharide-dipeptide is a key molecule, triggering resuscitation process.

Mycobacterial cell wall components – oligosaccharide-oligopeptides – are resuscitation inducers of the dormant mycobacteria

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Since the R. Koch's discovery of the causative of tuberculosis - *Mycobacterium tuberculosis* in 1882, the world tuberculosis pandemic is still a crucial problem of the society. In spite of the fact, that chemotherapeutic approaches have already been known for 50 years, one third of population carries tuberculosis infection in a latent state, being in a constant risk of transformation of the latent disease into the active form. Few years ago in our lab a protein Rpf (Resuscitation promoting factor), that stimulates reactivation of the "non-culturable" mycobacteria (incl. *M. tuberculosis*) into the active state was discovered. It had been proven, that Rpf possess enzymatic activity, namely – peptidoglycanhydrolase. But then the question arises how both activities - resuscitation and enzymatic are bound together. We supposed first, that low-molecular weight metabolites oligosaccharide-oligopeptides might be released in milieu as a result of peptidoglycan Rpf hydrolysis. These products presumably could serve as a signal directly toward the cell and the other surrounding cells. Though, it is still unclear, which compounds are being released under peptidoglycan Rpf treatment and which of them directly induce reactivation. In the current study we demonstrated the ability of Rpf to hydrolyze mycobacterial cell wall peptidoglycan, and it has been shown that, as low molecular weight as well as high molecular weight products were able to stimulate resuscitation of the dormant mycobacteria. High molecular weight products, as a matter of fact, served as a substrate for Rpf, with subsequent release of the products with the lower molecular mass. We identified, that the end product of mycobacterial PG joint degradation by Rpf with endopeptidase RipA (resuscitation promoting factor interacting protein) is N-acetylglucosaminyl – (ß1→4)-N-glycolyl-1,6-anhydromuramyl-L-alanyl-D-glutamin. Comparison of the resuscitation activity of some synthetic muropeptide-derivates allowed us to suggest, that disaccharide-dipeptide is a key molecule, triggering resuscitation process.

Fig. 1.

Keywords: Muropeptides, Mycobacterium tuberculosis, Resuscitation promoting factor

WED-144
Nanoparticle-based paper biosensors for visual detection of infectious diseases pathogens

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Paper-based sensing has emerged as an attractive analytical platform for pathogen diagnostics. Lateral flow paper biosensors (LFB) are optimal for accurate, rapid and sensitive analysis in research laboratory setups, but they have the potential to be used for field analysis. Especially the use of functionalized nanoparticles, as recognition labels has accelerated nucleic acids/ proteins sensing by LFBs, by increasing their sensitivity and specificity. LFBs are important for diagnostic purposes, such as to ascertain presence of infectious diseases, contamination with pathogens including biowarfare agents and presence of toxic compounds in food or the environment. Aim of the present study was to develop novel LFBs utilizing Au nanoparticles conjugated with specific antibodies or oligonucleotides for virus or parasite detection. Since aquaculture is one of the most important sectors of Greek and European economies with great environmental implications, we focused on viral nervous necrosis virus (VNNV) or Nodavirus; however adaptation of the proposed LFB for other viruses is feasible. In brief, Nodavirus particles were isolated from infected fish samples and applied on the LFB or mixed with specific polyclonal anti-Nodavirus antibodies before application. Specific anti-Nodavirus monoclonal or polyclonal antibodies were immobilized on the LFBs membrane, where antibody-conjugated Au NPs were also deposited. A red test line was formed when Nodavirus particles were present. The visual detection was completed within 30 min. In parallel, a lateral flow biosensor was developed for the parasite Leishmania detection. Infectious diseases caused by Leishmania affect humans with high killing rates in developing countries, but they also affect dogs, which are their main animal reservoirs. In this case, total DNA, isolated from canine samples was subjected to PCR amplification. The PCR products were mixed with specific oligonucleotide probe and applied next to oligonucleotide or antibody conjugated Au NPs. A red test line was formed when Leishmania product was present. The visual detection was completed within 20 min. Apart contributions on basic research, the proposed biosensors offer great potential for commercial kit development. This fact will have great impact on environmental safety and disease monitoring without time consuming and costly procedures.

Acknowledgements: The research project is implemented within the framework of the Action «Supporting Postdoctoral Researchers» of the Operational Program "Education and Lifelong Learning" (Action's Beneficiary: General Secretariat for Research and Technology), and is co-financed by the European Social Fund (ESF) and the Greek State.

Keywords: Biosensors, Infectious diseases, Nanoparticles.

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WED-145

Novel nano-coating surfaces based on hydrophobic polymers and phytostructures prepared by MAPLE with increased resistance to microbial colonization

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In recent years antibiotic resistant infections emerged and many Gram-positive and Gram-negative bacterial strains represents one of the major health and survival threats for hospital environment. This study reports novel surfaces based on hydrophobic polymers e.g. poly(lactic-co-glycolic acid), poly(lactic acid) and essential oils/phytostructures prepared by matrix assisted pulsed laser evaporation (MAPLE), to be used as anti-adherent coating for medical surfaces, in order to improve their resistance to microbial colonization and biofilm formation. The prepared surfaces were characterized by Transmision Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD), Differential Thermal Analysis (DTA) and Infrared Microscopy (IRM). Microbial biofilms developed by the tested Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) microbial strains were analyzed using SEM and culture-based assays for up to 3 days of incubation at 37°C, in order to establish the biofilm dynamic in different experimental conditions. *In vitro* citotoxicity was evaluated by the qualitative and quantitative analysis of the phenotypic changes of the treated eukaryotic cells up to 5 days, using flow cytomertry and MTT assay. *In vivo* biocompatibility was established by using a balb C mouse model, treated for up to 14 days with standardized suspensions of the tested bioactive nanostructures. SEM micrographs revealed uniform morphologies of the prepared coatings, while IRM proved the functional groups integrity and the homogeneity of the thin coatings. Microbiology results showed that the obtained thin coatings efficiently inhibited bacteria tested strains adherence and biofilm formation at different time points, in a strain and coating material type manner. These results, along with the great biocompatibility of the tested materials recommend the functionalized surfaces with hydrophobic polymers and essential oils/phytostructures prepared by MAPLE to be successfully used in the development of improved medical surfaces, resistant to microbial colonization.

Keywords: anti-biofilm surface, MAPLE deposited bioactive thin films, nano-coating.

WED-146

Photophysical properties of porphyrins as a selection criterion of porphyrins for study of their effectiveness against microorganisms

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One of the most promising directions of destruction of antibiotic-resistant microorganisms is photodynamic inactivation (PDI) of microorganisms by photosensitizers (basically porphyrins). Currently it is generally accepted that singlet oxygen is the main cytotoxic agent in phototherapy of cancer tissues and in PDI. Among the characteristics of photosensitizers the generation of singlet oxygen is the determining criterion to ensure the effective action of porphyrins. We studied photophysical properties of 16 new porphyrins and metalloporphyrins and has been shown that Zn-containing metalloporphyrins have higher values of quantum yields of singlet oxygen generation than the metal-free porphyrins (85–97% and 77–79%, respectively). The efficacy of these compounds was tested on a number of Gram (+) and Gram (-) microorganisms. Among the Gram (+) microorganisms one of the most dangerous is an antibiotic-resistant strain of the microorganism *Staphylococcus aureus* (*St. aureus*). In the U.S., the number of deaths due to methicillin-resistant strain *St. aureus* is equivalent to the total number of deaths from AIDS, tuberculosis and viral hepatitis. The objects of our study were the strain *St. aureus* 209 P and the strain *St. aureus*, methicillin-resistant. Using the criterion of the quantum yield of singlet oxygen generation we studied the effectiveness of four types of porphyrins: meso-tetra [4-N-(2′-oxyethtyl) pyridyl] porphyrin (TOEt4PyP)-(I); Zn-TOE4PyP-(II); Zn-meso-tetra [4-N-butyl pyridyl] porphyrin (Zn-TBut4PyP)-(III) and Zn-TBut3PyP-(IV). It was found that against both strains of *St. aureus* the highest activity has metalloporphyrin (IV) when the concentration of metalloporphyrins was 0.01 μg/ml, which correlates with the values of the quantum yields of singlet oxygen (97% for compounds III and IV). Destruction efficiency of microorganism *St. aureus* 209 P by compound (IV) in comparison with compound (I) was higher more than 8 times that would be expected from the values of quantum yield of singlet oxygen generation (for the compound (I) is 77%). Close results were also obtained for methicillin-resistant strain of *St. aureus*: in the same conditions, the highest efficiency was of the compound (IV) - 2.5 times higher than for the compound (I). Considering that the compounds (III) and (IV) are practically non-toxic, these compounds were recommended for studies *in vivo*.

Keywords: microorganisms, porphyrins, singlet oxygen.

WED-147

Poly (amidoamine) (PAMAM) dendrimers: physicochemical characterization for multifunctional applications

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In this work, we used dynamic laser scattering (DLS) and viscosity measurements to characterize in detail the properties of G6 PAMAM dendrimers in electrolyte solutions. These measure-

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ments were supplemented by electrophoretic mobility measurements, which allowed us to determine the isoelectric point and the uncompensated (electrokinetic) charge of the dendrimers. These results provide insight into the dynamic structure of PAMAM dendrimers in solutions.

The adsorption of PAMAM dendrimers onto silica surface was studied with quartz crystal microbalance (QCM-D) and surface plasmon resonance (MP-SPR) measurements. These measurements allowed the determination of both the kinetics of adsorption and the maximum coverage of PAMAM. We found that the thickness of the PAMAM films depends strongly on the pH and ionic strength of the solution that influences swelling of the PAMAM films. Our results supply the first compelling experimental evidence for significant swelling of G6 PAMAM dendrimer upon its protonation at the solid/solution interface. This phenomenon is a consequence of spatial relocation of the dendrimer amide groups due to the interactions of the positively charged amines with the oppositely charged condensed counterions and the penetrating water molecules. Insight into the interfacial phenomena is essential for designing materials dedicated to biological applications.

Our results have also a practical merit, because, they indicate that dendrimers may be used as stimulus-responsive drug delivery carriers, which can release a trapped drug upon a conformational transition induced in the dendrimer molecule by change in pH or the ionic strength.

Acknowledgement: This work was supported by Grant NCN OPUS4 2012/07/B/ST5/00767.
Keywords: Dendrimers Adsorption, Quartz Crystal Microbalance (QCM-D), Surface Plasmon Resonance (MP-SPR).

WED-148

Polyamines upregulate the formation of *E. coli* persister cells through the modulation of rpoS expression

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Background: Transition of a bacterial culture to stationary phase is accompanied by an increase in the number of persister cells - quasi-dormant variants of regular cells that are highly tolerant to antibiotics. This property of persister cells establishes requirements for the development of technologies and drugs targeted to their killing. Our previous investigations exhibited that a decrease in cell antibiotic tolerance can be achieved through the inhibition of key enzymes for the polyamine synthesis.
Objectives: In this work we therefore tried to discern if polyamines could promote the persister cell formation, which is one of cell strategies for the survival at lethal antibiotic impacts.
Methods: HPLC fluorescent method was used for determination of cell polyamines. In order to obtain the pure RpoS protein in the level enough for Western blot analysis, the overexpressing *E. coli* strain BL21DE3 pET19b rpoS was constructed. Western blot was used for assessment of cell RpoS protein. *rpoS::lacZ* operon fusion was transferred to *E. coli* HT306 with λRZ5. Gene expression was determined by β-galactosidase activity.
Conclusions: Data obtained in our experiments with the polyamine-deficient *E.coli* strain revealed that the formation of persisters tolerant to netilmicin was highly upregulated by polyamines in a concentration-dependent manner when the cells entered the stationary phase. Furthermore, the more tolerant persister fractions were formed on the later stages of stationary phase. We investigated dependence of persister formation on the expression of *rpoS*. It was shown that polyamine-dependent upregulation of *rpoS* expression reinforced persister cell formation during cell transition to stationary phase. These data were obtained with three independent experimental approaches: polyamine supplementation of polyamine-deficient mutant harboring *rpoS::lacZ* fusion, an ectopic expression of *rpoS* overexpressing plasmid and reciprocal experiments with the pair of isogenic *E. coli* K12 *rpoS*⁻ and *rpoS*⁺ strains. The latter approach enabled us also to assess quantitatively a contribution to persister cell formation of each of two players implicated in this process – RpoS and putrescine. Their complementary activity resulted in almost 1000-fold positive effect on persister cell formation.

This work was supported by the Program of Presidium RAS “Molecular and cellular biology” (project #12-П-4-1047) and grant from the Russian Foundation for Basic Research #13-04-96002.
Keywords: Antibiotic tolerance, Persisters, polyamines.

WED-149

Preparation of an iutA/pVax DNA cassette as a DNA vaccine candidate against urinary tract infection

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Urinary Tract Infection (UTI) is one of the most infectious diseases in human and Uropathogenic Escherichia coli (UPEC) strains are major agent of UTI. These strains colonize in bladder tissue and cause cystitis. Moreover, they could cause pyelonephritis if they get to kidneys. IutA protein has a major role during UPEC pathogenesis and consequently infection. In this study, IutA selected as a candidate immunogen. Genomic DNA was extracted from E.coli 35218 and *iutA* gene amplified by PCR. The amplicon was cloned to pBluescript vector and confirmed by sequencing. Subsequently, inserted to pVax expression vector and final pVax/iutA clone was verified by sequencing and transferred to COS7 cell line. Efficiency of pVax/ iutA expression in COS7 verified by RT-PCR. Mouse model divided to three groups and they injected with pVax vector, PBS and pVax/iutA cassette respectively in two stage (Days 1 and 14). One week after the second injection, bleeding from immunized mouse was performed and IgG, IgG1 and IgG2a sub classes were measured. Potency of Immunization was more in mouse that received DNA vaccine and shows higher cellular immune response. It signifies that houmoral immune response has a main role in prevention of bacterial colonization on bladder and cellular immune response could be efficient in preventing of recurrent infection. So, the current DNA cassette will be valuable for more trying to prepare a new vaccine against UTI.

Keywords: Genetic vaccination, Uropathogenic Escherichia coli, IutA

WED-150

Preparation of suitable culture medium for high level expression of human recombinant prethrombin-2 in *Escherichia coli*-based expression system

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Thrombin is a key enzyme of blood coagulation and its recombinant form is often used in pharmaceutical industry as a topical

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hemostatic reagent. *Escherichia coli*-based expression system is one of the most commonly used expression systems for heterologous expression of industrially important recombinant proteins. This expression system is characteristic of its simplicity and efficiency. In this work prethrombin-2, the smallest thrombin precursor, was used. Prethrombin-2 forms inactive intracellular inclusion bodies during the expression, therefore the refolding process to obtain an active conformation is necessary. Since refolding often does not lead to the desired yields of biologically active recombinant protein, the optimizing of protein expression is the first and key step. Here we demonstrate preparation of suitable medium composition for optimal cell growth in high cell density during the batch fermentation in bioreactor, which is crucially related to obtaining high yield of target protein. In this work a new optimized complex medium for batch fermentation was developed. Medium creation was based on the observation that the medium should not only contain essential ingredients, but it is also necessary to find a suitable concentration of the each component. Our results showed that addition of nutrients like a yeast extract and enzymatic casein hydrolysate to the defined medium components further supplemented with 4% glycerol had positive impact on protein expression. Using this medium, the amount of the target protein reached almost 50% of total cellular proteins

This work is result of project implementation: "Production of biologically active agents based on recombinant proteins" (ITMS 26240220048) supported by the Research and Development Operational Program funded by the ERDF.
Keywords: Culture medium composition, Escherichia coli, Human prethrombin-2.

WED-151
Production and characterization of exopolysaccharides synthesized by geobacilli isolated from an Armenian geothermal spring

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Exopolysaccharides (EPS) produced by microbes have found a wide range of applications in the food industry, medicine, agriculture, and wastewater treatment. During last decade a new approach to encountering EPS with novel properties is investigating different extreme environments, including thermal habitats. Among the various thermal ecosystems geothermal springs are proving to be a precious source of thermophiles that are able to produce EPS with unique characteristics.

The aim of this study was to determine composition and chemical-physical properties of EPSs produced by two geobacilli strains isolated from Arzakan, Armenia (located 40°26.902′N, 44°36.508′E, 44°C, pH 7.3) geothermal spring.

The phylogenetic analysis of 16S rRNA gene sequence of EPS producing isolates, designated as ArzA-6 and ArzA-8, referred them to *Geobacillus thermodenitrificans* (98% similarity) and *G. toebii* (98% similarity), respectively. The EPS yields using different carbohydrates (glucose, fructose and saccharose), added in the medium at the optimal growth temperature (65°C), were analyzed. Fructose displayed to be the most appropriate carbon source for EPS production. Maximum EPS production was reached in the stationary phase of growth. Indeed, the highest concentration of EPSs harvested after 48 h of culture were approximately 270 mg/g dry cells and 220 mg/g dry cells for *G. thermodenitrificans* ArzA-6 and *G. toebii* ArzA-8, respectively. Purified EPSs displayed a high molecular weight: 5×10^5 Da for *G. thermodenitrificans* ArzA-6 and and 6×10^5 Da for *G. toebii* ArzA-8. Chemical composition of the biopolymers, determined by using a high pressure anion exchange-pulsed amperometric detector (HPAE-PAD), showed the presence of Man/Gal/Ara/Fru/Glu/Sac in a relative proportion of 1/0,13/0,1/0,06/0,05/trace for the *G. thermodenitrificans* ArzA-6 and Man/Gal/Glu/Ara in relative proportion of 1/0,5/0,2/0,05 for the *G. toebii* ArzA-8. Optical rotation values $[a]_D^{25°C}$ (2 mg ml^{-1} H_2O) of EPSs were -142,135 and -128,645 for *G. thermodenitrificans* ArzA-6 and *G. toebii* ArzA-8, respectively.

The work was supported by the FEBS Short Fellowship-2009.
Keywords: Exopolysaccharides,, Geobacillus, thermophiles.

WED-152
Production of deleted form of human enterokinase light chain in *Pichia pastoris*

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Enterokinase, serin protease, highly efficiently catalyzes the hydrolysis of the polypeptide chain downstream of the N-terminal recognition sequence $(Asp)_4Lys$. Its high specific enzymatic activity and its tolerance to a wide range of reaction condition are the main characteristic features that make enterokinase a useful tool for an *in vitro* cleavage of various fusion proteins. The light chain represents catalytic domain of serin protease. Disadvantage of this enzyme is ability to cleavage besides canonical sequence. For increasing efficiency of enzymatic digestion is necessary eliminate unspecific proteolytic activity. This could be achieved by creating form with shorter amino acid sequence coding enterokinase light chain. In our work, light chain of enterokinase with deleted N-terminal and C-terminal end was developed. This modified form of enterokinase was produced in methylotrophic yeast *Pichia pastoris* in shake-flask. Subsequently, enzymatic activity of these two mutated form were compared with unmodified enterokinase light chain by digestion dermcidin 1 fused with thioredoxin. However, both of these mutated enzyme forms showed no activity in cleavage this fusion protein.

This publication is the result of the project implementation: "Competence center for research and development in molecular medicine" (ITMS 26240220071) supported by the Research and Development Operational Program funded by the ERDF.
Keywords: heterologous expression, human enterokinase light chain, Pichia pastoris.

WED-153
Production of recombinant human growth hormone in *Pichia pastoris* under constitutive promoter

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The growth deficiency in children is usually cured with a recombinant human growth hormone (rhGH) intravenous application. The hormone improves patient's condition in case of the Turner′s syndrome or cachexia associated with AIDS also. It was one of the first recombinant proteins approved for the usage in pharmaceutical industry. To this day, rhGH has been produced in different host organisms as *Escherichia coli, Bacillus subtilis, Saccharomyces cerevisiae* etc. The rhGH was successfully prepared in yeast *Pichia pastoris* under the strong inducible AOX promoter lately. In our study, the expression system in *P. pastoris*

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for the production of rhGH under a constitutive promoter was designed and implemented. The rhGH gene was integrated into genome of *P. pastoris*, in order to produce native rhGH into the extracellular media. The genome integration was validated through a PCR and expression in flasks. The SDS-PAGE results were confronted with a Western blot analyses. rhGH was then successfully produced in 1L fermenter and partially purified using anion-exchange chromatography. *P. pastoris* was chosen as the host organism because of its ability to grow to high cell densities and its strong promoters capable of the production of high yields of a recombinant protein. The system combines both, advantages of prokaryotes – high expression, simple scale-up, cheap production media; and advantages of eukaryotes – ability to perform various post-translation modifications.

This publication is the result of the project implementation: "Competence center for research and development in molecular medicine" (ITMS 26240220071) supported by the Research and Development Operational Program funded by the ERDF.
Keywords: constitutive promoter, human growth hormone, Pichia pastoris.

WED-154
Production of thermostable lipases by thermophilic bacilli

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Thermophilic microbes, thrived in the extreme environments, are interesting sources for the thermozymes. Particularly thermostabile hydrolases, including lipases are known as one of the most imperative biocatalysts in today's epoch. Thermostabile lipases were purified and characterized from thermophilic isolates mainly belonging to genus Bacillus and related genera, which nowadays are used in industry.

The present study was aimed to isolate extracellular thermostable lipase producing bacilli and to optimize their growth conditions affecting the lipase production and activity. Two bacilli strains namely Bacillus licheniformis Akhourik 106 and B. aestuarii Akhourik 107 isolated from geothermal spring of Akhourik (Armenia) served as subjects of study. The thermostable lipase was estimated by qualitative and quantitative analysis. The enzyme production was investigated in inducing medium containing Tween 40, 60, 80 (1%) and/or olive oil (1%), CaCl2x2H2O (0.05%), which visualize a halo close around the colonies. The lipase activity was measured by alkalimetric titration method at temperature range of 25°C–65°C and pH range of 5–10.

The temperature of 56°C, 0.5% salinity level and pH 6.5–7 were defined to be the optimal conditions for lipase activity. Maximal activity level was determined on the late stages of bacterial exponential growth. The activity values were 0.03 U ml-1 min-1 and 0.027 U ml-1 min-1 for B. licheniformis Akhourik 106 and B. aestuarii Akhourik 107, respectively. The thermal stability in aqueous solution of the crude enzymes was also assayed.

The work was partially supported by MES of RA and ANSEF-2011 Microbio-2493.
Keywords: lipase activity optimization, thermophilic bacilli, thermostable lipases.

WED-155
Protein-protein and protein-DNA interactions during replication proces of plasmid DNA

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Many plasmids are able to replicate in their original host and related species, and some of them have an extended host range which allows their establishment in several different bacterial species. At least two essential processes contribute to the host range phenotype: replication and partition Theplasmids became more interesting when scientist discovered that many plasmids contain genes responsible for antibiotic resistance and are responsible for the spread of antibiotic resistance among pathogen bacteria. Plasmids can give its host other kinds of resistance e.g. against heavy metals, bacteriocins, radiation and toxic anions. In this situation, the parasite host relation is more symbiotic, since the host gain some benefits. Many plasmids today are very important tools in gene technology and science e.g., as cloning vectors and expression vectors. In the previously isolated pAG20 plasmid from the Acetobacter aceti CCM 3620 strain, the Rep20 protein was characterized as a main replication initiator. The pAG20 plasmid origin was localized in the vicinity of the rep20 gene and contained two 21-nucleotide-long iteron sequences, two 13-nucleotide-long direct repeats, and a DnaA-binding site. Electrophoretic mobility shift assay and nonradioactive fragment analysis confirmed that the Rep20 protein interacted with two direct repeats (5′ TCCAAATTTGGAT 3′) and their requirement during plasmid replication was verified by mutagenesis. 9 amino acids of the third a-helix (between 58 and 66 amino acids) play a key role in the Rep20-specific binding to theoriregulation region. Similarly, the region (9 amino acids between 49 and 57 amino acids) in the middle of the second and the third α-helices, containing the β-sheet structure, appeared to be also important for DNA binding. Targeted mutagenesis of the Rep20 protein revealed the importance of the third a-helix and $_{63}$Lys, specifically during DNA binding.The second, closely adjacentb-sheet also took part in this process in which $_{52}$Asn played a significant role to binding Rep20 to DNA.

Keywords: protein – DNA interactions, Replication.

WED-156
Protonophores affecting *Rhodobacter sphaeroides* growth and hydrogen photo-production

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Purple non-sulfur bacteria has been established to produce hydrogen (H_2) under anaerobic conditions upon illumination that is mediated by nitrogenase catalyzing conversion of protons to H_2 by using energy from ATP [1]. It was shown that cyanide m-chlorophenylhydrazone (CCCP), a protonophore, which dissipates the proton gradient and inhibits the synthesis of ATP, suppressed nitrogenase activity in the other bacteria – cyanobacteria [2]. But effects of protonophores on purple bacteria are not known yet. In this study the comparative analysis of the effects of different protonophores such as CCCP and 2,4-dinitrophenol (DNF) on growth and H_2 production by *Rhodobacter sphaeroides* MDC6521 from Arzni mineral springs in Armenia was done.

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Both uncouplers affected the bacterial growth rate. *R. sphaeroides* was unable to grow in medium with CCCP and DNF, and addition of protonophores after 24 h growth of bacteria inhibited growth. The medium pH and redox potential are considered as the important factors evaluating bacterial growth under various conditions [1]. During the *R. sphaeroides* growth up to 24 h, pH of medium increased from 7.5 (initial pH) to ~8.0. By addition of CCCP (0.5–2 mM) pH decreased to ~7.4 during the growth up to 72 h. However, different kinetics of pH was observed after addition of DNF (5–50 mM): pH increased to ~9.0. Redox potential of *R. sphaeroides* control cells decreased down to -690 mV during the growth up to 72 h. CCCP (2 mM) delayed drop in redox potential, which was decreased to -135 mV. In the presence of 5 mM DNF redox potential dropped to -660 mV; whereas with 50 mM DNF potential drop was to -170 mV only. Both uncouplers inhibited H_2 production: H_2 yield lowered ~1.2-fold in the medium with 5 mM DNF, and was not observed in the presence of CCCP and 10–50 mM DNF. It is suggested that protonophores can decrease H_2 production by suppressing nitrogenase or proton-translocating ATPase activity. This study would be helpful for understanding the mechanisms of protonophores effect on H_2 production by purple bacteria and its application in H_2 biotechnology.

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Keywords: Hydrogen production, Protonophores, Redox potential.

WED-157
Real-time PCR optimization to identify *Mycobacterium tuberculosis* complex strains in clinical samples

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During recent years several molecular techniques have become available for *Mycobacterium tuberculosis* complex (MTC) detection, both for clinical samples and for isolates. One of the techniques more widely used is real time PCR in combination with nucleic acid amplification protocols. There are numerous studies based on PCR for the diagnosis of tuberculosis although the different protocols and primers used in the laboratory, together with the variability in the diagnostic performance of the methods tested, require that a comparative study be performed. Furthermore, the fact that the detection from clinical samples requires using highly sensitive targets suggests that this type of study should include multicopy targets to compare their efficiency with respect to the single copy. Our aim was to identify the members of the MTC using real-time PCR assays based on SYBR Green, among a large panel of isolated bacterial strains and clinical samples. We chose three targets (IS6110, senx3-regx3 and cfp32) and the optimal values for each PCR assay were empirically defined by testing in triplicate different concentrations of $MgCl_2$ and primer sets and different annealing temperatures. These conditions were determined based on the specific amplification reactions that showed a lower Ct value, higher fluorescence and absence of non-specific PCR products. The analytical sensitivity was evaluated by ten-fold serial dilutions of DNA from MTC and the specificity was tested by 62 different microorganisms, including bacteria related with the MTC. The diagnostic yield was evaluated in 66 specimens from patients with suspected tuberculosis; 30 had tuberculosis and 36 (control group) had different diseases. Under the conditions that resulted in optimization, standard curves showed that senx3-regx3 assay was the most efficient, followed by IS6110 and cfp32. However, the detection of bacterial DNA was faster with the repetitive element IS6110, with Ct values of up to 3 and 9 cycles of difference with respect to senx3-regx3 and cfp32. The analytical specificity, done only with the senx3-regx3 and IS6110 targets, was in the order of 100 and 93.5%, since IS6110 amplified various non-tuberculous micobacteria. For all the clinical samples studied, the sensitivity of both assays was identical (93.3%) but the specificity of senx3-regx3 (100%) was higher than that of IS6110 (94.7%). In conclusion, real time PCR assay-SYBR Green based on the targets senx3-regx3 is highly reproducible and more sensitive and specific than the assays based on IS6110 or cfp32. The protocol developed in this study provides an appropriate and rapid tool to identify the strains of MTC in different clinical isolates and specimens.

Keywords: Melting analysis, Molecular diagnostic, Tuberculosis.

WED-158
Regulation of flagellar motility by the PTS in *Vibrio vulnificus*

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Vibrio vulnificus is an opportunistic human pathogen that causes food-borne diseases such as gastroenteritis and primary septicemia, and its single polar flagellum-based motility is one of the potential virulence factors. Notably, it has been reported that glucose prevents the synthesis of flagella and hence swimming motility in some bacteria. Glucose is transported through the phosphoenolpyruvate: sugar phosphotransferase system (PTS) in most bacteria. The components of the PTS have multiple physiological roles as well as catalysis of the transport and accompanying phosphorylation of numerous PTS sugars. Here, we show that the dephosphorylated form of enzyme IIA of the glucose PTS, but not its phospho-form, interacts with a hypothetical protein (henceforth called Protein X) in *V. vulnificus*. A deletion mutation in the *protein X* gene resulted in loss of flagellum synthesis and reduced the expression of several flagellar genes, indicating that Protein X is essential for the flagellar motility. Taken together, we suggest that the interaction between enzyme IIA^{Glc} and Protein X regulates the flagellar motility by sensing glucose in *V. vulnificus*.

Keywords: Phosphotransferase system (PTS), Protein - protein interactions, vibrio vulnificus.

WED-159
Secondary metabolism of the cyanobacterium Oscillatoria sp. PCC 6506: from genome sequencing to cyanotoxin and metabolite biosyntheses

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Cyanobacteria are photosynthetic prokaryotes that are extremely diverse and certain species produce a wide range of secondary metabolites including potent toxins for animals and humans[1].

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Cases of animal death, due to cyanobacterial toxin exposure, are regularly reported worldwide. For instance, anatoxin-a and homoanatoxin-a, two cyanobacterial neurotoxins provoke the rapid death of animals by acute asphyxia on ingestion, because these alkaloids are potent agonists of the nicotinic acetylcholine receptor[2].

We have sequenced the genome of *Oscillatoria* sp. PCC 6506 which produces anatoxin-a and homoanatoxin-a and identified at least seven clusters of genes likely implicated in the biosynthesis of secondary metabolites, either polyketides, non-ribosomal peptides, or ribosomal peptides[3].

We have identified the *ana* cluster of genes that is responsible for the biosynthesis of anatoxin-a and homoanatoxin-a, and proposed an original biosynthesis for these neurotoxins involving polyketide synthases[4]. We have reconstituted in vitro this biosynthesis and solved the three dimentional strucutre of one of key enzyme of this pathway[5,6].

We have also identified the *cyr* cluster of gene responsible for the biosynthesis of cylindrospermopsin, a cytotoxin and showed that *Oscillatoria* sp. PCC 6506 produces cylindrospermopsin and 7-epi-cylindrospermospsin[7]. We have characterized CyrI a 2-oxoglutarate-dependent iron oxygenase that catalyzes the last step of this biosynthesis.

The characterization of the other clusters of genes involved in the secondary metabolism of *Oscillatoria* sp. PCC 6506 is under investigation, and so far we have identified a cluster responsible for the production of new cyanobactins, cyclic ribosomal peptides.

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Keywords: Biosynthesis, Polyketide synthase, Secondary metabolite.

WED-160
Structures of cell-wall glycopolymers of bifidobacteria

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Belarusian collection of non-pathogenic microorganisms (acronym BIM, WDCM 909) is authorized to deposit microbial strains for subsequent patenting procedure. Probiotic strains deposited at Belarusian collection are used in pilot plant production of probiotics, preventive – therapeutic veterinary compositions, bioactive supplements and substances. Bifidobacteria are thought to be important for health and their participation in the functions of the intestine, taxonomy and ecology has been reviewed. Several reports concerned the composition of the bifidobacterial cell wall and the structure of cell wall glycopolymers. It was shown that the cell wall glycopolymers of bifidobacteria possess anti-tumour

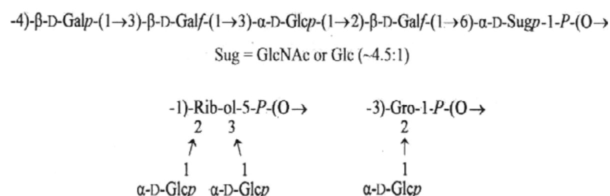

Fig. 1.

and immunopotentiating activities. A glycopolymers with oligosaccharyl phosphate repeats of two types and ribitol and glycerol teichoic acids were isolated from cell wall of *Bifidobacterium longum* BIM B-476 D (the patent application 20111815 BY) by stepwise extraction with 10% CCl_3CO_2H. The structures of the glycopolymers were established by sugar analysis, selective cleavage with aq 2% HOAc, dephosphorylation with 48 % HF, 2D NMR spectroscopy, and high-resolution ESI MS. The main chain of the ribitol teichoic acid is terminated with d-alanine.

Keywords: glycopolymer, structure, bifidobacteria.

WED-161
Substrate specificity in peptide synthetic reaction

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We previously reported that an amide bond is unexpectedly formed [1] by an acyl-CoA synthetase, AcsA, which plays an essential role in acid utilization in the nitrile-degrative pathway of *Pseudomonas chlororaphis* B23 [2]. Although AcsA essentially catalyzes the formation of a carbon-sulfur bond (the ligation of an acid with CoA), it surprisingly synthesized *N*-acyl-l-cysteine when a suitable acid and l-cysteine are used as substrates. Furthermore, this unexpected enzyme activity was also observed for acetyl-CoA synthetase and firefly luciferase, both of which belong to the same superfamily of adenylate-forming enzymes. Therefore, all enzymes in this superfamily of adenylate-forming enzymes are likely to synthesize *N*-acyl-l-cysteine when l-cysteine is used as one of the substrates.

Here, we succeeded in the synthesis of dipeptides (*N*-aminoacyl-l-cysteine) using a bacterial enzyme, which belongs to the same superfamily of adenylate-forming enzymes like acyl-CoA synthetases. In addition, we discovered that some amino acids and l-cysteine were also active as the substrates of the enzyme. These results suggest that the corresponding dipeptides would be produced. The structures of the products were determined by LC-MS/MS. Each fragmentation pattern showed that these products are *N*-aminoacyl-l-cysteine but not *S*-aminoacyl-l-cysteine, demonstrating that various dipeptides were formed as the reaction product. Furthermore, we calculated kinetic values for the synthesis of each peptide, because we found that peptides are synthesized along with the consumption of the amount of ATP and the increase in the amount of AMP. According to the catalytic efficiencies (k_{cat}/K_m), substrate specificity in peptide synthetic reaction of the enzyme was clarified.

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Keywords: None.

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WED-162

Swings in cell morphology, and expression of genes essential to cell adhesion, secretion, iron-chelation and transport, multi-drug resistance, and two-component signal transduction mechanism, from copiotrophic to oligotrophic lifestyle in a model strain, Klebsiella pneumoniae PB12

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Klebsiella pneumoniae although being a respiratory pathogen, the other sites of infection reported include urinary tract, digestive tract, surgical wound sites etc. The invasiveness of infection takes place generally via contaminated respiratory support equipment and urinary catheters or via other surgical/non surgical equipments. Interestingly, they are omnipresent. The model strain K. pneumoniae PB12, described in this study, isolated from river water possessing oligotrophic characteristics, was resistant to multiple antibiotics as well as resistant to human serum. PB12 can grow in an environment without nutrient-luxury. Whole genome transcriptomic responses of PB 12 to high and low nutrient medium was compared to reveal the regulation of gene expressions in relation to surface attachment, secretion, iron-chelation and transport, multidrug resistance, and two-component signaling system that might influence their adaptability to colonize diverse infection sites. The most highly up-regulated iron-transport genes in oligotrophic condition were afeABCD. Significant downregulation of dhaR, uhpA, evgS, basR, zraR, cusR, barA, sdiA, uvrY, and phoQ genes; and upregulation of six novel genes of two-component signal transduction system (phosho-relay) were observed under oligotrophic condition. Flow cytometric analysis has provided an understanding of the behavior of PB12 cells (and the presence of subpopulations) grown in oligotrophic medium enabling further insight into the phenotypic manifestation.
Keywords: Klebsiella pneumoniae, oligotrophy, whole genome transcriptomics.

WED-163

The allelic variant ctxB7 of cholera toxin is a wide spread challenge

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Vibrio cholerae the causative agent of cholera keep changing in biotype and antibiotic resistance pattern for better survival and infection. In 2007, a novel allelic variant of cholera toxin (ctxB7) was noticed in Orissa, India. The sequencing of *ctxB* revealed His20 to Asn mutation in signal peptide at -2 position of signal peptidase cleavage site. Later it rose in fame with Haitian ctxB (HCT) after causing a devastating epidemic in Haiti. The HCT producing strains were also reported from recent large cholera epidemics in Western Africa. In 2012-13, we have also noticed cholera outbreaks caused by HCT harbouring *V. cholerae* O1 El Tor strains in India. All the epidemics or outbreaks were associated with severe diarrhea and enhanced rate of mortality. An increased level of MIC for ciprofloxacin or reduced susceptibility for the drug was observed in recent strains. The use of doxycycline in moderate to severe cases of cholera for the management of outbreak like situation was found effective. Emergence of variant strains hampers the cholera control policies which can be a serious concern. There is pressing need to formulate and implement integrated multidisciplinary guidelines at national as well as at international level to combat the dissemination of novel variant strains.
Keywords: Cholera toxin, ctxB7/Haitian CT and epidemics.

WED-165

The comparison of macromolecular changes in different acinetobacter isolates upon neomycin exposure

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Species of *Acinetobacter* are ubiquitous in nature. Due to their versatile nature, *Acinetobacter* sp. has attracted considerable attention by many researchers. Bacteria of the genus *Acinetobacter* represent a group of opportunistic pathogens. They are of increasing importance because of their ability to rapidly develop resistance to the major groups of antibiotics. In this study, an environmental *Acinetobacter* isolate coming from a freshwater lake was compared to *Acinetobacter haemolyticus* ATCC 19002 in terms of neomycin resistance pattern. Also their whole cell alterations after sub-inhibitory concentration (<MIC) of neomycin exposure were examined with the help of attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy. Firstly minimum inhibitory concentration (MIC) values of neomycin were determined for both bacteria. Then these isolates were grown in nutrient broth medium with and without sub-inhibitory concentrations (<MIC) of neomycin. To take ATR-FTIR spectroscopy measurements the independent replicates for each of the *Acinetobacter* isolate neomycin treated groups and their controls were used. The results show that there were statistically significant changes on conformation of cell proteins and drastic changes in capsule and wall dynamics in both *Acinetobacter* isolates. The production of structurally different form of polyesters such as Polyhydroxybutyrate (PHB), which are induced under stress, was also shown in both bacteria. In addition, the significant alterations in lipid ordering of the membrane, in the amount of cell carbohydrates, the backbone of nucleic acids, and ribosomes were also shown for the environmental isolate. In conclusion, our results demonstrated that the metabolic responses of environmental *Acinetobacter* isolate differ from *Acinetobacter haemolyticus* even in the case of exposure to the same antibiotic.
Keywords: Acinetobacter, ATR-FTIR spectroscopy, neomycin.

WED-166

The large scale antimicrobial efficiency of a new multicomponent preparation for skin diseases treatment

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Human and animal skin diseases of bacterial, fungal and viral nature and their complications are widespread and cause serious trouble. The prevalence of these diseases is increasing continuously mainly due to acquired drug resistance phenomenon. Consequently, demand has increased for new antimicrobial drugs, which also could be less toxic, possess a wider spectrum of action and, on the other hand, would be economically more beneficial.

The goal of the study was to investigate antibacterial, antifungal and anti-bacteriophage activity of Petamcyn-A, new multicomponent preparation containing acetic acid, hexamethylenetetramine, phosphatidylcholine, tocopheryl acetate and glycerol [1].

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The investigations were performed using 9 bacterial, 3 yeast and 5 mould strains as test-organisms. T4 phage of *Escherichia coli* C-T4 was used to test the anti-bacteriophage activity. Nystatin (antifungal drug), Hexiloc (antiseptic drug) and 12% acetic acid (one of the active compounds of Petamcyn-A) were used as control agents for comparative analysis of Petamcyn-A activity.

The results have shown that Petamcyn-A has high activity against all used test strains. It has broader range of activity than control compounds. We found out that Petamcyn-A is more effective against tested gram-positive bacteria compared with gram-negative bacteria. Moreover, it has been demonstrated that Petamcyn-A has bactericidal activity against all used test strains. Minimal inhibitory, minimal bactericidal and minimal fungicidal concentrations of Petamcyn-A against different test strains were determined. MIC of Petamcyn-A was 14 μl/ml for *Staphylococcus aureus* WDCM 5233, 18 μl/ml for *E. coli* and 25 μl/ml for *Debaryomyces hansenii*. Both Petamcyn-A and 12% acetic acid inhibited the germination of tested fungal spores. Further determination of MIC showed that 12% acetic acid lost its activity at 20 μl/ml concentration, but Petamcyn-A was active till 15 μl/ml concentration against *Trichoderma viride* spores. In addition, the preparation demonstrated high activity against T4 phage of *E. coli* C-T4 completely inhibiting its growth. 1/5 dilution of the preparation also exhibited considerable activity reducing phage concentration by 2.6 Log10.

Thus, Petamcyn-A has the large scale of antibacterial, antifungal and anti-phage efficiency and is suggested to develop its production for skin diseases treatment. It is possible additional application of Petamcyn-A as disinfectant.

Keywords: Petamcin-A, skin infectious disease, antimicrobial activity.

WED-167

The study of interaction's effect between genes associated to drug resistance in *Mycobacterium tuberculosis* complex

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The key role of *M. tuberculosis* complex (MTBC) resistance formation is played by the SNPs in genes: *pncA* (Pyrazinamide, Z), *inhA, katG, oxyR-ahpC* (Isoniazide, H), *rpoCAB* operon (Rifampicin, R), *embCAB operon* (Ethambutol, E) and cluster of ribosomal genes *rrs, rpsL* and *gidB, tlyA, eis* (Streptomycin, S). The distribution and genetic frequency of mutations strongly depend on origin and geographical localization of isolates. Although, the harboring genetic processes might be affect on manifestation (e.g. epistastatic interaction, compensatory mutations).

The molecular genetics techniques and bioinformatical analysis were applied for detailed investigation in genes associated with phenomenon of resistance (*inhA, katG, oxyR-ahpC, ndh, rpoB, pncA, embB, rrs, rpsL, tlyA, gidB* and *eis*–promoter) from collection of 155 clinical phenotypic characterized MTBC isolates from Moscow region (Russia).

More over 5000 sequences have been revealed of 108 different SNPs in 13 genes, associated with loss of susceptible in MTBC, exc. *oxyR*, *ahpC*, *tlyA* genes and *eis*–promoter, where the mutations were not found. The significances of genetic variability ((Nm) from 0.75% to 27.7%) and genetic diversity ((h) from 3.7% to 73.1%) showed absolutely heterogeneity and were not correlated between themselves. This fact that may indicates on spontaneous character of mutagenesis. The concordance score between phenotypic and genetic data were varied from 89 to 100% for all drugs without ethambutol (CS = 43.2%). By the principal component analysis all genes were classified into four groups: *katG*, *rpoB* and *pncA* separately and other genes were united in distant group that correlated with comparability of genetic changes, which has been described in multidrug-resistance strains. The interaction between genomic replacements were reconstructed and visualized by Circos plot (Figure 1). The three general combinations of *embB-rpoB-pncA* (35%), *embB-rpsL-rpoB* (37%) and *embB-rrs-rpoB* (24%) were detected, where the central role of interaction has been belonged to *embB* gene. The two hypotheses could be discussed. On the one hand, the epistatic effect might be playing a general role as reflection on the central position of *embB* gene in the combination of rearrangements in extensively drug-resistance strains. However, the discordance results (according to publications) and specific metabolic pathway are closely related to barrier function in cell, might be induce the appearance of replacements in *embB* gene as response on other drugs action (H, R, Z and S), that appeared as complementarity and may not be related to ethambutol resistance.

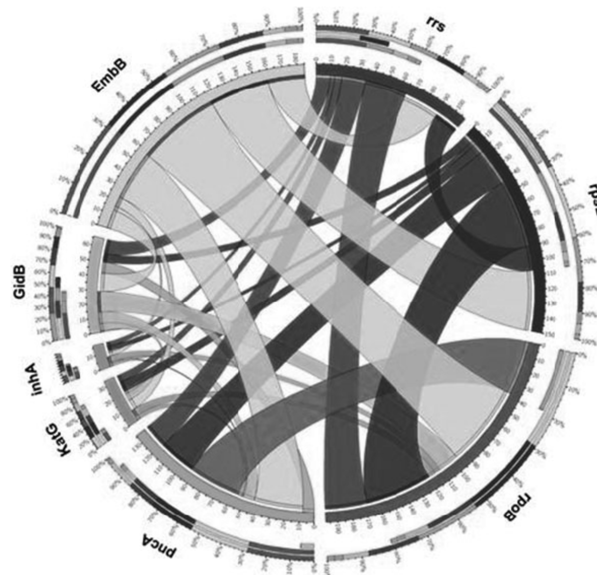

Fig. 1. Tlie reconstruction of interaction between genomic replacements in genes (*embB, rrs, rpsL, rpoB, pncA, katG, inhA* and *gidB*) associated to anti-tuberculosis drug resistance by Circos plot

Keywords: Comparative genomics, *Mycobacterium tuberculosis*, Resistance.

WED-169

Thermo-resistant microalgae for biodiesel production

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The rapid depletion of petroleum-derived fuels makes renewable energy sources more attractive in the world. Bio-diesel is a renewable fuel that is produced dominantly from agricultural waste, vegetable oils and animal fats. Moreover, biofuel is an alternative to diesel fuel, which is produced from oils via transesterification. Microalgae can be an attractive for biodiesel production because of its some properties such as growth rate, lipid content and bio-

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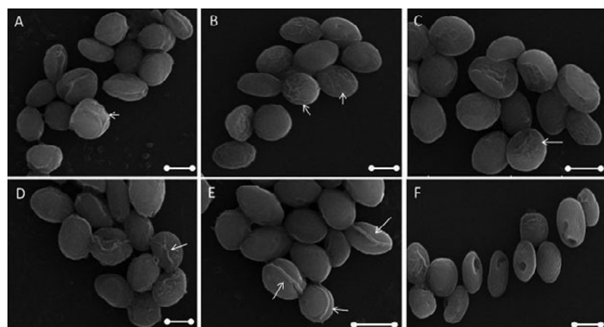

Fig. 1.

dieisel productivity. In this study, Samples were collected from three different hot spring locations from geothermal flora of Central Anatolia and we isolated microalgae by means of serial streaking cells across agar plate and dilution techniques. Then, we made their morphological and moleculer characterization via scanning electron microscopy (SEM) and sequencing 18S ribosomal RNA gene internal transcribed sequence 2 (ITS2)-rDNA of isolated samples, respectively. Scanning electron micrographs of the cells of microalgal isolates were given in Figure 1. ITS2 sequences of all isolates were analyzed and assembled using ContigExpress software of Vector NTI (Invitrogen). ITS2 secondary structure prediction of the consensus sequences of the isolates was done using the prediction algorithm implemented in the ITS2 database with the parameters of $E < 1e\text{-}16$ and minimum helical transfer of 75%. Next, we carried out lipid, carbohydrate and protein concentrations of isolated microalgae via hexane extraction method, anthrone method with minor modifications and Bradford method, respectively. One of our isolates identified as *Micractinium sp* displayed suitable properties for biodiesel production such as high photosynthetic rate, chlorophyll content, high growth rates and adaptibility to different temperatures, high biomass accumulation, desirable fatty acis methyl ester (FAME) profile and high biodiesel productivity. *Micractinium sp* has high growth rate, biomass concentration and biomass productivity as 5.2 ± 0.04, 0.76 ± 0.03 g.L^{-1} $^{\text{and } 0.17\pm0.007 \text{ g.L}-1}$. day^{-1}, respectively. In addition to this, *Micractinium sp* has high biodiesel production as $89 \pm 3.1\%$ and high amounts of oleic acid methyl esters (18:1), linoleic acid (18:2), palmitic acid methyl esters (16:0) for biodiesel production. In conclusion, *Micractinium sp* is a suitable candidate for biodiesel production and can further be improved for different industrial applications via genetic and metabolic engineering strategies.

Keywords: Biodiesel production, Micractinium sp., Microalgae.

WED-170
Thermostable α-amylase produced by *Anoxibacillus flavitermus* K103 isolated from an Armenian geothermal spring

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Thermozymes produced by thermophilic microbes have been of great scientific interest for the last decades and have found a number of commercial applications due to good correlation between their inherent stability and harsh industrial processes conditions. Thermostable starch-degrading enzymes are important for the production of glucose from starch and are widely used in food, feed, pharmaceutical and chemical industries. Thermostable α-amylases, mainly produced by thermophilic bacilli, have found a number of applications due to their overall inherent stability. Therefore, the isolation of novel thermostable amylase producers from natural sources is a priority research field.

The aim of this study was to isolate active producers of thermostable amylases from hot and warm mineral springs in Armenia and to select prospective producers of thermostable amylases and optimize the conditions for enzyme production. More than 160 thermophilic bacilli strains were isolated and 12 isolates with high amylase activity were selected. 16S rRNA genes analyses revealed affiliations to the genera *Geobacillus, Anoxibacillus* and *Brevibacillus*. The most active strain, designated *Anoxibacillus flavitermus* strain K103, was able to grow and produce amylase in the 50°C - 80°C temperature range and at pH 5.5 – 8.5, and showed maximum production of amylase at its optimum growth temperature (65°C) and pH 7. Amylase production started in early log phase and reached a maximum in late exponential phase with an activity of 205 U ml^{-1}. The enzyme fraction was subjected to zymographic analysis which revealed a single protein band. The molecular mass of the amylase was determined to 75 Kd by SDS-PAGE. The enzyme was stable at temperatures from 40 to 100°C and at a pH range from 5 to 10. It was stimulated by Ca^{2+} and Mn^{2+}, whereas inhibited by Cu^{2+}, Zn^{2+} and EDTA, suggesting metal dependence. High production rate, and activity and stability of the enzyme at high temperature indicate a strong industrial potential for *A. flavitermus* K103 and its amylase.

The work was supported by the CPEA-2011/10081 grant from the Norwegian Cooperation Programme in Higher Education with Eurasia and by a grant from the Armenian National Science and Education Fund ANSEF-2011 Microbio-2493 based in New York, USA.

Keywords: Anoxibacillus flavitermus, thermophilic bacilli, thermostable α-amylases.

WED-171
Transition to NC state is accompanied by increase of vapC toxin expression level

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Toxin-antitoxin (TA) loci are widely spread in bacterial plasmids and chromosomes. Toxins affect such important functions of bacterial cells as translation, replication and cell wall synthesis, whereas antitoxins are toxin inhibitors. Participation in formation of the dormant state in bacteria is also suggested to be a possible function of toxins.

To validate the role of VapBC TA in dormancy formation, we used a model of dormant, «non-culturable» (NC) *Mycobacterium smegmatis* cells obtained in potassium limited conditions. Under such conditions *M. smegmatis* cells lose their culturability on solid medium transiting into the NC dormant (but reversible) state. Early we have found that overexpression of VapB antitoxin prevented transition to dormancy in these conditions [Demidenok et al., 2014]. In this work, we demonstrated that transition to NC state is accompanied by increase of vapC toxin transcription level.

Alteration of *vapC* expression level in *M. smegmatis* cells during transition to NC state was examined with qRT-PCR. It was revealed that the *vapC* transcription level was increased (ca. 20 times) before formation of NC state. Detected level of *vapC* expression in NC cells was decreased (ca. 6 times) in comparison with active cells. The lower level of *vapC* expression in NC dormant cells is obviously due to their low metabolic level [Shleeva et al., 2004].

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In summary, these findings point to involvement of a VapBC TA system in the development of dormant state.

Keywords: dormant state, toxin-antitoxin function.

WED-172
Trends in next generation bioactive lead compounds from microbes

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Microbes are the large repositories of bioactive compounds with different structural and functional forms viz. sugars, acids, esters, ethers, terpenoids, peptides, proteins and nucleo peptides. Which are meant for the immense applications in the treatment of the wide range of disease types, such as anticancer, anti-inflammatory, antimicrobial, antiviral and antifungal etc., As long as the microorganisms are treated with the classical antibiotics they are supposed to be developing the nature of resistance to the specific disease types. Now it is time to prepare the new drugs and makes to modify in the structural characteristic for the target based drug design for the specific disease.

A significant number of interesting natural novel biomolecules have been reported from the microbial source like, Rhodethrin, Rubrivivaxin, Sphestrin and Rhodophestrol (Ranjith et al). These compounds exhibit HTP activities such as antimicrobial, anticancer, anti inflammatory, COX-I & II inhibition, cytotoxic and phytohormonal activities.

The utilization of these vast resources is poorly understood in microorganisms. However, the microbial screening provides an opportunity to manipulate new generation drugs for efficient therapeutic approach.

Keywords: None.

WED-173
Unravelling the molecular structure and function of VirB4-like ATPases in nucleoprotein import and export

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Bacterial type IV secretion systems (T4SS) transfer macromolecules like nucleic acids and proteins from bacteria to eukaryotes or between bacterial cells. VirB4-like proteins are associated with all T4SSs described to date. These signature ATPases function in assembly of the T4SS channel and biogenesis of extracellular pili in Gram-negative systems. They are also required for translocation of secretion substrates and nucleoprotein uptake during pilus-mediated phage infection. Very little is known about the regulation of VirB4-like ATPases in protein trafficking. It is also not known whether these proteins are involved in recognition of substrates destined for secretion. Our paradigm to study control of VirB4-like ATPases is TraC of the F-like conjugative plasmid RI.

TraC was modified with a strep-tag and its function verified by complementation of conjugative transfer of a *DtraC* derivative of R1. However, purified strep-TraC did not exhibit ATPase activity in vitro. One possible explanation may be that ATPase activity is controlled by oligomeric state. Hexamers of VirB4-like protein TrwK hydrolyze ATP in vitro and ATP binding forces the formation of hexamers. Strep-TraC was incubated with a 20-fold molar excess of non-hydrolysable ATP and analyzed by gel filtration. The chromatograms indicate that strep-TraC behaves as a monomer in solution with and without ATP. The ATPase activity might be controlled by interaction(s) of TraC with other relaxosome proteins. Possible interaction partners are currently under investigation.

Enzymatic activity of VirB4-like ATPases can also be controlled intra-molecularly. The C-terminus of the VirB4-like protein TrwK possesses alpha helices, which were shown to have an auto-inhibitory activity. Secondary structure prediction of TraC shows four conserved alpha helices. The significance of these alpha helices for enzyme regulation is under investigation. Interestingly, truncated versions of TraC, lacking individual alpha helices are unable to complement the function of wild type *traC* in conjugative transfer.

To test the hypotheses that TraC is subject to auto-inhibition and that the alpha helices mediate protein-protein interactions, truncated versions of TraC will be purified. The ability of truncated TraC to hydrolyze ATP will be tested with and without other potential binding partners. Also, the purified truncated versions of TraC will be used in protein-protein interaction studies with other relaxosome proteins. Furthermore, these TraC truncated variants are used in phage-infection assays to study nucleoprotein uptake by T4SS.

Keywords: ATPase activity, type iv secretion systems, VirB4.

WED-174
Vibrio vulnificus HPr stimulates pyruvate kinase A activity to protect cells against H_2O_2 stress

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The bacterial phosphoenolpyruvate (PEP):sugar phosphotransferase system (PTS) consists of two general energy-coupling proteins (enzyme I and HPr) and several sugar-specific enzyme IIs. In addition to the phosphorylation-coupled transport of sugars, the PTS components participate in many physiological processes. In this study, we have identified pyruvate kinase A (PykA) as a binding partner of HPr in *V. vulnificus*, which is an opportunistic human pathogen. The interaction between HPr and PykA was dependent on the presence of inorganic phosphate and only unphosphorylated HPr interacted with PykA. Domain swapping experiments between PykA and its *E. coli* ortholog revealed a requirement of the C-terminal domain of *V. vulnificus* PykA for specific interaction with *V. vulnificus* HPr. Unphosphorylated, but not phosphorylated, HPr decreased the Km of PykA for PEP about 4 fold without affecting Vmax. A *pykA* mutant became more susceptible to H_2O_2 than wild-type *V. vulnificus* and this sensitivity was completely rescued by the addition of pyruvate to the culture medium. Based on these data, we suggest that PykA plays an important role in H_2O_2 stress response in the presence of PTS sugars.

Keywords: Hydrogen peroxide, Phosphotransferase system (PTS), Pyruvate kinase.

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CSIV-06 – Transcription & RNA processing

WED-175

Role of vitamin E on PPARgamma and Nrf2 related signalling pathways in in vivo model of atherosclerosis

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Increased serum cholesterol and oxLDL are implicated in the pathogenesis of atherosclerosis. We have previously reported a significant increase in mRNA levels of the scavenger receptor CD36 in aortas of cholesterol fed rabbits and shown that vitamin E treatment attenuated increased CD36 expression (1). In the present study, we investigated redox signaling pathways involved in the cellular defence against cholesterol diet induced atherogenesis and the effects of vitamin E on related signaling pathways in in vivo model of atherosclerotic rabbits. Male albino rabbits were assigned randomly to three groups fed: (i) vitamin E deficient diet, (ii) vitamin E deficient diet containing 2% cholesterol, and (iii) vitamin E deficient diet containing 2% cholesterol with daily intramuscular injections of vitamin E (50 mg/kg). After four weeks, supplementation with cholesterol resulted in ~30-fold increase of plasma cholesterol while vitamin E treatment increased serum vitamin E levels 11-fold (mean ± SD, $n = 7$, $p < 0.001$). When ROS generation was detected by lucigenin chemiluminescence, a significant increase in cholesterol group determined and vitamin E treatment inhibited cholesterol-mediated increase in ROS generation. (mean ± SEM, $n = 3$, $p < 0.001$). The effects of vitamin E supplementation on altered cellular signaling events in aortae were further examined by determining peroxisome proliferator-activated receptor gama (PPARγ), ATP-binding cassette transporter A1 (ABCA1), and matrix metalloproteinase-1 (MMP-1) mRNA levels (n = 5 per each group) by quantitative RT-PCR and protein levels of MMP-1, nuclear factor-erythroid 2-related factor 2 (Nrf2), and glutathione S-transferase α (GSTα) (n = 3 per each group) by immunoblotting. Increased MMP-1 and, decreased GSTα expession demonstrated that a cholesterol-rich diet contributes to the development of atherosclerosis, whereas vitamin E treatment affords protection by decreasing MMP-1 and increasing PPARγ, GSTα and ABCA1 levels in aortae from rabbits fed a cholesterol-rich diet for 4 weeks. Notably, Nrf2 protein expression was increased in both the cholesterol fed and vitamin E supplemented group. Although Nrf2 activation can promote CD36-mediated cholesterol uptake by macrophages, the increased induction of Nrf2-mediated antioxidant genes is likely to contribute to decreased lesion progression. Thus, our study demonstrates that Nrf2 can mediate both pro- and anti-atherosclerotic effects.

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1. Ozer, N.K. *et al.* (2006). *Atherosclerosis* **184**, 15–20.

Keywords: Atherosclerosis, Nrf2, vitamin E.

WED-176

(Mis)regulation of nuclear import of disordered proteins in neurodegenerative disease and cancer

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"Function requires structure" – this is a common statement throughout structural biology. Indeed, most proteins need to adopt a defined 3D structure to carry out their function. However, recent studies have indicated that a large fraction of the genome of any organism encodes proteins that do not adopt a defined 3D structure but are nevertheless essential for cellular function: the so-called intrinsically disordered proteins/regions (IDP/Rs). IDP/Rs are highly abundant in nature and their functional repertoire complements the function of ordered proteins. Here, I will present our recent published and unpublished results on the molecular mechanism of (mis)regulation of nuclear import involving IDP/Rs, their essential role in signal transduction, as well as their link to ageing and age-related diseases such as cancer, diabetes, and neurodegeneration [1–3].

One prominent example of a largely disordered class of proteins are transcription factors. The *bona fide* tumor suppressor Forkhead box O transcription factors (FOXO) are of vital importance in cell-cycle control, oxidative stress resistance, tumor suppression, and organismal lifespan. FOXO activity and cellular localization are regulated through an intricate code of post-translational modifications (PTMs). However, how FOXOs re-localize to the nucleus as response to oxidative stress remains enigmatic. Using a combination of structural and *in vivo* techniques we have discovered a novel mechanism of oxidative stress-mediated nuclear import involving the formation of an inter-molecular disulfide bond between the disordered region of FOXO and the nuclear import factor transportin-1 [2]. This highly conserved mechanism not only connects redox signaling directly to the longevity and tumor suppressor protein FOXO but provides first evidence for a novel nuclear import mechanism specific for redox proteins.

Furthermore, I will present our recent results for the molecular mechanisms of (mis)regulation of the protein Fused-in-Sarcoma (FUS) and Tau involved in the neurodegenerative diseases amyotrophic lateral sclerosis (ALS)[3], and Alzheimer's [1], respectively.

Summarizing, our recent results demonstrate the key role of IDP/Rs in transcription factors (FOXO), RNA-binding proteins (FUS), and other disordered proteins (Tau). We find that these IDP/Rs are essential for regulation in signaling – they act as interaction hubs for a plethora of co-factors and platforms for an intricate code of PTMs. Given that misregulation linked to these regions is the cause of a plethora of diseases, our structural and functional studies provide the first insight into the underlying molecular mechanisms.

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Keywords: intrinsically disordered protein, neurodegenerative diseases, structural biology.

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WED-177
A functional analysis of regulatory regions that differ between Neandertals and present-day humans

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The closest evolutionary relatives of present-day humans are the Neandertals and their Asian sister group, Denisovans. They share a common ancestral population with modern humans about 553,000 and 589,000 years ago. Because high-quality genomes from a Neandertal and a Denisovan individual are available, it is possible to identify changes in the genome that are present in all or almost all present-day humans but where the Neandertal and Denisovan genomes carry the ancestral, ape-like variants. This is an interesting class of changes since some of them may be of relevance for functional traits where modern humans differ not only from the great apes but also from extinct earlier forms of humans.

In order to begin to investigate such genetic changes that may be of regulatory significance, we focus on well-described transcription factor binding sites that are conserved among primates, Neandertals, and Denisovans yet changed in modern humans. We have cloned the ancestral and derived variants of 25 such binding sites into vectors containing a minimal promoter. After transfection into several cell lines we use the amounts of transcripts produced to assess the activity of the two variants. A number of constructs show transcriptional activity above background and differences in activity between the ancestral, Neandertal-like variant and the variant specific to present-day humans. The results will be presented and discussed.

Keywords: enhancer, human evolution, transcriptional regulation.

WED-178
A new flexible approach to model the structure of large macromolecular assemblies with cross-linking data. Application to RNA polymerase III

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Cross-linking/mass spectrometry has been recently demonstrated to allow modeling of large macromolecular assemblies based on structures of individual subunits. Although modeling methods based on cross-links have been proposed, the current methods do not deal well with conflicting cross-links. Moreover, current methods usually do not allow using cross-links that link regions missing in the starting structures, thus excluding many potentially useful modeling restraints. Here, we propose a novel semi-automated computational method to model the structure of large protein assemblies based on cross-links. To automatically deal with conflicting cross-links, we use a log-normal distance potential and a re-weighting scheme inspired by methods for structure determination from NMR data. Furthermore, the method allows modeling subunit structures as flexible molecules and including missing regions of the starting structures explicitly. To demonstrate the method, we performed cross-linking of 17-subunit yeast RNA Polymerase III (Pol III) and built its atomic model. The model agrees with published cryo-electron microscopy reconstruction and previously published cross-links not used in the modeling.

Keywords: Molecular modeling, MS cross-linking data, RNA polymerase III.

WED-179
Archaeal protein aFib and Nop5p heterodimer methylates 16S rRNA site-specifically, independently of C/D guide RNA

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Archaeal fibrillarin (aFib) is a well-characterized RNA 2′-O-methyltransferase that acts in C/D RNP complexes. It has been established that the methylation target is selected via base-pairing of the guide RNA of the complex and aFib finds it only by interactions with the other proteins of the complex: L7Ae and, especially, Nop5p (1). Nevertheless, we investigated functions of C/D RNP components and showed that *Pyrococcus abyssi* aFib in a heterodimer with Nop5p, and without other components of a C/D RNP, effectively methylates 16S rRNA site-specifically *in vitro*. We further identified methylated positions using tritium-methyl incorporation into RNA assay, mass spectrometry, reverse transcription stop analysis, alkaline hydrolysis. This aFib-Nop5p heterodimer activity could be an example of proteins that evolved from stand-alone enzymes to C/D RNP components acting in RNA-guided manner.

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Keywords: C/D RNP, Fibrillarin, RNA 2′-O-methylation.

WED-180
Asn53Ser SNP alters human RNase κ enzymatic activity

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Single nucleotide polymorphisms are single base changes found in both coding and noncoding regions of the genome. They represent the most frequent type of DNA variation throughout the human population. It is thought that non-synonymous coding SNPs along with SNPs in regulatory regions have high impact on phenotype. RNase κ belongs to a recently identified highly conserved in all metazoans protein family whose biological role is currently under investigation. Human RNase κ is a novel endoribonuclease expressed in all developmental stages of normal and malignant tissues [1].

In this report, we present data on the biochemical and enzymatic characterization of the single nucleotide polymorphism Asn53Ser of human RNase κ. This polymorphism is the only one found within the coding region of the gene that is identified exclusively in heterozygotes. Site directed mutagenesis was employed for the construction of the mutant Asn53Ser RNase κ. The recombinant mutant enzyme was expressed in the *Pichia pastoris* eukaryotic expression system and the secreted heterologous protein was partially purified using an anion exchange chromatography. Focusing on the study of the ribonucleolytic activity of the mutant molecule, a fluorescent substrate was used. The rate of ApG bond hydrolysis was compared with the ribonucleolytic activity of the wild type enzyme. Our data indicated that the mutant RNase κ exhibited a significantly lower activity towards the synthetic substrate used for both molecules. The obtained results could partially explain the presence of the Asn53Ser polymorphism only in heterozygotes.

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Keywords: polymorphism, Ribonuclease.

WED-181
CAT3 gene expression profiles in response to copper stress in tolerant and non-tolerant *Brassica nigra* ecotypes

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Phytoremediation is being offered as an environmentally friendly and cost-effective means of reducing metal contaminated soil and other substrates worldwide. Illumination of the defense mechanism in response to heavy metal stress in plants is essential for convenient phytoremediation approach. Our knowledge about transcriptome response to heavy metal stress is limited. The purpose of this study is to evaluate the potential of *Brassica nigra* (Black mustard) for phytoremediation of Cu from contaminated soils. The majority of differentially induced genes in our Affymetrix ATH1 microarray experiments were functionally related to metal transport, accumulation and osmotic stress response mechanism. One of these genes the Catalase (CAT3), an important enzyme of antioxidant system, was investigated for the potential role in preventing the plant from Cu-induced oxidative stress caused by reactive oxygen species. RT-PCR approach was used in order to determine the variety of expression levels of CAT3 gene in different tissues of Cu-tolerant and non-tolerant *B. nigra* ecotypes. We showed different CAT3 regulatory mechanism in response to copper stress. qPCR experiments showed that CAT3 expression level increased by copper in leaf tissue of tolerant ecotype and decreasing expression pattern by Cu was shown in shoot tissue. Conversely, in non-tolerant ecotype, maximum expression level was observed in 25 μM Cu treated shoot tissue and increasing CAT3 expression level by Cu concentration was detected in leaf tissue. CAT3 was down regulated in root tissue. All our findings strongly support that overexpressing CAT3 in black mustard could enhance the tolerance under Cu stress.

Keywords: *Brassica nigra*, CAT3, Phytoremediation.

WED-182
Characterisation of microRNA promoters in the murine genome

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The promoter of protein coding genes is conventionally considered to be located a few kilobases around their transcriptional start site (TSS). However – given the short half-life of miRNA primary transcripts (pri-miRNAs) – the TSS of miRNA is in most cases not annotated, therefore the position of the promoter is usually not know and potentially located tens or hundreds of kilobases upstream of the annotated miRNA gene (pre-miRNA). Several attempts have been made to identify miRNA TSSs and promoters in human, mouse, worm and plants. Nevertheless, to our knowledge, most of the studies in mouse only made use of a limited number of experimental techniques and/or sources of information available at the time of publication. To overcome these limitations we implemented an integrative approach based on computational predictions, sequence annotations and high-throughput chromatin modification data generated by the ENCODE project to produce an accurate and comprehensive annotation of miRNA promoters in the mouse genome.

For each murine miRNA annotated in miRbase version 18 we scanned a genomic region of 100 kb upstream of it looking for features indicative of promoters. We found on average 15.7 candidate promoters for each miRNA with a mean size of ~1.4 kb in accordance with the typical size of known promoters. We also found that they were typically located 5 kb upstream of the miRNA, supporting the validity of our approach and in accordance with previous reports. When we inspected what chromatin modifications supported each of the candidate promoters we found that nearly all of them have a DNase Hypersensitivity Site (DHS), more than 50% have both a DHS and H3K4 trimethylation (H3K4me3), and more than 30% have a DHS mark, an H3K4me3 mark and a CpG island. We also found that the highest scoring promoters are on average more conserved than all other promoters and more conserved than random genomic locations of the same size, strongly supporting the validity of our approach. Finally, by chromatin immunoprecipitation assay (ChIP) and methyl-CpG immunoprecipitation (mCIP) we validated H3K4me3 and CpG methylation of the predicted promoters of selected miRNAs, confirming the validity of our approach. Taken together, these results provide a valuable resource that will help to shed light on the transcriptional regulation of miRNAs in the murine genome.

Keywords: None.

WED-183
Characterisation of the human URG-4 (Up-Regulated Gene 4) gene promoter

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Upregulated gene 4 (URG4), a novel gene located on 7 chromosome (7p13), was firstly found as natural effector of HBxAg protein. It is reported that, this gene contributed to hepatocarcinogenesis regulating the expression of cyclin D1 protein, associated with the onset of tumorigenesis and cell cycle regulation. Due to the proliferative activity of URG4 in osteosarcoma cells, URG4 may be a valuable prognostic marker for certain types of human cancer. Also, there is no information about transcriptional activity of this gene. In this study, promoter activity and relation with transcription factors of URG-4 promoter site was clarified.

Initially, URG-4 promoter site was detected by bioinformatically and amplified based on PCR strategy. *In silico* analysis of URG-4 promoter site was showed that, it has 80% GC content and secondary structure. Therefore, different PCR strategies such as enhancers (Ethylen glycol, DMSO, Betain and 7-DeazaGTP), $MgCl_2$ and gDNA concentration gradients were tried. 5′truncated promoter fragments of URG-4 (-109/+63, -344/+63, -261/+63 and -109/+63) were cloned into pMetLuc Luciferase Reporter vector to detect core promotor activity by using transient transfection assay. Lusiferase activity of promoter fragments were measured by utilizing luminometer with Reporter Assay kit. Transient transfection analysis indicates -109/+63 promoter fragment is sufficient to drive transcriptional activity. In addition, expression plasmids containing SP1, USF and C/EBP transcription factors were cotransfected with URG-4 promoter fragments. Cotransfec-

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tion analysis showed Sp1 and USF transcription factors upregulate URG-4 promoter activity.

Keywords: promoter analysis, transcriptional regulation, URG-4 gene.

WED-184

Characterization of ARF1 paralogs in the nodular structure formation of *Medicago truncatula*

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ADP-ribosylation factors (Arf), a family of small GTP-binding proteins, have been shown to be important for housekeeping functions; e.g., basic cellular trafficking in yeast, mammalian, and plant systems. Although these genes are very well conserved both functionally and sequentially throughout Eukaryotae, some members of these genes have been reported to be sub-or neofunctionalized in plant kingdom. In this study, we investigated the possible lineage-specific functional evolvement of four *Arf1* paralogous genes in the development and maintenance of nodular structures of *Medicago truncatula*. Even though all of *Arf1* paralogs expressed in every tissue of *M. truncatula,* but the expression level of them was higher in nodular tissues. Also, we further investigated the temporal expression profile of *MtArf1* paralog genes by looking into the expression at early stages of after the infection of the roots with *S. melliloti*. The previously identified gene, *Mtr13597775,*solely expressed at 72 hpi (hour post inoculation) and *Mtr27524313* significantly expressed at 6 dpi (day post inoculation), We further confirmed the temporal expressional characteristics of Arf paralogs at protein level by Western blot analyses. That is, Arf1 protein expression level was much higher in the infected roots and nodules in comparison to shoots. The findings in this study could suggest that Arf genes likely play an important role in the nodule formation of *M. truncatula*.

Keywords: ADP-ribosylation factors, medicago, nodule.

WED-185

Characterization of group I introns in bacterial flagellin gene and homing endonuclease from thermophilic *Bacillus* sp. Kps3

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Bacterial flagellum is a rotary motility device that consists of three parts: a basal body, a hook, and a long helical filament. The flagellar filament is composed of a single protein subunit, flagellin, which is abundantly, expressed when flagellar filament is elongated. The self-splicing group I introns were identified in flagellin genes of thermophilic *Bacillus* species, which interrupt highly conserved 3′ region. These group I introns can be spliced at wide range temperature. *In vitro* splicing efficiency was increased with temperature, and the most efficient splicing was observed around the optimal growth condition of these thermophiles, 60–70°C.[1,2)]

We demonstrated the presence of two introns in the gene encoded flagellin in thermophilic *Bacillus* sp. Kps3. In addition, free-standing homing endonuclease gene (HEG) was found in downstream of the flagellin gene. Homing endonuclease (HEase) is a restriction enzyme which recognizes non-palindromic DNA sequence (14–40 bp). HEG is usually encoded within their group I or group II introns, and rarely located at the outside of the introns as an independent open reading frames (free-standing). HEG play an important role in the beginning of the intron transition (intron homing). It is known that many of the introns are found in archaea and eukaryotes. However bacterial introns are mostly found in non-protein coding genes, such as tRNA or rRNA. We analyzed the HEase to elucidate the function in detail. We found that the HEase catalyzes double-strand break of the gene encoded flagellin whose two introns were eliminated. Furthermore, we revealed that cleavage site of the HEase is 5′-GGCTCAAGC↓CAACC-3′ which located downstream of the insertion site of intron II.

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Keywords: flagelline intron, group I self-splicing intron, homing endonuclease.

WED-186

Characterization of *Streptococcus thermophilus* Csm complex mediated CRISPR interference in vitro

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Immunity against viruses and plasmids provided by CRISPR-Cas systems relies on a ribonucleoprotein effector complex that triggers the degradation of invasive nucleic acids. Effector complexes of Type I (Cascade) and Type II (Cas9-dual RNA) target foreign DNA. Intriguingly, the current knowledge regarding Type III systems maintains that Csm (III-A) complexes target DNA, whereas Cmr (III-B) complexes target RNA. Here we aimed to investigate nucleic acid targets and their degradation by the *Streptococcus thermophilus* (St) Csm (Type III-A) complex. We expressed the Type III-A St CRISPR-Cas locus in *Escherichia coli*, isolated the Csm effector complex and characterized its activity *in vitro*. Further, we demonstrated that Csm complex may be reprogrammed to cleave the NA substrate of interest. This work was funded by the Research Council of Lithuania (grant MIP-40/2013 to G.T.).

Keywords: CRISPR-Cas, Csm, effector complex.

WED-187

Characterization of the mechanisms of transcription termination by the helicase Sen1p

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Pervasive or hidden transcription is widespread in eukaryotes and needs to be controlled in order to avoid interferences with gene expression. A key actor in controlling pervasive transcription in *Saccharomyces cerevisiae* is the Nrd1-Nab3-Sen1 (NNS) complex, which elicits early termination of non-coding RNAs and targets them for degradation/processing by the nuclear exosome. Nrd1p and Nab3p are RNA binding proteins that interact with specific sequences on the nascent transcript while Sen1p is a putative RNA and DNA helicase. We have recently developed a

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transcription termination *in vitro* assay using purified RNA polymerase II and NNS components to dissect the mechanism of termination. We have shown that the helicase Sen1p alone can elicit dissociation of elongation complexes *in vitro*. In addition, we have found that, akin to the bacterial terminator factor Rho, Sen1p interacts with the nascent RNA and hydrolyses ATP to provoke transcription termination.

In this work, we have pursued the study of the mechanisms of action of Sen1p. We have first performed a biochemical characterization of Sen1p activities to explore its capacity to bind several types of nucleic acids, as well as to unwind RNA-DNA and DNA-DNA duplexes. Finally we have also performed a functional dissection of Sen1 protein to identify the minimal regions that are required for ATP hydrolysis and for transcription termination. Our results provide important insights into the function of Sen1.

Keywords: Sen1p helicase, transcription termination.

WED-188
Deciphering the cellular role of HelD, a helicase-like protein associated with bacterial RNA polymerase

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Bacterial RNA polymerase (RNAP) is a central enzyme of gene expression. The activity of RNAP can be affected by a number of factors. We identified a new interaction partner of RNAP in *Bacillus subtilis*, a helicase-like protein termed HelD. Recently, we showed that HelD binds to RNAP and stimulates its activity in an ATP-dependent manner. Here, we will present data addressing the global effect of HelD on the transcriptome, a structural/functional analysis of HelD domains, and molecular details of HelD interaction with RNAP. A model of HelD's functioning will be presented and its cellular role(s) will be discussed.

This work is supported by grant No. P302-11-0855, from the Czech Science Foundation.
Keywords: Bacillus subtilis, HelD, RNA polymerase.

WED-189
Deletion of the maltase gene regulatory regions in yeast *Candida utilis*

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Candida utilis is an important organism with a significant potential in pharmaceutical and biotechnology applications. In the last few years it has been demonstrated that the yeast system *Candida utilis* represents a promising expression host, generating relatively high levels of recombinant proteins. However a basic knowledge of regulation of gene expression, which is needed for more extensive use of this organism, is lacking. We have successfully adapted the Cre-*loxP* system for the use in *C. utilis* and applied it to obtain a null mutant of the maltase gene by multiple gene disruptions. The first step was to introduce a mutagenesis cassette harboring a marker gene between two *loxP* sites into the target cells. By disruption of maltase gene and regulatory regions, we managed to prepare mutants without marker gene. After the disruption procedure both native and mutated alleles still existed even at high concentration of selective antibiotic. The deletion mutants exhibited a reduced level of α-glucosidase activity. When tandemous mutagenesis procedure was used on clone with reduced α-glucosidase activity, the clone with no detectable α-glucosidase activity and only with mutated allele was obtained.

This work is result of project implementation: "Production of biologically active agents based on recombinant proteins" (ITMS 26240220048) supported by the Research and Development Operational Program funded by the ERDF.
Keywords: candida, deletion, glucosidase.

WED-190
Desulfovibrio gigas HcpR is required for growth under nitrosative stress conditions

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Desulfovibrio gigas is an anaerobic microorganism that belongs to the group of sulfate reducing bacteria (SRB). SRB are metabolic versatile microorganisms widespread in nature as well as in the human gastrointestinal tract. The byproduct of sulfate respiration, hydrogen sulfide gas, is highly toxic and corrosive and thus has a major negative impact in economy and health. In their natural environments, SRB are often exposed to reactive nitrogen species (RNS), produced by other bacteria or by the human innate immune system. RNS interact with several cellular targets causing great damage to microbial cells. In order to overcome those deleterious effects microorganisms have developed several mechanisms that protect them against nitrosative stress. Although *Desulfovibrio* is the most studied genus of SRB, the mechanisms and regulatory elements involved in this protection are still poorly understood. Using *D. gigas* as a model organism we addressed the importance of HcpR, a transcriptional regulator belonging to the family of FNR/CRP regulators, in nitrosative stress response. We found that the growth of a mutant lacking HcpR is severely impaired under nitrosative stress conditions. An *in silico* search in *D. gigas* genome revealed several putative targets of HcpR whose dependence on this transcriptional regulator was experimentally validated by quantitative real-time PCR. Furthermore, we showed for the first time in *Desulfovibrio* spp. that recombinant HcpR is able to bind the consensus sequence present in the promoter region of *hcp* gene. We also found that *D. gigas* genome contains a related HcpR regulator, HcpR-like, that has orthologs in several other *Desulfovibrio* spp.

Keywords: Desulfovibrio gigas, HcpR, Nitrosative stress.

WED-191
D-foci – sites for RNA decay in human mitochondria

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RNA decay is usually mediated by protein complexes and can occur in specific foci like P-bodies in the cytoplasm of eukaryotes. In human mitochondria little is known about the spatial organization of the RNA decay machinery and the ribonuclease responsible for RNA degradation was searched for many years. We demonstrated that silencing of human polynucleotide phos-

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phorylase (PNPase) causes accumulation of RNA decay intermediates and increases the half-life of mitochondrial mRNA. A combination of fluorescence lifetime imaging microscopy with Förster resonance energy transfer (FLIM-FRET) and bimolecular fluorescence complementation (BiFC) experiments proved that PNPase and hSuv3 helicase form a complex in vivo. This complex, referred to as the degradosome, is formed only in specific foci (named D-foci) which co-localize with mitochondrial RNA and nucleoids. Notably, interaction between PNPase and hSuv3 is essential for efficient mitochondrial RNA degradation. This provides indirect evidence that mitochondrial RNA decay takes place in foci present in the mitochondrial matrix. We present results showing that D-foci are sites for RNA decay in human mitochondria.

Keywords: mitochondrial RNA, RNA degradation, spatial organisation of RNA transactions.

WED-192
DNA electrostatics: its role in transcription regulation in prokaryotes, a new natural selection factor, and a base for DNA phenotype and biophysical bioinformatics

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Genome DNA physical properties (PP) define its shape in the functional space and influence its interactions with proteins, esp. for transcription regulation (TR). DNA is highly charged and electrostatics (E.) contributes greatly to the subject. DEPPDB was developed to provide all available information on these properties of genome DNA combined with its sequence and annotation of biological and structural properties of genome elements and whole genomes, organized on a taxonomical basis.

DEPPDB and its tools [1,2] (deppdb.psn.ru or electrodna.psn.ru) were used to carry out the analysis.

E. potential (EP) is distributed non-uniformly along DNA molecule and correlates, though not corresponds exactly, with GC content, strongly depending on the sequence arrangement and its context (flanking regions). Binding frequency of RNA polymerase to DNA along the genome, measured in direct experiment, correlates to the calculated EP.

TR areas have EP and other PP peculiarities. Binding sites of transcription factors of different protein families in different taxa are located in long areas of high EP. Promoters in average have high value and heterogeneity of EP profile. The transcription starting sites of prokaryotic genomes are characterized by extensive (hundreds of bp) zone of high EP and some peculiarities directly around TSS. This is associated with protein binding and formation of PP due to transcription machinery. Specific details of the TSS EP architecture are similar in related taxa. Promoters up-element demonstrates electrostatic nature.

E. effects on genome functioning interact with other PP of DNA, in particular - bending, thermal stability, supercoiling. They may interact with E. in both, formation, and TR.

E. plays important and universal role in TR in prokaryotes, affecting proteins binding probability and positioning accuracy. It may influence horizontal gene transfer, TR systems evolution and contribute to genome regulatory regions high AT content.

PP formation principles affect such fundamental problems as Chargaff's II rule, redundancy of the genetic code, neutrality of synonymous substitutions; and justify the fundamental idea of DNA phenotype, defining the new principle of biophysical bioinformatics.

Acknowledgements: The authors are grateful to IMPB RAS for hosting the DB.

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Keywords: biophysical bioinformatics, DNA electrostatic properties, transcription regulation.

WED-193
Domain organisation and functional analysis of small RNA methyltransferase HEN1

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Small, 21-33-nucleotide RNA molecules are essential for post-transcriptional gene regulation in eukaryotic organisms including humans. All types of small interfering RNAs (siRNAs) and microRNAs (miRNAs) in plants, piwi-interacting RNAs (piRNAs) in animals require 2′-O-methylation on the 3′-terminal nucleotide for their stabilization, preventing them from a 3′-end uridylation and degradation. This specific modification is carried out by the S-adenosyl-L-methionine–dependent small RNA 2′-O-methyltransferases, which are also found in metazoan, fungal, and bacterial proteomes. The best studied representative of them is *Arabidopsis thaliana* HEN1.

Analysis of tertiary protein structure revealed that *Arabidopsis* small RNAs methyltransferase consists of five domains. To elucidate experimentally the function of each domain, miRNA/miRNA* and siRNA/siRNA* binding analysis, steady-state and pre-steady-state kinetic studies of truncated variants of methyltransferase and HEN1 mutants with point mutations were done. The obtained data indicate that: the methyltransferase domain of HEN1 is important for methyl group transfer; the first double-stranded RNA-binding domain is required for substrate recognition and its tight binding; the second dsRNA-binding domain as well as the extra loop between beta sheets β1 and β2 in R1 (residues 31 to 49), which communicates with methyltransferase domain, are the essential factors decelerating the decay of ternary complexes after methylation reaction. Similar binding and methylation parameters observed with siRNA and miRNA substrates suggest that the HEN1 does not encompass any domain necessary for distinguishing two types of small non-coding RNAs *in vitro*. As the central part of HEN1 is not responsible for the interaction with substrates, it was supposed that this part can be important for binding others biogenesis proteins in plants. This hypothesis was confirmed by data obtained using electrophoretic mobility shift assay, yeast two-hybrid system and pull-down method. The obtained results provide valuable insights into the enzymatic mechanism and biological role of an abundant class of small RNA 2′-O-methyltransferases, which share similar catalytic domains, and are widely distributed in all biological kingdoms except archea.

Keywords: Methyltransferase, HEN1, microRNA biogenesis.

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WED-194
Drought stress alleviate boron toxicity by enhancing stress related gene expressions

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Boron (B) is an essential micronutrient for plant growth and it is toxic in high concentrations. Also, drought is an important abiotic factor effects plant growth and development. To understand plant stress responses in high B condition with drought, we focused on transcript accumulation of three stresses related genes: *GR1*, *MT2*, and *Hsp90*. Quantitative real-time PCR technique was used to determine stress responses of these genes in B and drought stressed tomato leaves. Also, MDA content of plants were detected to observe stress level. Results showed that the genes were over-expressed upon B and drought-stressed induction. The highest relative fold change value was measured on *GR1* transcript (7–8 times comparing with control), indicating the activation of oxidative stress enzyme to tolerate B and drought stresses. Also, *Hsp90* transcript was over-expressed. However, *MT2* expression was not significantly changed. The results showed that the stresses were not enough to trigger the stress responsive genes alone. Dual effect, B with drought stress, can cause to activation of those genes. Hence, we proposed that drought stress may alleviate the B-toxicity by activating the stress-related genes in tomato.

Keywords: None.

WED-195
Electrostatic properties of bacteriophage lambda genome and its elements

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Bacteriophage lambda (L.) is a classical model object and its genome is extensively studied. However, little is known about physical properties of its genome and its elements. Here for the first time we study their electrostatic potential (EP) properties.

Methods and Algorithms: DEPPDB and its tools [1,2] were used to carry out the analysis.

EP global distribution along L. genome corresponds to the localization of its main regulatory elements in the restricted area with high negative EP. Binding frequency of RNA polymerase to DNA along the genome, measured in direct experiment [3], correlates to the calculated EP. Strong lambda phage promoters have pronounced up-element compared to the absence of it in weak promoters. Promoters with intermediate strength possess weak up-element. Lambda-like phages strong pL promoters possess strong electrostatic up-elements, the sequence texts of which are quite different. As shown earlier [1], strong early T7-like and E.coli ribosomal promoters with pronounced up-element have high levels of the EP within it. Strong E.coli promoters such as rrnB with eliminated up-element (and thus greatly reduced strength) do not have pronounced EP valleys in the corresponding area. Mutated up-elements with enhanced promoter strength exhibit deep EP valleys and peculiarities of some other physical properties. This may indicate the direct role of EP in promoters functioning. Attachment sites of L. and E.coli have high EP for integrase recognition. Rho independent lambda and E.coli terminators have the same M-like EP profile, reflecting their palindrome nature, with the same EP scale in three-fold different annotated palindrome length. Rho dependent terminators have no common EP.

Almost all lambda genome elements exhibit E. peculiarities of different kind, that reflect their structural properties and may play role in their biological functioning. Overall genome EP reflects its transcription and host-integration regulation architecture.

Acknowledgements: The authors are grateful to Saveljeva E. G. for technical support and the Institute of Mathematical Problems of Biology of RAS for hosting the Database.

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3. Y.Harada et al., (1999) Single-Molecule Imaging of RNA Polymerase-DNA Interactions in Real Time, Biophys. J., 76 (2): 709–715.

Keywords: DNA electrostatic properties, promoter up-element, terminators.

WED-196
Electrostatic properties of natural DNA palindromes – transcription factors binding sites and terminators

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Electrostatic properties of genome DNA are important to its interactions with different proteins, in particular those related to transcription - RNA polymerase and transcription factors.

During our studies of electrostatic properties of bacteriophage lambda (a classical model object), binding sites of different families of transcription factors and transcription start sites in different prokaryotic taxa, we revealed some common properties of the electrostatic potential distribution around the natural DNA palindromes, such as transcription factors binding sites and terminators, as well as around transcription start sites.

Rho independent lambda and E.coli terminators have the same M-like EP profile, reflecting their palindrome nature, with the same EP scale in three-fold different annotated palindrome length. Rho dependent terminators have no common EP.

The averaged profiles of the binding sites centers exhibit the pronounced rise in the negative potential value with the characteristic symmetrical profile in the consensus area. The same overall properties, though vary in particular details, are typical to binding sites of other families of transcription factors in a diverged range of bacterial taxa. The same pronounced rise in the negative potential value characterize TSS, that have M-like profile, although it is not symmetrical.

Although they share the same main property – symmetry, that reflects the palindrome nature of DNA, they may differ in the details due to different underlying sequences, that carry out their biological functions. Interestingly, terminators also have the base surrounding electrostatic potential valley, as the TFBS and TSS.

DEPPDB and its tools [1,2] were used to carry out the analysis.

Acknowledgements: The authors are grateful to Saveljeva E. G. for technical support and the Institute of Mathematical Problems of Biology of RAS for hosting the Database.

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Keywords: DNA palindromes, electrostatic properties, genome elements.

WED-197
Electrostatics of prokaryotic transcription factors match that of their binding sites

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Transcription regulation is well known to be influenced by physical properties of genome DNA, especially by its electrostatic interactions with different regulative proteins. We studied different families of transcription factors in different prokaryotic taxa together with their binding sites to reveal the role of these properties in the transcription regulation proteins binding.

DEPPDB and its tools [1,2] were used to carry out the analysis.

Electrostatics (E) distribution on TF surface reflects the DNA E of their binding sites. The averaged profiles of the DNA E potential aligned around the binding sites centers exhibit the pronounced rise in the negative potential value with the characteristic profile in the consensus area (often being a palindrome). The extensive (around 100–300 bp long), symmetrical overall potential rise can not be explained by the influence of the consensus alone and reflects the sequence organization of the flanking regions, contributing to the high potential area formation. Apparently this sequence organization was selected evolutionary to support the binding site recognition by the regulation protein molecule and its retention. TF have strong electropositive patch at the DNA binding area, that is surrounded by mostly electronegative or neutral surface. This may lead to proper orientation of TF molecule and facilitate DNA TF site recognition and binding.

The high potential area is relatively AT-enriched, which is reflected in that different other physical properties, especially energy-related, exhibit similar behavior, though the size and parameters of peculiarities are different. This may facilitate binding and accompanying DNA bending. The same overall properties, though vary in particular details, are typical to binding sites of other families of transcription factors in a diverged range of bacterial taxa.

The role of electrostatics in the regulations of cell genome functioning and evolution is significant and universal. This may facilitate the horizontal gene transfer and adaptation of new regulatory circuits for the pan-genome evolution.

Acknowledgements: The authors are grateful to Saveljeva E. G. for technical support and the Institute of Mathematical Problems of Biology of RAS for hosting the Database.

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Keywords: binding sites, electrostatic properties, transcription factors.

WED-199
Expression analysis of putative small GTPase in *Medicago truncatula*

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The recent studies on genomes of *Medicago truncatula* and symbiont nitrogen fixing bacterium, i.e., *Sinorhizobium meliloti*, have contributed to the better elucidation of the symbiotic associations between rhizobial bacteria and leguminous plants at molecular level. Even though many genes have been reported to be evolved at Fabaceae lineage, possibly playing role in the development and function of the nodular structures, the nodulation has not been yet fully illuminated at molecular level. Small GTP-binding proteins purported to be involved mainly in basic cellular functions such as molecular switches in signal transduction, cell proliferation, cytoskeletal organization, and intracellular membrane trafficking. It is very likely that these genes could have been subfunctionalized in legume-specific manner to regulate processes related to nodulation. To assess the validity of this assumption, we have investigated the spatiotemporal expressional characteristics of the previously identified (*Yüksel and Memon, 2008) small GTP-binding genes, namely, Arf (*MtrGB13780538*, *MtrIMGAAC144806_7.1*), Rab (*MtrGB13781173*, *MtrGB13781681*, *MtrPUT45174*, *MtrPUT18368*, *MtrPUT155272*, *MtrIMGAAC1221648.2*), Rob (*MtrIMGAAC140545-16.1*). In brief, the quantitative reverse transcription PCR analyses indicated that these putative small GTPase genes have different expression profiles in different plant tissues of *Medicago truncatula*. All genes had significant level of expression in the root, nodules, petioles, stems, and leaves. However, the expression level of particular putative nodule-specific gene, i.e., *MtrPUT45174, MtrGB13781681, MtrPUT155272, MtrIMGAAC1221648.2 MtrPUT18368* were noticeably higher a in the infected roots and nodular structures. To further more detailed experiments will be needed to better assess the possible key role of these genes in the establishment and maintenance of nodular structures.

* YÜKSEL, B., MEMON, A.R., 2008, Comparative phylogenetic analysis of small GTP-binding genes of model legume plants and assessment of their roles in root nodules, *Journal of Experimental Botany*, 59, 14, 3831–3844.

Keywords: medicago, quantitative reverse transcription PCR, small GTPase.

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WED-200
Expression patterns of catalase genes in maize plants colonized with the bird cherry-oat aphid (*Rhopalosiphum padi* L.)

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The purpose of this study was to investigate impact of *Rhopalosiphum padi* L. (bird cherry-oat aphid) colonization on relative expression of *cat1* and *cat2* genes encoding the relevant catalase isoforms in tissues of maize (*Zea mays* L.) plants. The biotests were accomplished on 14-d-old seedlings of Zota Karowa (aphid-susceptible) and Waza (aphid-relatively resistant) genotypes. Four levels of insect infestation were tested: 5, 10, 20 and 40 wingless parthenogenetic females per plant. Transcriptional activity of the two catalase genes was analysed after 1, 2, 4, 8, 24, and 48 h post-initial aphid infestation (hpi). Real-time qRT-PCR was applied to quantify the amount of *cat1* and *cat2* transcripts, and the data were normalized to glyceraldehyde 3-phosphate dehydrogenase (*gapdh*) gene. The insect attack led to profound alternations in gene expression levels compared to the non-treated controls. The highest transcriptional reprogramming of both examined genes in seedlings of investigated maize plants was evidenced after 24 and 48 hours of the aphid feeding. Importantly, it was demonstrated significant intervarietal differences in the scale of insect-elicited modulations in expression of the targeted catalase genes. The obtained results suggest a pivotal role of catalase isozymes in counteracting the redox imbalance occurring within *R. padi*-attacked maize plants.

Keywords: Catalase genes, Maize genotypes, Rhopalosiphum padi.

WED-201
Functional analysis of a gene encoding a protein of Spen type, in *Arabidopsis thaliana* L. (Magnoliophyta: Brassicaceae)

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The family of Spen proteins (split ends) greatly varies in size (90–600 kD). These proteins are characterized by the presence of at least one RRM domain (RNA recognition motif) that allows them to bind to RNA, and a SPOC domain that allows them to interact with other proteins. RRM domain contains 90 amino-acid residues and it is found in a variety of hnRNPs proteins (heterogeneous nuclear ribonucleoproteins) which are implicated in the RNA alternative splicing, and in components of snRNPs proteins (small nuclear ribonucleoproteins). SPOC domain contains approximately 165 amino-acid residues and it is implicated in signaling pathways in development, facilitating protein-protein interaction. Spen proteins are involved in different biological processes (1).

The At4g12640 gene encodes a 823 aa putative protein containing two RRM motifs (25–92 aa 152–220 aa) and a SPOC domain (471-567 aa). We generated gene constructs in which a segment of 1000 base pairs of the promoter was fused to the marker gene *uidA::GFP* using the Gateway® technology, *A. thaliana* plants were transformed by the modified floral dip method (2), using *A. tumefaciens* strain pGV2260. Expression associated to the vascular tissue was observed in the transformed plants in the following structures: root, cotyledon leaf, true leaf and stamens. In the gynoecium the expression was detected in the developing embryos.

The second gene that encodes a protein of type spen, in Arabidopsis is *FPA*, which participates in the regulation of the autonomous pathway of flowering (3). However, the expression patterns of both genes are different, so we consider that At4g12640 participates in the development of the embryo and in vascular tissue. Moreover, inactivation and overexpression of the gene in the phenotype of *A. thaliana plants* are being analyzed to infer aspects of its biological function, which will be discussed in more detail.

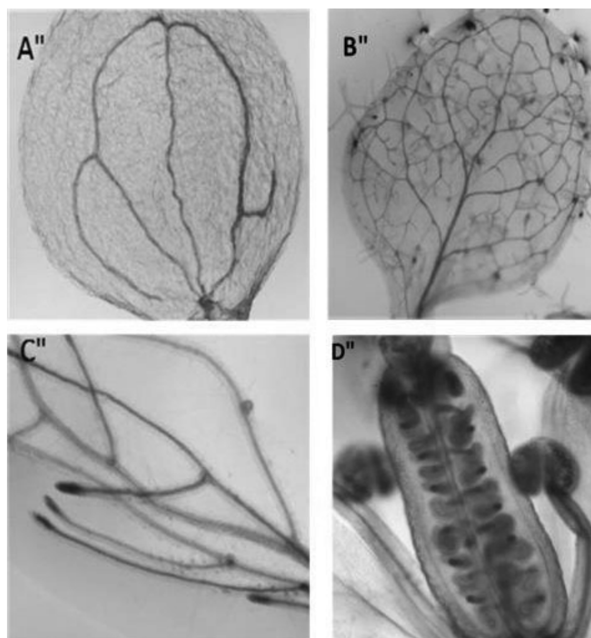

Fig. 1. Expression conferred by the promoter of the At4g12640 gene in transformed plants of *Arabidopsis thaliana*. (A) cotyledon leaf, (B) true leaf, (C) Roots, (D) Gynoecium with developing embryos.

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Keywords: Arabidopsis, function, protein, Spen.

WED-202
Functional promoter analysis of the developmentally regulated A/B.L9 chorion gene pair in *Bombyx mori*

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In the silkmoth *Bombyx mori*, chorion genes of the same developmental specificity are organized in divergently transcribed α/β gene pairs which share a common 5′ flanking promoter region. To date, the suggested model describing the developmental regulation of these genes implicates both *cis*-elements, harbored in their bidirectional promoters and their corresponding transcription factors (BmC/EBP, BmHMGA, BmCHD1, BmGATAβ).

In order to investigate the *A/B.L9* promoter (pL9) architecture (figure 1) and its contribution to temporal gene specificity, several pL9 constructs, bearing different *cis*-element mutations, were cloned upstream of the *lac*Z reporter gene in both orientations, so that the *lac*Z gene stood in for either the α- or the β- chorion gene. These were used in an electroporation-based transient

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expression method for *ex vivo* developing follicles. Constructs containing the complete promoter were used as control.

Mutation of the HMGA recognition site rendered pL9 completely inactive as expected, since HMGA is responsible for recruitment of transcription factors on the promoter. Promoter constructs with functional only one of the C/EBP1, C/EBP3 or C/EBP4 sites were not capable of initiating transcription in both directions. However, the pL9 construct with functional only the C/EBP2 site could initiate transcription in both directions, while mutation of the C/EBP2 binding site resulted in complete inactivation of pL9. Thus, the C/EBP2 site was required for promoter activation. The promoter construct with functional only the C/EBP3 site made pL9 completely inactive while mutation of the C/EBP3 binding site did not change the expression pattern in either orientation compared to the control. Therefore, the C/EBP3 binding site appeared to be "neutral". On the other hand, expression patterns produced from other constructs revealed the importance of the C/EBP4 binding site for fine tuning of *A/B.L9* gene expression. Finally, mutation of the GATA recognition site shifted the expression pattern to earlier stages - compared to the control - regardless of promoter orientation, confirming that BmGATAβ acts as an early repressor on the expression of middle specificity L9 chorion genes.

Combining the data described above and previous data from our lab we propose a more detailed model describing the events which control the developmentally accurate *A/B*.L9 gene pair expression.

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Fig. 1. Wild type L9 promoter architecture which involves the number of and distance between transcription factor recognition sites. C/EBP binding sites are numbered from 1 to 4 beginning from the closest to the α-gene. Arrows represent the orientation of each binding site.

Keywords: Bidirectional promoter, C/EBP binding site, Choriogenesis.

WED-204
Functional studies of CrNIP7 from *Chlamydomonas reinhardtii*: a protein involved in the ribosome biogenesis

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Ribosome biogenesis is a complex and highly regulated process, in which a primary transcript is processed to form mature ribosomal RNAs. This process is best characterized in yeast, interestingly several factors which were first described in *Saccharomyces cerevisiae* have shown divergent functions in humans. One of these divergent factors is NIP7, a highly conserved protein encoded by the essential single-copy *nip7* gene, that acts in the formation of 60S and 40S ribosomal subunits in yeast and human, respectively. To advance our understanding of ribosome biogenesis in other eukaryote models, we have performed some functional analysis of NIP7 ortholog from the algae *Chlamydomonas reinhardtii*, an ancestor organism to plants. In this context, the *CrNip7* gene was amplified by PCR from total cDNA and subcloned in plasmids for functional complementation assays, using three different strains of *S. cerevisiae*. Our results revealed that CrNIP7 complements the function of yeast Nip7p, since the expression of CrNIP7 in a temperature-sensitive mutant (DG130) strain and in the *nip7Δ* strain (DG442) recovered the growth of both strains, similarly to the positive control strain. Furthermore, yeast two hybrid assays were also performed in order to identify potential protein interactions of CrNIP7. Some potential protein partners were identified, including a protein with a G-path domain, and these interactions are currently under validation. Taken together, our results will contribute to clarify the role that CrNIP7 plays in the ribosome biogenesis of *Chlamydomonas reinhardtii* in comparison with other eukaryote models.

Keywords: Chlamydomonas reinhardtii, ribosome biogenesis, yeast complementation assay.

WED-205
Genome wide identification of natural antisense transcripts in *Plasmodium falciparum* clinical isolates

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Antisense transcription is pervasive among all kingdoms of life. Natural Antisense Transcripts (NATS) are transcribed from the strand opposite to that of the sense transcript of either protein-coding or non-protein coding genes. Initially, it was considered by many as the product of transcriptional noise but now it has taken its position as an important regulator of gene expression. In an organism, diverse species of NATs may exist in different developmental and environmental conditions and their level of expression may also change in response to various environmental cues. As the role of NATs in modulation of gene expression is becoming evident day by day, it is crucial to identify NATs in organisms like *Plasmodium falciparum*, where the understanding of genome regulation is limited. Although, NATs have been identified in different intra erythrocytic developmental cycle stages and gametocyte stages of *P. falciparum* in culture condition, its diversity in natural parasite population has not been documented.

We report here the diversity of NATs in this parasite, using isolates taken directly from patients with differing clinical symptoms caused by malaria infection. Using a custom designed strand specific 244K Agilent microarray, a total of 797 NATs targeted against annotated loci have been detected. These were compared with preciously reported studies from cultured parasites. Out of these, 545 NATs are unique to this study. The majority of NATs were positively correlated with the expression pattern of the sense transcript. The antisense transcripts map to a broad range of biochemical/ metabolic pathways. Importantly, NATs from 91 annotated gene loci were found to be differentially regulated in complicated malaria (n = 9) compared to uncomplicated malaria (n = 2). Detection of antisense transcripts and their differential expression for selected number of genes (n =6) was further validated using strand specific reverse transcriptase and quantitative real-time PCR experimentations. Moreover, position wise genome wide scanning and experimental studies were carried out to under-

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stand the possible mechanisms of antisense production. The results detailed here call for studies to deduce the possible mechanism of action of NATs, which would further help in understanding the *in vivo* pathological adaptations of these parasites.

Keywords: Complicated malaria, Natural antisense transcripts, Plasmodium falciparum.

WED-206
Global transcriptional profiles of the copper responses in the cyanobacterium *Synechocystis* sp. PCC 6803

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Copper is an essential element implicated in fundamental processes like respiration and photosynthesis. However, it becomes toxic when is present in excess, which has forced organisms to control its cellular concentration. With the exception of the photosynthetic cyanobacteria, which contain an intracellular organelle called thylakoid which hosts the Cu-requiring process, photosynthesis, there are no known bacterial species that have intracellular copper-containing enzymes. All known copper-containing proteins in gram-negative bacteria are either periplasmic enzymes or located in the cytoplasmic membranes, making cyanobacteria in an important bacteria group to study copper metabolism. Copper resistance in the photosynthetic cyanobacterium *Synechocystis* is mediated by the CopRS (hik31-rre34) two-component system. This two-component regulates its own expression and expression of a RND metal transport system (CopBAC) in response to copper in the media [1]. The system is also able to respond to changes in internal copper pools when plastocyanin is degraded in response to photosynthetic electron transport block [1]. Furthermore CopS is able to bind copper directly with high affinity and is partially localized to thylakoid membranes where it could detect copper released from plastocyanin. Here we have used microarrays to interrogate the global responses to copper additions at non-toxic (0.3 μM) and toxic concentrations (3 μM) of copper in WT and in the copper sensitive *copR* mutant strain. Addition of the non-toxic copper concentration stimulated the metabolism and induced the switch in the use from cytochrome c_6 to plastocyanin. In contrast, toxic copper catalyse the formation of ROS, lead to a general stress response and induced expression of Fe-S cluster biogenesis. According to this, a *copR-sufR* mutant strain, that expresses constitutively the *sufBCDS* operon, tolerated higher copper concentration than the *copR* mutant strain, suggesting that Fe-S clusters are direct targets of copper toxicity in *Synechocystis*. Finally, global gene expression analysis of the copR mutant strain revealed that CopRS only controls the expression of *copMRS* and *copBAC* operons in response to copper.

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Keywords: copper resistance, cyanobacteria, genome-wide analysis.

WED-207
Identification of RNA editing targets using NGS data

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RNA editing was first discovered in 1984 by Benne et al in trypanosomatid mitochondria. Since this time different types of RNA editing were discovered in many phylogenetically distant groups of organisms (plants, animals, dinoflagellates, myxomycetes).

In trypanosomatid mitochondria U are extensively inserted in or deleted from primary transcript by editosome – large multienzyme complex. Editing results in formation of functional ORF, including creation of start codon.

Out lab focuses on evolution of edited genes structure and function. Recently we began to use NGS approach to obtain and analyze full transcriptome from trypanosomatid's mitochondria. But still discovery of edited sites remains a challenging task. As example, ND8 edited transcript has length about 450 nucleotides and ND8 pre-edited mRNA has only about 250 nucleotides. So about 200 Us are inserted in the process of editing. Surely, it is not possible to map a read corresponding to edited from of RNA using the existing software.

We have modified Bowtie2 short read mapper to suite RNA edited reads mapping task and tested it on our data. The modified Bowtie2 version successfully maps even extensively edited reads on reference genome and produces correct high-quality alignment.

We think our approach can be used to identify new targets of RNA editing of different types not only in trypanosomatids, but in other organisms too. Furthermore we think that such search of new targets of RNA editing is an important step in understanding gene expression and regulation and especially nowadays when huge amounts of RNAseq data are generated in many labs.

Keywords: mitochondria, NGS, RNA editing.

WED-208
Investigating RNA editing in the pathogenesis of amyotrophic lateral sclerosis

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The susceptibility of motor neurons to injury in amyotrophic lateral sclerosis (ALS) may result from excitotoxicity and subsequent dysregulation of intracellular calcium homeostasis. Post-transcriptional editing of the AMPA glutamate receptor (GluR) subunit(s) may alter the calcium ion permeability of the receptor pore, leading to neurodegeneration.

Objectives: The aims of the study are (i) to investigate GluR RNA editing in ALS compared to non-neurological controls, and (ii) to characterise TDP43 and p62 expression in ALS.

Methodology: RNA extraction and cDNA preparation followed by amplification of cDNA by PCR was used to generate amplified $GluR_2$, $GluR_5$ and $GluR_6$. These products were digested to produce cuts in the internal site of GluR mRNA and differentiate edited from non- edited GluR, and analysed by agarose gel electrophoresis and using the Agilent Bioanalyser. TDP-43 and p62 expression was assessed by immunohistochemistry.

Results: $GluR_2$ was fully edited in all motor cortex of ALS cases and controls. $GluR_5$ was 83% edited in motor cortex of ALS cases and 82% edited in controls. Whereas, $GluR_6$ editing in motor cortex was 88% and 91% in the ALS cases compared to 75% and 62% in controls, respectively. $GluR_2$ was 100% edited in the motor neurons isolated by LCM.

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Phosphorylated TDP-43^{+} and p62^{+} skein-like bodies and compact inclusions were detected in C9ORF72/ALS positive cases and C9ORF72/ALS negative cases whereas no immunoreactivity was detected in the control healthy subjects.

Conclusions: Our data on whole tissue sections suggests that $GluR_2$ receptor of motor cortex in both sALS and control individuals is fully edited whereas partial editing has been identified in $GluR_5$ and $GluR_6$ receptors. Full editing of $GluR_2$ in dissected motor neurons isolated by LCM was confirmed.

Motor neurons in ALS cases, both C9ORF72^{+} and C9ORF72^{-} expressed pathogenic inclusions of phosphorylated TDP-43 and p62, which are a hallmark for ALS

Keywords: ALS, RNA editing.

WED-209

Ki-1/57 and CGI-55 ectopic expression impact cellular pathways involved in proliferation and stress response regulation

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Ki-1/57 (HABP4) and CGI-55 (PAIRBP1) are regulatory proteins and paralogs with 40.7% amino acid sequence identity and 67.4% similarity. Functionally they have been implicated in the regulation of gene expression on both the transcriptional and mRNA metabolism levels. Both localize to the cytoplasm but also shuttle to the nucleus and are modified by several post-translational modifications. However, to date there is no knowledge on the target genes, which expression may be regulated by Ki-1/57 and CGI-55 and detailed insight in their possible cellular function is missing. Here, we were interested in characterizing the alterations of the global transcriptome profile after Ki-1/57 or CGI-55 over-expression in HEK293 cells by DNA microchip technology in order to obtain further insights in these proteins functions. We were able to identified 363 or 190 down-regulated and 50 or 27 up-regulated genes for Ki-1/57 and CGI-55, respectively, of which 20 were shared. The great majority of the genes with altered expression are associated to proliferation, apoptosis and cell cycle control processes, prompting us to further explore these contexts experimentally. We observed that over-expression of Ki-1/57 and CGI-55 results in reduced cell proliferation, mainly due to a G1 phase arrest. In case of Ki-1/57 over-expression we found protection from apoptosis after treatment with the ER-stress inducer thapsigargin. Furthermore, we found that CGI-55 and Ki-1/57 co-localize with cytoplasmic stress granules under different stress conditions. Our global transcriptome analysis after Ki-1/57 and CGI-55 over-expression, together with a correlation of the published protein interactome data suggest that both proteins act mainly as repressors that affect the expression of genes that are involved in proliferation and cell cycle as well as apoptosis regulation. Protein interactome data and additional experimental data confirmed these functional contexts and further established a link with stress responses, in which both proteins tend to localize to cytoplasmic stress granules.

Keywords: CGI-55, Gene repressor, Ki-1/57.

WED-210

Licensing mRNP for nuclear export: a role for chromatin remodelers

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The harmonious production of export competent messenger ribonucleoparticles (mRNP) is linked to the tightly orchestrated transcription. Indeed, RNA polymerase II coordinates the ordered recruitment of mRNA processing and export factors resulting in the assembly and release of 5′capped, spliced and polyadenylated mRNPs. In yeast, the conserved mRNA export receptor Mex67 mediates nuclear exit of fully mature mRNPs through sequential interactions with nuclear pore complex (NPC) proteins lining the pore. The nuclear export machinery already associates with nascent transcripts during the transcription and maturation steps and insure the tight connection between mRNA biogenesis and transport. Owing to its complexity, mRNA biogenesis is error prone and mistakes taking place at any step of the biogenesis process lead to nuclear accumulation and degradation of "faulty" mRNPs. This is the consequence of quality control systems that combat the nuclear exit of aberrant mRNPs, which may be harmful if translated into defective proteins. The accuracy of eukaryotic gene expression relies on the balance of mRNPs assembly and surveillance. This competition therefore implies the existence of nuclear export "checkpoints" to prevent futile synthesis/degradation cycles.

Intriguingly, our recent results evidence a central role for a conserved ATP-dependent chromatin remodeling complex in licensing nuclear mRNPs for export. Indeed we found that inactivation of this complex was able to partially suppress the growth and mRNA export defects of a certain category of mRNA export mutants. Chromatin remodelers have the faculty to slide, eject or reposition nucleosomes thereby providing the transcription and replication machineries with dynamic access to the genome. However, the complex we identified was reported to have very little influence, if any, on the transcription process per se. In this context, our identification of strong genetic, physical and functional interactions between the mRNP biogenesis machinery and a chromatin remodeler might well be key to unravel its previously unsuspected role in gene expression. Our working hypothesis is that this chromatin remodeler would exert a nuclear export licensing function already at the transcription site.

We are currently attempting to decipher the molecular mechanism this complex to the licensing of newly synthetized mRNPs for nuclear export. Whereas the dynamics of chromatin structure is increasingly recognized as playing an essential role in the control of DNA-associated processes (transcription, replication, repair), our present findings open up the possibility that chromatin remodelers might also influence the fate of newly synthesized transcripts.

Keywords: chromatin, licensing activity, mRNA export.

WED-211

Maf1, a negative regulator of RNA Pol III, mediates yeast response to amino acids starvation and inhibition of translation

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Transcription and translation play a crucial role in regulation of gene expression. Cross-talk between these processes is still far from being understood. Treatment of yeast *Saccharomyces cerevi-*

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siae with cycloheximide leads to inhibition of translation elongation and a decrease in rRNA levels. Interestingly, we and others found that under amino acid starvation the presence of cycloheximide can stimulate tRNA synthesis. Analysis of tRNAs by Northern blotting indicates that cycloheximide treatment augments the level of tRNA precursors, which suggests enhanced activity of the Pol III machinery. Activity of Pol III is known to be controlled by a set of factors, including a negative regulator Maf1. We showed that cycloheximide treatment of amino acid-starved cells partially suppressed Pol III repression by Maf1. In contrast to the previously observed dephosphorylation and nuclear localization of Maf1 under stress conditions, we observed that in cycloheximide-treated starved cells a fraction of Maf1 is in the cytoplasm. Moreover, cycloheximide treatment resulted in the increase of phosphorylated Maf1 fraction. Finally, a decreased interaction of Maf1 and Pol III was shown by co-immunoprecipitation. A possible mechanism controlling tRNA synthesis in conditions which repress translation elongation will be discussed.

Keywords: cycloheximide, Maf1, RNA polymerase III.

WED-212
Modulation of HIV-1 gene expression by binding of UHM-containing splicing factors to a ULM motif in the Rev protein

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Human immunodeficiency virus type 1 (HIV-1) is a lentivirus encoding the Gag, Pol and Env proteins common to all retroviruses and several specific regulatory proteins. The HIV-1 regulatory protein Rev is essential for virus replication and ensures the expression of partially spliced and unspliced transcripts. Rev binds to the Rev-responsive element (RRE) in viral mRNAs in the nucleus and recruits factors required for the export of these viral mRNAs to the cytoplasm. Additional functions have been shown for Rev, among them the regulation of viral transcript splicing.

We identified a ULM-like motif in the RRE-binding region of the Rev protein. ULMs (UHM ligand motifs) mediate protein-protein interactions and modulate spliceosome assembly through their binding to UHMs (U2AF homology motifs). We therefore investigated whether the Rev ULM can interact with UHMs present in host splicing factors. Isothermal titration calorimetry and NMR titration experiments showed that Rev ULM binds to the UHMs of SPF45 and U2AF65, alternative and constitutive splicing factors, respectively. The crystal structure of the SPF45-UHM bound to the Rev ULM reveals that the Rev ULM adopts an extended conformation upon binding to SFP45. Structural analysis and biochemical experiments demonstrate that the highly conserved W45 in the Rev ULM is crucial for Rev-UHM interactions. Moreover, Rev coprecipitates with U2AF65 in human cells. Finally, we show, that W45 in Rev ULM is important for proper processing of HIV transcripts and Rev-mediated increase of unspliced RRE-containing RNA transcribed from a HIV-derived reporter plasmid (pDM128).

We propose that Rev-ULM interactions with UHM-containing splicing factors contribute to the regulation of HIV-1 transcript processing, possibly at the level of splicing.

Keywords: HIV-1, mRNA processing.

WED-213
NMR conformational dynamics of La and RRM1 motifs of La protein

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La/SSB protein serves as an important mediator of RNA polymerase III transcripts folding. It has been well established that guides accurate 5′ end maturation of pre-tRNAs by RNase P, while protecting at the same time their 3′ ends from degradation. La/SSB is found in abundance within nuclei, and studies have shown that is up-regulated during oncogenesis, a cellular perturbation which requires elevated protein synthesis rates. Originally discovered in patients with systemic lupus erythematosus and Sjögren's syndrome, it contains a characteristic conserved, predominantly helical structure, termed "La motif" and two RNA binding motifs (RRM1 and RRM2). So far, the structure of full-length La protein remains elusive. Scattered structural data of the La and the RRM motifs from few eukaryotes (including human) with synthetic oligoribonucleotides bound provide only limited information on the possible roles of the La/SSB protein as a whole, in a more dynamic tRNA-dependent cellular network. In the present study we initiated an extensive structural and functional characterization of a "domain library" of La/SSB which includes several domains of different length and structural signature. Here, we present the NMR-derived structure of La and RRM1 motifs. Both motifs were found to be well-folded in "winged-helix" and classical RRM structures, respectively, as revealed by high resolution NMR spectroscopy. In addition, the RNA binding properties of the La motif were investigated and the interaction interface was identified through chemical shift perturbation of amide groups in ^{1}H-^{15}N HSQC spectra. Interestingly, both NMR analysis and biochemical experiments indicate that La motif alone can mediate interaction with pre-tRNAs, an observation which raises questions on the actual role of La motif in combination with the RRM motifs, during tRNA biogenesis.

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Keywords: Lupus Antigen, NMR, tRNA.

WED-214
On the track of novel mechanisms controlling iron storage in yeast

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Disruption of iron homeostasis originates significant damage in cells. Organisms have evolved sophisticated mechanisms that, on one hand avoid the drastic consequences of iron scarcity and, on the other hand circumvent the toxic effects of iron overload. The budding yeast *Saccharomyces cerevisiae* is able to grow under a wide magnitude of Fe accessible environments and can survive large fluctuations in Fe bioavailability. Unlike vertebrates, but similar to plants, the yeast cell vacuoles function as iron reser-

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voirs. Ccc1 is the vacuolar transporter that mediates iron storage in yeast. In a high-Fe milieu, *CCC1* deletion is lethal and Yap5 - one of the eight members of the Yeast AP-1 like proteins (Yap) family - regulates its expression. We have previously shown that besides the iron vacuolar transporter *CCC1*, Yap5 also directly controls the expression of glutaredoxin *GRX4,* known to be involved in the regulation of the nuclear localization of Aft1 (the major transcriptional activator in Fe deficiency)[1]. Consistently, we showed that in the absence of Yap5, Aft1 nuclear exclusion is slightly impaired. In addition, we demonstrated that Yap5-mediated regulation of *CCC1* is not essential for cells to overcome iron overload. This new finding brings forward the hypothesis that another yet unidentified factor is regulating iron storage in yeast. This hypothesis is further corroborated by the unexpected observation that Yap5 is degraded in the presence of iron. Interestingly, the removal of *CCC1* 3′ untranslated region (3′UTR) renders yeast cells more sensitive to growth under Fe overload, suggesting that *CCC1* is also post-transcriptionally regulated. Overall, this work provides further evidence that cells avoid iron overload by using multiple pathways.

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Keywords: Ccc1, Iron, Yeast.

WED-216
Regulation of EhTMKB1-9 gene is through lipid (s) via PI3 kinase signaling pathway in protozoan parasite *Entamoeba histolytica*

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Amebiasis is a major health problem in developing countries and the third leading cause of death due to parasitic diseases. It is mainly spread through contaminated food and water. The protist parasite *Entamoeba histolytica* is the causative agent of amoebiasis. More than 90% of the infected individuals do not display symptoms of invasive disease (intestinal and extraintestinal) and under certain hitherto unknown conditions it invades tissues. It is believed that amoeba needs appropriate signal to turn from non virulent to virulent form. Our laboratory has been investigating different signaling pathways of this pathogen in order to understand mechanisms of this transition from a commensal to a pathogen. In this study we describe a signaling pathway initiated by an unsaturated fatty acid that is involved in regulating expression of a trans membrane kinase that may participate in proliferation of this parasite. Transmembrane kinases of *E. histolytica* are known to have diverse roles ranging from virulence, phagocytosis and stress response. Transmembrane kinase EhTMKB1-9 is one of the early response genes and is regulated by bovine serum, one of the components necessary for cellular proliferation *in vitro*. In our attempt to understand the nature of the signal given by serum, we tested different components of serum and found that bovine serum albumin can replace serum in stimulating EhTMKB1-9 expression in serum starved cells. Further analysis, showed that unsaturated fatty acid, such as oleic acid can replace bovine serum albumin in stimulating expression of EhTMKB1-9. Promoter element of EhTMKB1-9 was identified and found to be between -817 to -768. Both BSA and oleic acid stimulated EhTMKB1-9 expression through activation of the promoter as observed for whole serum suggesting that the activation is through transcriptional activation of promoter due to binding of specific factors. Inhibitors that block different signaling pathways were tested and the results showed that activation is through PI3 kinase mediated pathway. Inhibitor studies also indicated that Class I PI3 kinases are involved in the regulation of this gene. Our results also show that actin dynamics is not involved in this activation suggesting that uptake of lipids may not be linked to activation. Dietary fats are known to influence fate of infection and our results provide a possible explanation for this.

Keywords: cell proliferation, PI 3 kinase signaling, transcription regulation.

WED-217
Regulation of slamf1 gene encoding co-stimulatory receptor CD150 in human B cell lines

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SLAMF1 (CD150) receptor serves as co-activator molecule expressed on a variety of hematopoietic cells, controlling innate and adaptive immune responses. This receptor is a type I transmembrane glycoprotein of the CD2 family and immunoglobulin (Ig) superfamily. Disorders in regulation of SLAMF1 expression have been identified as important factors for development of autoimmune diseases. Expression of CD150 is low in naive human B cells and becomes upregulated after B cell activation via BCR, CD40, CD180 and signaling after LPS and IL-4 treatment.

In our study we investigated the expression of the gene slamf1 (CD150) in human B - lymphoblastoid cell lines. We found that there are two main mRNA isoforms in these cells, one of which contains a number of short upstream open reading frames (uORF) in its 5′- untranslated region (5′UTR) impeding effective translation. It was established that B-cell lines with high expression levels of CD150 protein (MP-1 line) preferably synthesized the short mRNA isoform containing no short uORF, whereas the long mRNA isoform is mainly expressed in the cell line with a relatively low expression level of CD150 (Blin-1 line).

The promoter of slamf1 gene was also studied. Using deletion analysis, we showed that the most significant area for CD150 promoter activity is 220 nucleotides upstream of the transcription start point of the short mRNA isoform. This sequence contains binding sites for transcription factors PU.1, IRF4, AP- 1, Oct2, EBF1, NfkB, SP1. Mutation of EBF1 binding site has reduced promoter activity by 80%; mutations of several other binding sites (PU.1, IRF4, NfkB, SP1 binding sites) also had significant effects on the promoter activity. Additionally, four putative enhancer sequences that can enhance the activity of the putative slamf1 gene promoter were found and functionally tested.

Keywords: gene expression, promoter analysis, slamf1.

WED-218
Role of AhR in the myelination of the central nervous system

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The aryl hydrocarbon receptor (AhR) is a transcription factor known to mediate most of the toxic effects of widely persistant organic pollutants such as dioxin (TCDD). This receptor belongs to the basic helix-loop-helix Per/ARNT/Sim family whose members are involved in physiological processes such as circadian cycle, neurogenesis and organs development. Historically, the AhR has been described as a xenobiotic-activated transcription

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factor, which promotes the elimination of xenobiotics through the regulation of the expression of genes involved in xenobiotic metabolism. Recently, studies conducted in invertebrates have demonstrated that this receptor played a role in the development and homeostasis of the nervous system regardless of exposure to xenobiotics. However, the physiological roles of AhR in mammals remain unclear. A behavioral study conducted in our laboratory showed that AhR knockout mice had eye instability named horizontal pendular nystagmus. Our goal is to identify and characterize the biological mechanisms involved in the development of this phenotype. Nystagmus is often associated with myelin disorders. In this context, we conducted experiments with TCDD, an AhR ligand and siRNA targeting this receptor used independently to block its physiological functions, and demonstrated in a murine oligodendrocyte lineage, that both protocols changed the expression of Wnt/ ß-catenin target genes coding for myelin or cell cycle proteins. Meanwhile, a real-time cell analysis (Xcelligence®) showed that treatments with AhR agonist or pharmacological antagonists altered the cell index values suggesting changes of cell morphology, alterations of the cell adhesion and/ or proliferation. Cytoskeleton labelling by immunofluorescence showed that these treatments produced early morphological changes.

Keywords: Aryl hydrocarbon receptor, dioxin, oligodendrocyte.

WED-219
Roles of nElavl in Kif2a pre-mRNA splicing and functional roles of KIF2A protein isoforms

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RNA binding proteins (RNABP) are cytoplasmic and nuclear proteins that contain RNA recognition motifs (RRM) which binds to either single or double-stranded RNAs and form a ribonucleoprotein complex. *Elavl2*, *Elavl3* and *Elavl4* are family members of highly abundant nElavl (neuronal Embryonic lethal abnormal vision-like) RNABP proteins. nElavl proteins were discovered as autoantigens in a multisystem neurologic disorder termed paraneoplastic encephalomyelopathy and are exclusively expressed in neurons. Previously, it has been found that nElavl proteins regulate alternative splicing of numerous neuronal pre-mRNAs, by binding to specific intronic sequences. One of the targets of nElavl is *Kinesin superfamily protein 2a (Kif2a)* pre-mRNA, encoded by a gene with roles in axonal branching, pruning and cortical development. It has been shown that deletion of Elavl3 and 4 affect *Kif2a* alternative splicing at exon 18 and consequently isoform abundance. Our results demonstrate that nElavl proteins bind directly to intronic elements in the intronic regions flanking the *Kif2a* alternative exon 18. Moreover, it has been previously shown that KIF2A represses axon branching and elongation, such that deletion of *Kif2a* in neurons causes ectopic axonal branching and extra synapses at target regions. Currently, our understanding of the functional roles of different KIF2A isoforms is completely unknown. Our results demonstrate that exon 18 alternative splicing does not regulate the subcellular localization of the different Kif2a protein isoforms. Our efforts continue to focus on identifying different functional roles for Kif2a protein isoforms.

Keywords: Alternative Splicing, Kif2a, nElavl.

WED-220
SCL and RUNX1 regulate RUNX1 gene expression by association to RUNX1-P1 promoter

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RUNX1 is a transcription factor essential to hematopoiesis process. Its gene is located in chromosome 21 and two promoters, P1 and P2, regulate its transcription. Analysis of P1 promoter's sequence identified several consensus sites for different hematopoietic transcription factors include SCL, RUNX, and others, suggesting a possible role in transcriptional regulation of RUNX1 gene. Moreover several report have shown that these two transcription factors are associated to regulate target genes. To test if P1 promoter is regulated by SCL and RUNX1 we cloned P1 in a luciferase-reporter vector, and we measured reporter activity to SCL response. We observed increased reporter activity in a doses-dependent manner. To determinate association of SCL to P1 promoter we performed chromatin immunoprecipitation (ChIP) in hematopoietic cells (HL-60 and Jurkat cells), our results show that SCL binds to SCL site. Interestingly, we also detect SCL enrichment in RUNX sites present in P1 promoter. Using ReChIP assays we demonstrate that RUNX1 and SCL simultaneously associate to P1 promoter through RUNX binding sites. Taken together our results demonstrate that in HL-60 and Jurkat cells SCL regulate P1 promoter activity and associates to RUNX1 protein.

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Keywords: gene expression regulation, RUNX1.

WED-221
SREBP-1 is a transcriptional regulator of secreted frizzled-related protein 2 in chondrogenic cells

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Secreted frizzled-related protein 2 (Sfrp2) is highly expressed in developing limbs and is associated with skeletal malformation such as Syndactyly and Brachydactyly. However, the molecular mechanism underlying the transcriptional regulation of Sfrp2 in chondrogenic cells is largely unknown. In this study, we demonstrated the mechanism of transcriptional activation of the Sfrp2 gene in chondrogenic cells. We found that overexpression of SREBP-1 led to stimulation of Sfrp2 promoter activity and increase of Sfrp2 mRNA in chondrogenic ATDC5 cells. Results of reporter gene assays using deleted Sfrp2 promoter constructs showed that the SREBP-1-responsive region is localized at the position between -1150 and -840 and is responsible for transcriptional activation of the Sfrp2 gene. Mutation of a SREBP-1-binding site in this region resulted in elimination of SREBP-1-mediated Sfrp2 promoter activity. Results of promoter enzyme immunoassay and chromatin immunoprecipitation assay showed that SREBP-1 binds directly to the SREBP-1-binding site at region -935/-926 of the mouse Sfrp2 promoter. Collectively, these results demonstrated that SREBP-1 is a positive regulator of Sfrp2 regulation in chondrogenic cells.

Keywords: Sfrp2, SREBP-1, Transcriptional regulation.

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WED-222
The effects of rotenone on ischemia-reperfusion injury in rat testis

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Introduction: Testis is sensitive to ischemia-reperfusion injury, therefore, ischemia and consecutive reperfusion cause an enhanced formation of reactive oxygen species that result in testicular cell damage and apoptosis. The aim of this study is to investigate the effects of rotenone on testicular tissue.
Methods: 24 rats were randomly divided into3 groups: control, sham (I/R) and testis torsion+Rotenone group. Testicular ischemia caused by spermatic cord torsion of 720°. 1 h torsion and 2 h detorsion of the testis were performed. 10 µg/kg Rotenone (i.p) was administered to the I/R+rotenone group 30 minutes after ischemia.
Results: Thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), catalaz (CAT) and Glutatyon S-transferaz (GST) activities were evaluated. Data were analyzed with SPSS test, One way ANOVA was used. TBARS and SOD levels are significantly different, but CAT levels are not significantly different between groups. For immunohistochemistry p53, Bax, NfKB and E2F primary antibodies were used. While HRP detection kit was used for secondary antibodies, AEC was used for chromogen and Mayers Hematoxylin for counter staining. Immunopositive cells were counted under NIS Elements Image Analysis Software (Nikon, Japan) and all the data analyzed statistically by SPSS for Windows 16.0. For statistical analysis One Way ANOVA with Tukey HSD test was used. In the evaluation of all slides it was found that p53, Bax, NFkB and E2F expressions were significantly increased when compared Control and IR groups .On the other hand, in the evaluation between Control and I/R+Rotenone groups, it was determined that there were no significant differences in E2F and NFkB expressions At last, although p53 and Bax expressions were decreased, there were also significant differences in these groups
Conclusion: The present results suggest that rotenone is a potentially beneficial agent in protecting testicular I/R in rats.
Keywords: None.

WED-223
The expression of splicing factors in digestive system tumours and cell lines

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The removal of introns and joining together of exons through pre-mRNA splicing is an essential part of eukaryotic gene expression. The most of human genes pre-mRNAs undergo alternative splicing, which is a very precise process and plays a major role in the regulation of gene expression and the generation of proteomic and functional diversity. Recently was showed that splicing also is essential for one of alternative microRNA biogenesis when short intronic miRNA, mirtrons, are produced. While hundreds of splicing-derived mirtrons have been recognized by bioinformatics analysis, only three mirtrons (hsa-mir-877, hsa-mir-1224, hsa-mir-1226) are experimentally proved.

In the cell, splicing takes place in nucleus within a large macromolecular complex, the spliceosome, which consists of five small nuclear ribonucleoproteins (snRNPs) and numerous proteins, the expressions of which are different. Changes in the expressions of splicing factors affect tumour formation. MicroRNA also have been implicated in the suppression and induction of oncogenesis. Almost all cancers displayed differentially expressed miRNA compare with normal cell, but until today practically nothing known about mirtrons expression profiles in tumours or cancer cell lines.

In this study, we aim to compare the expression of various splicing factors in the digestive system tumours and cell lines, and define how these factors can influence mirtron hsa-mir-1226 expression.
We report that: (1) The expression of splicing factors between the digestive system healthy and cancer tissues are different. Obtained results revealed that expression of splicing factors in pancreas, stomach and colon tumours are decreased compare to healthy tissue; the expression of mirtron hsa-mir-1226 is decreased in colon, increase in stomach and no changes were detected in pancreas tumours compare to healthy tissues.

(2) In pancreas, stomach, kidney, colon cancer cell lines compare to 293A cell line, the expression of splicing factors and the expression of mirtron hsa-mir-1226 are decreased.

(3) The expression of splicing factors and mirtron hsa-mir-1226 in colon tissue and the colon cancer cell line correlates, therefore the cell line can be used as colorectal carcinoma model system.

Influences of altered expression of splicing factors for cancer associated gene splicing are under further investigation.
Keywords: splicing factors, mirtron, cancer.

WED-224
The first complete transcriptome study of the two mitochondrial genomes of a species with Doubly Uniparental Inheritance (DUI) of mtDNA: unique features unveiled

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The majority of mitochondrial transcriptome studies conducted to date come from maternally transmitted mtDNAs. However, certain mitochondrial genomes are exclusively paternally transmitted. Such genomes occur in a group of bivalvian mollusks, which are known to have Doubly Uniparental Inheritance (DUI) of mtDNA. These organisms have an egg-transmitted (F) and a sperm-transmitted (M) mitochondrial genome in stable co-existence for over 200 million years.

We studied the start/end points of mRNA, rRNA and tRNA transcripts of F and M genomes of *Mytilus galloprovincialis*, the most extensively studied species with DUI. All coding genes are transcribed from the same DNA strand. The primary transcript is cleaved into ten mRNA transcripts, eight of which are monocistronic, one is tricistronic and one is most likely, but not certainly, bicistronic (see Figure 1). Cleavage is mediated either by the excision of a tRNA or in some cases, by the presence of a stem-loop structure. The identification of a tricistronic transcript is a novel finding for metazoan mtDNA. The three co-transcribed genes participate in the same respiratory chain complex. The cleavage signals of each gene probably existed in the past, but were lost due to the replicative advantage of smaller mtDNA molecules.

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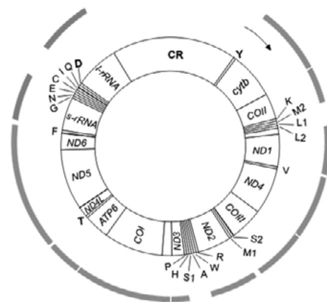

Fig. 1. Gene map of *Mytitus galloprovincialis* mtDNA. All genes are transcribed clockwise. The one-letter amino acid code is used for tRNA designation. Designation: L1, L2, M1, M2, S1, S2**.** tRNAs recognizing codons CUN, UUR, AUA, AUG, AGN and UCN, respectively; *ATP6,* ATP synthase subunit 6; *COI-III,* cytochrome c oxidase subunits I, II, and III; *Cytb,* cytochrome b apoenzyme; *ND1-6* and *ND4L* nicotinamide adenine dinucleotide dehydrogenase subunits 1–6 and 4L; *s-rRNA* and *l-rRNA,* small and large ribosomal RNA genes; CR, main control region. In blue are the mRNA transcripts, while in orange – the rRNA transcripts.

We also found polyadenylated and non-adenylated transcripts for both rRNAs and tRNAs, with heterogenous 3′ ends. The l-rRNA 3′end was found 48 nucleotides upstream from the one assigned by previous annotation, which makes the adjacent main control region (CR) correspondingly longer. We observed poly-adenylated tRNA transcripts carrying the CCA trinucleotide, mRNA, s-rRNA and l-rRNA transcripts with truncated 3′ ends and polyadenylated RNA remnants carrying the sequences of the control region, all of which strongly suggest RNA degradation activity and thus the presence of degradosomes in *Mytilus* mitochondria.

Keywords: degradosome, mitochondrial DNA, tricistronic mitochondrial transcript.

WED-225

The identification and characterization of heavy metal stress responsive genes in *Brassica juncea*

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Heavy metals are important environmental pollutants, and many of them are toxic even at very low concentrations. *Brassica juncea* is a dry-land plant with developed root system known to hyperaccumulate certain heavy metals such as copper, zinc. In this study, lead (50 μM $Pb(NO_3)_2$) and cadmium (25 μM $CdSO_4$) were applied to *B. juncea* var. P78 for 24 hours. *B. juncea* var. P78 was identified as a good accumulator of Pb and hyperaccumulator of Cd. Even though the elucidation of the genes which show expressional differentiation upon exposure to heavy metals is important for understanding the phytoremediation capacity, there has been limited number of reports on this subject. We conducted microarray analysis on the roots of *B. juncea* grown in a media containing 50 μM $Pb(NO_3)_2$. Since there is no *Brassica*-specific microarray, interspecific hybridization was done with *Arabidopsis thaliana* Affymetrix gene chips for the expression profiling. A total of 183 genes were identified to be differentially expressed. However, only 20 of the up-regulated and 18 of the down-regulated genes among them statistically significant ($p < 0.05$) expressional difference. The majority of 38 differentially- expressed genes were significantly conserved at cross-species level. These results suggested that cross-species hybridization approaches are not convenient for the expressional profiling. To further gauge the utility of interspecific expressional profiling approaches, we also conducted comparative in silico studies between *Arabidopsis* Affymetrix probes and *Brassica* EST databases. Furthermore, the validity of the microarray data was confirmed by expressional profiling of 6 genes obtained by microarray analyses with quantitative reverse transcription PCR. Thus, the identified and expressionally characterized genes in this study contributed significantly to better comprehension of metal metabolism in plants paving the way for the improvement of plant remediation capacity for the studies in the removal of heavy metals from different environments.

Keywords: cross-species hybridization, microarray, quantitative reverse transcription PCR.

WED-226

The impact of stress factors on the induction of violaxanthin cycle in diatom *Phaeodactylum tricornutum*

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Diatoms are a major group of microalgae found in both marine and freshwater environments and are responsible for about 40% of the global primary photosynthetic production. Due to their ecological success, as well as fast-evolving and novel biological processes absent in other model organisms, diatoms are considered among the most important living organisms, however, still poorly understood. An unusual physiological feature of diatom cells is the presence of two types of xanthophyll cycles. Apart from diadinoxanthin cycle, which is a major mechanism protecting their photosynthetic apparatus from photodamage, accumulation of zeaxanthin is also observed under high light conditions.

We chose *Phaeodactylum tricornutum* to our studies as it is an ideal model diatom of known genome and great ecological importance. Changes in amount of xanthophylls were analyzed during the exponential phase of growth under optimal (15°C) and higher (20°C) temperature, osmotic stress (100, 150 mM NaCl) and light stress (700 μE for 6 h a day and 1250 μE continuously). Concurrently, we studied differences in relative steady state mRNA transcript levels.

It was found that at both temperatures proportions of xanthophylls are comparable with only slightly higher pigment level observed at 15°C. While violaxanthin cycle was induced neither in samples from elevated temperature conditions nor in those treated by osmotic stress, light intensity of 700 μE for 6 h a day caused significant increase in Ddx cycle pigments and induction of Vx cycle (strong increase in xanthophylls amount during the first day of stress). Continuous light (1250 μE) had an adverse impact on the diatoms survival. Not only Vx cycle was strongly induced but it increased until the death of diatoms. Ddx cycle that was enhanced at first, showed decreased rate after few days of strong light treatment. At optimal temperature, relative steady state mRNA transcript levels (six genes of xanthophylls cycle) were higher than at 20°C. Light stress caused an increase in VDL2 and ZEP3 transcript levels, which are normally almost

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undetectable. Alterations in mRNA are not directly proportional to changes in amount of xanthophylls.

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Keywords: diatom, xanthophyll cycle.

WED-227
The influence of NFAT transcription factor on CacyBP/SIP gene expression

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The CacyBP/SIP protein binds several ligands and seems to be involved in different cellular processes such as ubiquitination, proliferation, differentiation, tumorigenesis or cytoskeletal rearrangement (Schneider and Filipek, 2011). The most important function discovered quite recently is its phosphatase activity towards ERK1/2 kinase and tau protein (Kilanczyk et al. 2011; Wasik et al., 2013). Studies concerning distribution of CacyBP/SIP show that it is present in various mammalian tissues with a particularly high level in brain and spleen. The level of CacyBP/SIP is also up-regulated in cells exhibiting a differentiating potential such as neuroblastoma NB2a, SH-SY5Y or pheochromocytoma PC12 cells (Filipek et al., 2002) as well as in cells highly proliferating, such as colon cancer HCT116 (Kilanczyk et al, 2012).

Due to the tissue and cell specific distribution of CacyBP/SIP as well as its involvement in different cellular functions it seems very important to determine the regulation of CacyBP/SIP gene expression. Thus, in this work we focused on identification of factors, external stimuli, signaling molecules or transcription factors, which might affect CacyBP/SIP expression. For that we examined the sequence of CacyBP/SIP gene promoter (1,6 kb upstream from the Transcription Start Site, TSS) using both the MatInspector and TESS programs to search for binding sites of potential transcription factor(s). We found the binding sites, among others, for factors such as NFAT (Nuclear Factor of Activated T cells), CREB (cAMP response element-binding), E2F transcription factors and DREAM (downstream regulatory element antagonist modulator). We found that among those transcription factors, NFAT seems to modulate the activity of CacyBP/SIP gene promoter and to evoke changes in CacyBP/SIP mRNA and protein level in the colon cancer HCT116 cells. Our results show that the effect of NFAT on the CacyBP/SIP protein level might depend on intracellular calcium concentration.

This work was supported by grant from the National Science Center (NZ1/00595) and by statutory funds from the Nencki Institute of Experimental Biology.
Keywords: CacyBP/SIP, NFAT, transcription factors.

WED-228
The localization and mRNA expression of poorly characterized members of SDR superfamily in human tissues

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The SDR (the short-chain dehydrogenase/reductase) superfamily represent one of the largest and oldest groups of carbonyl reducing enzymes, whose members occur in all life forms including mammals. In humans, these enzymes are involved in the metabolism of a large variety of compounds containing carbonyl groups, including endogenous (aldehydes, steroids, prostaglandins, retinoids and lipids) and xenobiotic (NNK, alfatoxin B1, quinones, doxorubicin and daunorubicin) compounds (Skarydova L. and Wsol V. 2012). The SDR enzymes also play an important role in some serious pathological diseases as some types of cancer, obesity, metabolic syndrome and endometriosis (Jansson A. 2009, Morton N.M. 2010). Over 75 SDR genes have been identified within the genome of human beings (Persson B. and Kallberg Y. 2013).

The most of the known information refers about the role of cytosolic forms of carbonyl reducing enzymes in the metabolism of xenobiotics (e.g. CBR1, AKR1C1-4). The knowledge about microsomal forms is limited and only one well-known microsomal form involved in biotransformation of xenobiotics - 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) - has been reported so far (Maser E. et al. 2006). But it is probable that other microsomal members of SDR superfamily participate in biotransformation. This study has investigated expression and tissue localization of mRNA of four human poorly characterized microsomal enzymes included to SDR superfamily - DHRSX, DHRS1, DHRS8 and DHRS12.

The total of 16 tissues were collected from humans. The RNA was extracted using Trizol reagent from all tissues and subsequently RNA was reversely transcribed to cDNA. The expression and tissue localization of mRNA was determined using real-time SYBR Green absolute qPCR with specific primers.

Tissue localization together with knowledge of enzyme activity of these four carbonyl reducing enzymes will open the way for studies of its potential role in the metabolism of important endogenous and xenobiotic compounds in human.

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Keywords: expression, mRNA, SDR enzymes.

WED-229
The predictive role of S100A4 as a pro-metastatic factor in pancreatic ductal adenocarcinoma

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Pancreatic cancer is characterized by an aggressive biology being a rapidly disseminating disease with a dismal prognostic and survival rate. There is an urgent need to identify early molecular diagnostic markers that could contribute in improving patients' survival. S100A4 is a member of calcium-binding protein family

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and has a functional EF-hand domain mediating its activity. The gene S100A4 encodes a calcium-binding protein involved in invasion and metastasis by binding to several intracellular target proteins and modulating their function. The aim of this study was to evaluate the clinical significance of S100A4 mRNA and protein expression in pancreatic ductal adenocarcinoma and their role as early biomarker in PDAC.

Materials and methods: Pairs of non-tumoral and tumoral tissues from a total of 73 patients with PDAC were included in our study. Gene expression of S100A4 was assessed by two-step qPCR using TaqMan hydrolysis probes. Protein expression of S100A4 was evaluated by immunohistochemistry and nanoLC – MS.

Results: Gene expression values of S100A4 increased with advances stages of PDAC, with degrees of tumors differentiation and with lymph node status. Kaplan Meier curves showed a statistically significant difference in survival outcome of patients according to their S100A4 protein expression by immunohistochemistry as either lower or higher than 10% (P-value=0.043). The same statistical test showed statistically semnificative correlations with cliniopathological parameters: presence of metastasis (P-value=0.08), lymph node ratio (cut-off=0.16, P-value=0.06), type of resection (P-value=0.003) and CA19-9 level (cut-off=1000 ng/ml, P-value=0.054). Multiple regression Log Rank (Mantel-Cox) test confirmed that resection type (P = 0,07) and S100A4 protein expression (P = 0,057) has statistically significant value correlated to PDAC. NanoLC-MS identified S100A4 as being repetitively higher expressed in tumoral compared to non-tumoral tissues.

Conclusions: Our results showed that S100A4 plays an important role in PDAC and might be a valuable marker by addressing the problem of early diagnostic. Identification of S100A4 in early stages of the disease could represent a real benefit for public health care.

Study financially supported by the research grant PNII-PT-PCCA 90/2012.

Keywords: molecular biomarker, pancreatic cancer.

WED-230
The SRI domain family: a common scaffold for RNA polymerase II CTD binding

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The carboxyl-terminal repeat domain (CTD) of the largest subunit of the RNA polymerase II (RNAPII) serves as docking platform for a wide range of nuclear factors at different stages of the transcription cycle *(Egloff and Murphy, 2008)*. A wide variety of folds are involved in CTD recognition *(Meinhart et al. 2005)* including a small domain discovered in the histone methylytansferase Set2, called SRI after Set2 Rpb1 Interacting, which alters RNAPII elongation *(Kizer et al. 2005)*. It was recently showed that SRI domain is also present in the C-terminal domain of the RECQ5 helicase, which is critical for maintaining genome integrity *(Li et al. 2011)*. The purpose of this work is to search and study the conservation of the SRI domain during evolution in different protein families and highlight the structural and/or functional features of key residues.

We combined here original tools we previously developed for detecting hidden relationships between remote sequences: (1) SEG-HCA *(Faure et Callebaut, 2013b)* delineates foldable domains (*i.e* domains which may form stable 3D structures) from the only knowledge of a single amino acid sequence; (2) TREMOLO-HCA *(Faure et Callebaut, 2013a)* adds to the results of sequences similarities searches information on domain architecture of the aligned sequences extracted from the Conserved Domain Database *(Marchler-Bauer et al. 2013)* as well as on the conservation of hydrophobic core residues. I-TASSER threading program *(Roy et al. 2010)* was used to predict the 3D structure models.

We show that SRI domains are found outside the Set2 and RECQ5 proteins, in which they are also involved in RNAPII CTD or RNAPII CTD-like recognition. Interestingly, SRI domains are always located in the C-terminal extremity of all the proteins of the SRI domain family. The whole family showed a large sequence divergence, especially within the loop between the first 2 helices that is much longer in SET2 proteins than in other members of the SRI family. Despite this divergence that made this domain undetectable by the present SRI CDD profile, it is worth noting that important amino acids for RNAPII CTD binding are highly conserved in the whole family as well as the core hydrophobic residues responsible of maintaining the left-handed three helix bundle fold.

These results allow getting insights into the diversity of this family of domains and into its critical structural and functional features.

This work is supported by a grant from INCa (DIREP).

Keywords: Domain architecture, Protein families, Remote homology.

WED-231
The zinc finger protein lastin-mediated inhibition of cell proliferation in a cell density-dependent manner

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Zinc finger proteins are involved in transcriptional regulation through their zinc finger domain, and regulate various cellular functions, including cell proliferation and differentiation. Approximately one third of the zinc-finger proteins contain the Krüppel-associated box (KRAB) domain, which is involved in transcription repression. Lastin is a member of KRAB-containing zinc finger proteins. However, the functions of the most of the KRAB-containing zinc finger proteins, including lastin, are largely unknown. In this study, we examined functions of lastin.

First, we examined the subcellular localization of lastin. We found that lastin was mainly localized to the nucleus. Furthermore, to examine a region in lastin responsible for its nuclear localization, we constructed two lastin mutants lacking the KRAB domain (lastin-ΔKRAB) and the zinc finger domain (lastin-Δzinc). Lastin-Δzinc was localized to the cytoplasm, whereas lastin-ΔKRAB was localized to the nucleus. These results suggest that lastin is localized to the nucleus in its zinc finger domain-dependent manner. Next, to examine the involvement of lastin in cell proliferation, we generated cell lines inducibly expressing lastin, lastin-ΔKRAB, or lastin-Δzinc. Intriguingly, lastin expression drastically inhibited cell proliferation at low cell density but slightly inhibited cell proliferation at high cell density. Lastin-Δzinc expression did not inhibit cell proliferation, whereas lastin-ΔKRAB expression did inhibit cell proliferation. The nuclear localization and the expression levels of lastin were not altered both at low cell density and high cell density. These results suggest that lastin is involved in inhibition of cell proliferation at low cell density in its zinc finger domain-dependent manner.

In conclusion, we show that lastin plays a critical role in inhibition of cell proliferation in a cell density-dependent manner. The

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zinc finger domain of lastin is involved in its nuclear localization and inhibition of cell proliferation. Now, we are trying to identify the lastin-target genes and to clarify the mechanism of inhibition of cell proliferation in a cell density-dependent manner.
Keywords: cell density, proliferation, zinc finger.

WED-232
Transcription factors from Sox family regulate zebrafish Gla-rich protein 2 gene

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Gla-rich protein (GRP) is a vitamin K-dependent protein previously identified in our group with orthologs described in all taxonomic groups of vertebrates and a paralog (GRP2) in bony fish. It was initially appointed as specific for cartilage but later also detected in blood, soft tissues and bone. Because of the extensive number of Gla residues and the absence of other identifiable functional domains we hypothesized that GRP might be a physiological modulator of soft tissue calcification. However, conflicting information is available from mouse and fish deletion experiments. While in mouse the knockout did not show any phenotype, in zebrafish the morpholino-mediated knockdown showed a mild phenotype affecting cartilage development recovering after few days. Although *GRP* expression was repressed in retinoic acid treated dedifferentiated chondrocyte and in chondrocytes exposed to either BMP-2 or TGF-β1, no data is available indicating a direct transcriptional regulation of the GRP gene by any of those potential regulators.

We chose to use zebrafish as our model organism and we cloned and analyzed *grp2* promoter transcription using p*grp2*-Luc and a serial of 5′ promoter deletion constructs in transfection experiments. The entire *grp2* promoter showed high levels of luciferase activity when compared with 5′ deletion constructs. This result was confirmed *in vivo* by microinjection of zebrafish embryos. To identify putative transcription factor binding sites (TFBS), an *in silico* analysis was performed and several putative binding sites for SOX, ETS1 and MEF2 were found. These TFs are known to affect transcription of several skeleton and cartilaginous genes. Co-transfection assays were performed indicating an up-regulation by Sox9b and Sox10. The transactivation of *grp2* promoter by the Sox10 was confirmed in co-transfection assays using a Sox10 mutated construct. The results were confirmed *in vivo* following microinjection of xenopus eggs with p*grp2*-Luc construct and Sox9b and Sox10 mRNA.

Data obtained provide the first relevant information demonstrating that *grp* is a target of these TFs thus providing new insights towards understanding the function of this gene.

CF and NC are supported, respectively, by a doctoral and a post-doctoral grant from FCT (SFRH/BD/66745/2009 and SFRH/BPD/48206/2008).

This research was partially supported by the ERDF through the COMPETE and national funds through FCT, under the project “PEst-C/MAR/LA0015/2011”.
Keywords: gene regulation, GRP, SOX.

WED-233
Transcriptional regulation of the starch branching enzyme isoforms in the leaf and the stem under long and short photoperiod in lentil

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Starch is the predominant carbohydrate reserve in many plants and is observed in both photosynthetic and non-photosynthetic tissues. In addition, starch constitutes most of the dry matter accumulating in the harvested organs of crop plants and therefore is not only the primary source of calories in the human diet, but can also be regarded as renewable resource that may be utilized in many industrial applications.

Starch branching enzyme catalyze branch point formation by the cleavage and reattachment of a-1,4-linked Glc chains to -1,6 branch points in the growing starch molecule. Branching enzymes are proposed to interact with starch synthases in formation of amylopectin. In all plant species examined so far, although more than two starch-branching enzyme (SBE) isoforms often occur, all isoforms can be separated into two classes based on their predicted primary protein sequences deduced from primary amino acid sequences. A recent stdy suggested that transcriptional control of the starch biosynthetic enzymes is under the control of circadian clock. Therefore, in this study, we explored the transcriptional regulation of SBEI and SBEII in stem and leaf under different photoperiods length in lentil. To this end, we first isolated and characterized BEI and BEII of lentil, then we performed quantitative real time PCR (qPCR) to see the effect of photoperiod length on the transcription of SBEI and SBEII in lentil. Our preliminary results indicated that transcriptions of the BEs are under the control of the circadian clock.
Keywords: Lentil, Real time-PCR, Starch Branching Enzyme.

WED-234
Transcriptional regulation of the starch synthases isoforms in the leaf and the stem under long and short photoperiod in lentil

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Starch is the predominant carbohydrate reserve in many plants and is observed in both photosynthetic and non-photosynthetic tissues. Starch is a staple in the diet of much of the world’s population and is also widely used in different industries as a raw material. Starch biosynthesis requires three enzymes: ADP-glucose pyrophosphorylase, starch synthase and branching enzyme.

Among those enzyme starch synthases (SS) catalyze the transfer of the glucosyl moiety from ADP-glucose to the nonreducing end of an α-1,4-glucan. Collectively, the starch synthases are able to extend α-1,4-glucans in both components of starch, amylose, and amylopectin *in vitro*. Based on sequence comparisons, they can be divided into at least four different classes. These are designated GBSS, for granule-bound starch synthase, SSI, SSII, and SSIII.

Recent studies showed that transcription of the starch biosynthetic enzymes under the control of circadian clock. Therefore, in this study, we explored the transcriptional regulation of SSI and SSIII in stem and leaf under different photoperiod’s length in lentil. To this end, we first isolated and characterized SSI and SSIII of lentil, then we performed quantitative real time PCR (qPCR) to see the effect of photoperiod length (duration of the

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dark and light photoregiems) on the transcription of SSI and in lentil. We have seen that the differential regulation of the lentil SS at transcriptional level. Our results will be highlighted the importance of photoperiod length, in turn circadian clock, on the transcriptional regulation of SBE isoforms in stem and leaves.
Keywords: Lentil, Real time-PCR, Starch Synthase.

WED-235
Transcriptomic signature of mature endocrine pancreatic cells

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Pancreas is a mixed gland composed of endocrine and exocrine tissues and plays a crucial role in the metabolism of all vertebrates. The endocrine cells are mainly grouped into the islets of Langerhans and secrete distinct hormones, such as glucagon (α-cell), insulin (β-cell), somatostastin (δ-cell) and ghrelin (ε- cell). Diabetes occurs when insulin production by the β-cells is unable to counteract increase of glycemia. Better knowledge on pancreatic cell differentiation, regeneration and physiology is needed to design novel therapies for diabetes. While several transcription factors have been identified as pivotal for the differentiation of the various pancreatic cell types, there is still no comprehensive list of genes presenting cell type-specific expression. In a first step, we have determined the transcriptional landscape of the zebrafish mature alpha, beta and delta endocrine cells as well as exocrine cells. This was achieved by dissecting pancreas from the transgenic insulin:GFP, glucagon:GFP, somatostatin:GFP and ptf1a:GFP lines, dissociation and sorting GFP cells by FACS, RNA extraction, synthesis of cDNA and sequencing on Illumina platform. Experiments were performed in duplicates for each cell type and about 40 millions of sequenced reads were obtained per sample. Sequences were aligned to the zebrafish genome and expression level per-gene was measured using HTSeq software. Heatmap plot and principal component analysis show that samples belonging to the same cell type cluster together, indicating that transcriptome profiles are characteristics to different cell types. Cell type specific transcripts were identified using the DEseq and EBSeq software. Among these genes are transcription factors known to control pancreatic cell differentiation (i.e. pdx1, nkx6.2, ptf1a). Some new cell type specific transcripts were selected based on their GO annotation and studied by ISH. In order to define a common vertebrate signature, comparative transcriptomics for endocrine and exocrine tissue were performed using previous published data. This cross-species comparison should highlight genes having an evolutionary conserved action distinguishing them as important for the pancreas physiology.
Keywords: development, Pancreas, Transcriptomics.

WED-236
Transforming growth factor-β1 represses testicular steroidogenesis through ALK5/Smad3 signaling

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Transforming growth factor-β1 (TGF-β1) has been reported to be an autocrine/paracrine factor that inhibits LH mediated-steroidogenesis in testicular Leydig cells. However, the mechanism by which TGF-β1 controls the steroidogenesis in Leydig cells is not well understood. In this study, we investigated the molecular mechanism for TGF-β1-mediated repression of testicular steroidogenesis. In primary Leydig cells and rat R2C cell line, TGF-β1 treatment inhibited mRNA expression of cAMP-induced steroidogenic genes, decreasing the production of testosterone. The repressed mRNA expression by TGF-β1 treatment was rescued by the treatment of ALK5 inhibitor, SB431. Moreover, overexpression of ALK5 TD, a constitutively active TGF-β type I receptor, repressed the cAMP-induced promoter activity of steroidogenic genes. Interestingly, the transactivation of steroidogenesis-related transcription factors such as Nur77 was significantly repressed by the overexpression of both ALK5(TD) and Samd3. Taken together, these results suggest that TGF-β1/ALK5/Smad3 signaling represses the expression of steroidogenic genes in mouse Leydig cells probably via the suppression of the transcriptional activity of steroidogenesis-related transcription factors.
Keywords: gene regulation, steroidogenesis, TGF-β1.

WED-237
Cross-talk between the two helicase cassettes of the spliceosomal RNP remodeling enzyme, Brr2

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Splicing entails the removal of non-coding intervening sequences from eukaryotic pre-mRNA and the ligation of the neighboring coding regions and is carried out by the spliceosome. An active spliceosome evolves on the substrate by a stepwise assembly that is driven by members of superfamily 2 ATPases/RNA helicases. Major conformational and compositional RNP remodeling is required to convert an initially inactive complex into an active spliceosome. The Brr2 protein is the key player in this catalytic activation process. Brr2 belongs to a unique group of nucleic acid helicases, whose members are exceptionally large and contain two expanded helicase units fused in tandem. Unlike other spliceosomal helicases, Brr2 is preassembled with its substrate, the U4/U6 di-snRNP, before incorporation into the spliceosome. After catalytic activation, Brr2 remains associated with the spliceosome throughout the splicing cycle until spliceosome disassembly. The enzyme has been suggested to be required again during splicing catalysis and spliceosome disassembly, but presumably does not act as an ATPase/RNA helicase during these stages. Thus, both before recruitment to the spliceosome and after spliceosome catalytic activation, Brr2 may have to be shut off. Recently, we have determined the crystal structure of an active, 200 kDa portion of Brr2, showing that its two helicase units intimately interact and functionally cooperate. The C-terminal unit, while inactive in ATP hydrolysis and RNA duplex unwinding, strongly stimulates the N-terminal helicase *via* an extensive interaction interface between the two cassettes. However, it is not clear how the communication channels between the two helicase cassettes of Brr2 modulate the N-terminal cassette activity. Using pre-steady state kinetics, we probed the nucleotide binding preferences and worked out possible nucleotide binding mechanisms of each cassette. We also observed that the affinity of the active N-terminal cassette towards ATP and RNA is affected when the inactive C-terminal cassette carries interface or ATP binding pocket mutations or is pre-bound to different nucleotides. Interestingly, mutations in the ATP binding pocket of the C-terminal cassette not only reduce Brr2 helicase activity in vitro but also induce growth defects in yeast. We suggest a

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possible mechanism by which Brr2 interactors and ligands may exploit the C-terminal cassette as a "remote control" to regulate the N-terminal helicase of the enzyme.

Keywords: Pre-mRNA splicing, RNA helicase, spliceosome catalytic activation.

WED-238

Yap1-mediated repression of the yeast low affinity iron transporter gene, FET4, confers cadmium tolerance

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Cadmium is a well-known mutagenic metal, which can enter cells via non specific metal transporters, causing several cellular damages and eventually leading to death. In the yeast *S. cerevisiae*, the transcription factor Yap1 plays a determinant role in the regulation of several genes involved in metal stress response. Recently we have shown that Yap1 negatively regulates *FET4* gene, encoding a low affinity iron transporter[1]. In the present work we study the relevance of this repression in cell tolerance to cadmium. Our results indicate that *yap1* mutant exhibits increased *FET4* protein and mRNA levels compared to the wild-type strain. This data correlates well with the increased intracellular levels of cadmium observed in the mutant, as measured by ICP-AES. These results suggest that Yap1 repression of *FET4* prevents cadmium uptake. Nevertheless, Yap1 is not a direct repressor of this gene, as no Yap1 consensus site was found in its promoter. Our previous microarray data suggested that Yap1 regulates *ROX1* expression[1], a well known repressor of *FET4*. Using Chromatin-Immuno Precipitation and Real-Time PCR approaches, we show that Yap1 directly regulates *ROX1*, which in turn represses *FET4*. After cadmium induction we observed an abrupt decrease of *FET4* mRNA levels in the wild-type and *yap1* mutant strains. We also show that *FET4* transcript stability after stress relies on the 5′-3′ exoribonuclease Xrn1. Together, our results highlight the role of Yap1 in mediating cadmium tolerance revealing a new route for cell protection.

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Keywords: Yeast, Cadmium, FET4.

WED-239

Yeast YIL096C binds and methylates 25S rRNA

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The ribosomal RNA contains 109 specific modifications that affect translation and ribosome biogenesis. Only few of them correspond to base methylations catalyzed by methyltransferases (MTases). In eukaryotes, most of the rRNA modifications are maintained by enzymes that require guide snoRNA. In this work we identify a specific substrate for *Saccharomyces cerevisiae* YIL096C (BMT5) [1, 2], a snoRNA-independent MTase, using *in vitro* isotope dependent assays. We show that YIL096C binds and methylates yeast 25S rRNA and possesses substrate specificity to oligoRNA containing already known methylation site m^3U2634. This is highly consistent with the recent study by independent group showing that BMT5 is a new m^3U2634 MTase [3]. Our results contribute to better understanding of rRNA base methylation process, ribosome biogenesis and function. Presented approach should be helpful in further studies on substrate specificity of rRNA base MTases.

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Keywords: base methylation, in vitro assay, rRNA methylation.

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CSIV-07 – Education, training and career planning in molecular life sciences

WED-241
Education and public engagement spotlight on "Genomics and Bioinformatics"

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The 361° Division at The Genome Analysis Centre (TGAC) specialises in Scientific Training, Education and Learning. Here we share our initiatives and impact from our first year as well as future efforts. We illustrate four key projects from our Education and Public Engagement Team:

1. TGAC4Kids, an online series of interactive activities aiming to introduce key areas of molecular biology and bioinformatics, reflecting current research and its impact on our daily lives. It is a free resource geared towards children aged 4–11. Our objective is to empower teachers to first acquaint themselves with current trends as well as to facilitate their teaching of such concepts in the classroom.

2. TGAC4Kids on the road, where schools apply for a TGAC4Kids visit, and we also conduct formative evaluation, collecting views and ideas about TGAC4Kids effectiveness in its current state to inform future developments. The schools are invited to help build TGAC4Kids with us.

3. SIB Swiss Institute of Bioinformatics: Creating new activities around the exhibition ChromosomeWalk.ch based on plants and animals

4. DNA Learning Centre (DNALC), Cold Spring Harbor Laboratory: The Genomics and Bioinformatics of Stem Cells. The DNALC is the world's first science center devoted entirely to genetics education and is an operating unit of Cold Spring Harbor Laboratory, an important center for molecular genetics research. DNALC pioneered the concept of providing genetics labs and bioinformatics experiences to large numbers of students and teachers. The DNALC will work with us to develop a complete teaching module aimed at middle school (US Grades 6–8) students using tissue culture techniques developed at Kew Botanical Gardens and Science and Plants for Schools (http://www.saps.org.uk/). The goal is to deploy a hands-on lab in which cauliflower cuttings (which contain stem cells) can be propagated into clonally reproduced plants. The collaboration will be an opportunity to gain knowledge from the DNALC's expertise in developing Internet multimedia that compliment classroom learning. Possible extensions of the laboratory could include an online lab notebook with detailed protocols, and reagent recipes. Expanding on the topic of stem cells and genomics in general, computer exercises relevant to the topic could also leverage DNALC's expertise in developing classroom-friendly bioinformatics workflows.

Keywords: Bioinformatics education, genomics, public engagement.

WED-242
ELIXIR: The UK node

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ELIXIR's UK Node contributes the country's substantial expertise in bioinformatics training for life science researchers and computer scientists. With the advent of next-generation sequencing and other high-throughput techniques, the major bottleneck in the life sciences has shifted from data production to data analysis and interpretation. ELIXIR-UK considers sustained professional training to be central to building data analysis capacity in Europe.

Plugging the skills gap: In a rapidly changing technological environment, researchers working in the clinic, on model organisms or crunching large datasets each use bioinformatics in different ways and have distinct training needs. Accordingly, supporting widely differing disciplines is one of ELIXIR-UK's core values. ELIXIR-UK will serve all of Europe through a flexible mix of learning opportunities. Face-to-face training at different centres of excellence in the UK remains the highest-impact training, complemented by a wide-reaching online learning programme. In all its activities, ELIXIR-UK will exploit the most effective tools available to help an international and diverse audience get to grips with all manner of biological data.

A critical mass of training excellence: ELIXIR-UK adopts a multi-agency approach, leveraging internationally recognised UK expertise in the biomedical, bioscience and environmental sectors.

Keywords: bioinformatics training, data analysis, skills.

WED-243
Training in data, tools and resources for molecular life scientists

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With the wealth of data being generated by laboratories worldwide, and the depositing of such data into publically accessible databases, there is an increased need for bench biologists to gain the skills required to mine, analyse and interpret this data. EMBL-European Bioinformatics Institute develops and maintains a variety of data tools and resources for application across the life sciences, but also provides a number of mechanisms for scientists to learn how to become better users of biological data through a range of training opportunities.

EMBL-European Bioinformatics Institute develops and maintains a variety of data tools and resources for application across the life sciences, but also provides a number of mechanisms for scientists to learn how to become better users of biological data through a range of training opportunities.

Our trainees are diverse, many are from a bench background and at PhD / Postdoctoral level, but whilst they have expertise in their area of scientific interest they are often lacking in computational skill and knowledge of the tools available to them. Our aim is not to produce a new generation of bioinformaticians, but rather to develop a group of scientists who are more confident users of data tools and resources.

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We have created a programme that delivers training in three modes – on-site, off-site and on-line. This enables us to provide training to a wide audience, and to provide different focus for courses, from subject specific analysis through to advanced use of a specific resource.

Providing such a programme is challenging due to the diverse nature of our potential trainees, but we present here the approach we take to meeting these challenges and to providing training that enables life scientists to become more confident data users.

Keywords: data analysis, Life scientists, Training.

WED-244

Posters at academic/scientific conferences: meeting the needs of viewers, presenters and society

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Generally, posters are used to give a visual aspect to opinions or findings, with the aim of engaging and swaying a wider audience. Posters are the second most prolific form of knowledge dissemination in the sciences and academia, after journal article publication, and offer an established format for large numbers of researchers to present and discuss their research at conferences. Since their mainstream inception in 1969, poster practice itself has changed very little. This is incongruent with the developing needs of poster users, be they presenters, viewers, conference organisers or members of the global society. As such, a paradox has arisen where although we continue to present posters in ever-increasing numbers, we also take issue with the lack of appreciation given to the medium, the lack of exposure and longevity posters receive, and the ill-applied conception of a 'second-rate publication'.

Posters are an under-researched medium of scientific / academic communication, especially when we consider their predominance in professional practice. This poster looks to raise the awareness of this paradox, and to ask the question: How can we better develop a popular medium to meet the needs of users in the 21st century ? Answers may lie in the way we conduct future poster sessions, the way institutions appreciate and value the medium as a marker of professional practice and engagement, or perhaps how we enable others to engage with our work ... long after the last conference delegate has gone home.

Of course, of perhaps primary note is the wealth of subject information presented in the poster medium, and further development may help to ensure that considerable amounts of hard work and enquiry do not get consigned to 'undiscovered public knowledge' – a dustbin from which great discoveries have been gleaned.

Nicholas Rowe is currently conducting research into the development of posters at scientific/academic conferences – please feel free to contact him at: nrowe@ulapland.fi .

Keywords: knowledge dissemination, Poster presentation, scientific communication.

WED-245

The characteristic of microfertilizer for increasing of the productivity of cereals plants and their tolerance to stress factors

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The preparation of microfertilizer (PMF) was purified from green seeds of *Ulmus parvifolia* by hydrophobic chromatography on octyl-sepharose column and reverse phase chromatography on column type RP 18 column. It was developed the new specific test for PMF. It is property to activate of NADPH specific – glutamate dehydrogenase (NADPH-GDh) in aleuronic layer of the wheat seeds. Among all phytohormones only fusicoccin has the same ability. The experiments which were carried out on the fields of the North Kazakhstan were established that MPF increases the productivity of the winter rye on 35%. PMF also increases the tolerance of the wheat and barley to cold stress and to salinity. The most convenient for ecology of the property of PMF is ability to stimulate the germination of seeds which have lost the ability to germinate. As well as PMF is well convenient for vegetative reproduction of the fruit and forest trees plants. PMF stimulates the formation of the main and adventitious roots whereas auxin stimulates only the formation of adventitious roots. The main difference of PMF in comparison with auxin is next PMF stimulates the formation of the new leaves and stems whereas auxin inhibits formation of the above-ground part of plants.

Keywords: The preparation of microfertilizer, NADPH specific – glutamate dehydrogenase, auxin.

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CSV-01 – Autophagy

WED-247
Autophagy is positively regulated by 17β-estradiol and progesterone during functional development of alveolar structures formed by bovine mammary epithelial cells in 3D culture

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Sex steroids: 17β-estradiol and progesterone, play a crucial role in the process of mammary gland development. Their regulatory actions, mediated mainly by specific steroid receptors are necessary to fulfill proper lobulo-alveolar structure formation. The growing importance of their role during mammogenesis lies in the ability to regulate crucial cellular processes such as apoptosis and autophagy, which were proven to determine the proper development of mammary alveoli. Immunofluorescence staining of p62 in BME-UV1 bovine mammary epithelial cells cultured on reconstituted basement membrane (rBM) demonstrated induction of autophagy in the centrally localized cells of developing spheroids, and this process was enhanced by sex steroids. The same 3D culture model was used to elucidate the mechanism of autophagy regulation by estradiol and progesterone. Real Time PCR analysis of autophagy related genes (ATGs) showed that both steroids increased the expression of *ATG3*, *ATG5* and *BECN1* when acted singly, and this effect was accelerated in the presence of estradiol and progesterone together. The functional interaction of sex steroids was also reflected by increased levels of autophagy proteins Atg5, Atg3 and LC3-II determined by Western Blot analysis. To explore the nongenomic actions of sex steroids, we examined the activity of Akt, ERK, AMPK and mTOR kinases, which are known to be involved in autophagy regulation. We observed a slightly increased level of phosphorylated AMPK in parallel with diminished phosphorylation of Akt and mTOR. Our observations clearly indicate, that synergistic action of 17β-estradiol and progesterone enhance autophagy in bovine mammary epithelial cells forming acini by upregulation of ATGs and their protein products, and partially by regulation of AMPK and MAPK signalling pathways. All collected data indicate, that physiological role of 17β-estradiol and progesterone in the process of mammary alveoli formation is based on complex interplay of their individual actions.

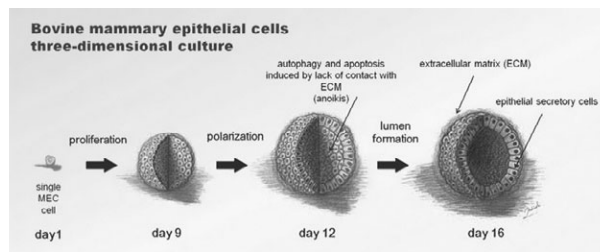

Fig. 1.

Keywords: autophagy, mammary gland, sex steroids.

WED-248
Autophagy regulates cell differentiation and proliferation within germline tumors in *C. elegans*

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Teratomas are germ cell derived tumors that result from precocious differentiation of somatic cells in the gonads or in the embryo. A teratoma model can be induced in *C. elegans* germline by depletion of GLD-1, a major suppressor of translation similar to mammalian Quaking [1]. Recently, it has been shown that reducing nutrient/energy supply triggers a proliferation arrest in teratomas [2], although the mechanism has not yet been clarified. Here we show that autophagy-related proteins are expressed in a subset of teratoma cells, in particular differentiated neuronal cells. Interestingly, knocking-down autophagy impairs neuronal differentiation and increases germ cell number in the teratoma. Accordingly, autophagy depletion leads to a faster invasion of the head and vulva regions and an increase in the percentage of animals displaying a tumor prolapse. On the other hand, autophagy induction by fasting or LET-363 depletion leads to a reduction in the germ cell number in the tumor and a decrease in the percentage of animals presenting a tumor prolapse. Furthermore, fasting significantly delays death caused by teratomas in an autophagy-dependent manner. In conclusion, we propose that autophagy plays a role in regulating the balance between cellular differentiation and proliferation within the teratoma.

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Keywords: autophagy, C. elegans, germline tumor.

WED-249
Structure of the human Rod-Zw10-Zwilch (RZZ) complex

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Faithful chromosome segregation lies at the heart of mitotic cell division. The fidelity of this process is of unquestionable importance, failing which cells are left with irreparable consequences. Kinetochores are dynamic multi-protein assemblies, built on the chromosomes, which are at the helm of above process. On one hand, they help chromosomes bind to microtubules and modulate their stability, and, on the other they oversee that these attachments are not faulty by a complex mechanism called spindle assembly checkpoint. The 3-subunit Rod-Zw10-Zwilch (RZZ) complex is a crucial component of the spindle assembly checkpoint (SAC) in higher eukaryotes. It is required for kinetochore localization of the Mad1-Mad2 checkpoint complex and of the microtubule motor dynein, thus contributing not only to kinetochore-microtubule attachment but also to the dynein dependent stripping of SAC components upon checkpoint satisfaction.

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Although much is known about spatial and temporal organization of the RZZ complex, it remains poorly understood from a purely structural perspective. We used single particle Cryo-Electron Microscopy (cryo-EM) to determine the first 3D structure of RZZ. We also elucidated the architecture of the complex by interweaving cross-linking mass spectrometry and antibody labeling studies. We aim, ultimately, to unravel the molecular basis of SAC.
Keywords: Kinetochores, Single particle cryo-EM.

WED-250
Ceramide-activated protein phosphatase Sit4p deregulates macroautophagy, mitophagy and mitochondrial dynamics in isc1Δ cells

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Sphingolipids regulate crucial biological processes, such as stress response and apoptosis. The budding yeast *Saccharomyces cerevisiae* has been pivotal to uncover the impact of sphingolipid dynamics on eukaryotic cell physiology and metabolism. The bioactive sphingolipid ceramide can be generated by the *de novo* biosynthetic pathway or through the hydrolysis of complex sphingolipids, the last catalysed by the inositol phosphosphingolipid phospholipase C protein, Isc1p (the yeast orthologue of the mammalian neutral sphingomyelinase 2). Cells lacking Isc1p display shortened chronological lifespan (CLS), oxidative stress sensitivity and severe mitochondrial dysfunctions. We demonstrated that Isc1p acts upstream of Sit4p, the catalytic subunit of mammalian type 2A protein phosphatase (PP2A). The deletion of *SIT4* restores mitochondrial function in *isc1Δ* cells, increasing oxidative stress resistance and extending CLS.

The regulatory role of macroautophagy in mitochondrial function has been demonstrated in *S. cerevisiae*. Since *isc1Δ* cells exhibit mitochondrial dysfunctions, we intended to assess any defect on macroautophagy impacting on mitochondrial function. The results show that the process is impaired in *isc1Δ* cells, ultimately leading to mitochondrial dysfunction. Importantly, *SIT4* disruption re-established the autophagic flux in the mutant strain, which correlates with the restoration of mitochondrial function in *isc1Δ sit4Δ* cells.

Mitophagy and mitochondrial dynamics are key quality control mechanisms by maintaining a healthy mitochondrial network sustaining overall cellular energetics. We demonstrate that mitophagy was impaired during ageing and this was correlated with mitochondrial fragmentation and decreased cell viability in *isc1Δ* cells. Sit4p had also a regulatory role on mitophagy and contributed to mitochondrial fragmentation in the mutant strain.

Overall, our work demonstrates that Isc1p-driven ceramide signaling is crucial for proper regulation of macroautophagy, mitophagy and mitochondrial dynamics by Sit4p-dependent mechanisms.
Funding: This work was financially supported by FEDER (Fundo Europeu de Desenvolvimento Regional) through the program "Programa Operacional Fatores de Competitividade-COMPETE", by FCT (Fundação para a Ciência e Tecnologia) and by "Programa Operacional Regional do Norte (*ON.2 – O Novo Norte*)", through the projects NORTE-07-0124-FEDER-000001 and FCOMP-01-0124-FEDER-028210.V.T. (SFRH/BD/72134/2010) and R.V. (SFRH/BD/48125/2008) were supported by FCT fellowships.
Keywords: Macroautophagy, Mitophagy, Sphingolipids.

WED-251
Cross-talk between autophagy and apoptosis of tumor cells by knockdown of antisense non-coding mitochondrial RNAs (ASncmtRNAs)

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The novel family of non-coding mitochondrial RNAs (ncmtRNAs) displays a differential expression pattern according to proliferative status. Normal proliferating cells express the sense (SncmtRNA) and antisense ncmtRNAs (ASncmtRNAs), whereas tumor cells express the sense transcript and down-regulate de ASncmtRNAs. By using chemically modified oligonucleotides directed against the antisense ncmtRNAs, we have achieved a massive degree of death of tumor cells, while normal cells are unaffected by the same treatment, suggesting a potential application of this treatment for the development of selective cancer therapy. Tumor cell death elicited in this manner exhibits molecular and morphological features typical of cell death type I (apoptosis), such as phosphatidylserine translocation, loss of mitochondrial membrane potential (MOMP), caspase activation, nuclear condensation and DNA fragmentation. At earlier post-transfection times, we have observed accumulation of autophagic vacuoles, together with LC3-I to LC3-II conversion only in cells treated against the antisense ncmtRNA. Electron microscopy revealed double-membraned structures and mitochondrial collapse only in tumor cells. Inhibition of the autophagic pathway does not necessarily block the onset of apoptosis. Our results indicate that knockdown of ASncmtRNAs in tumor cells elicits autophagy as a cytoprotective mechanism cross-talked to a point of no return towards cell death by apoptosis.
Acknowledgment: Dr. Sergio Lavandero, Universidad de Chile.
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Keywords: Autophagy, mitochondria, non-coding RNA.

WED-252
Expression of the autophagy genes in cancer cells overexpressed constitutively active PKC epsilon

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Introduction: Protein kinase C epsilon (PKCε), a novel PKC isotype is characterized as a calcium-independent and phorbol ester/diacyloglycerol sensitive serine-threonine kinase. PKCε is involved in many signaling pathways including adhesion, migration, proliferation, secretion, differentiation, gene expression and apoptosis. PKCε, among all PKC isozymes, shows the greatest oncogenic potential. This protein participates not only in tumor development but also in tumor invasion and metastasis. Overexpression of PKCε was found in cancers of the colon, breast, prostate, thyroid and lung.

Autophagy is an evolutionarily conserved, a multi-step, lysosomal degradation pathway that eliminates long-life proteins and converts damaged or old organelles into basic biomolecules which are returned from lysosomes to the cytoplasm. Autophagy plays an important role in the regulation of survival and death

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signalling pathways in a variety of human diseases, including cancer.

Aim: The aim of this study was to investigate the expression of the autophagy genes in cancer cells overexpressed constitutively active PKCε.

Material and methods: A human epitheloid cervix carcinoma cells HeLaPKCεA/E (transfected with pBl-PKCεA/E) were tested. In the present study the technique of qPCR was used to shown the expression of genes contributing to autophagy pathways using Real Time Primers ready Human Autophagy Primer Library 96 (HATPL-1) (Biomol, Germany) that enables profiling of 88 target genes and 4 reference genes. The amplification products of crucial autophagy regulators were resolved by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining. Moreover the technique of electrophoresis, WB, immunoidentification with appropriate antibodies were used to shown the crucial autophagy proteins level.

Results: In HeLaPKCεA/E cells treated with doxycycline (inductor of *PKCεA/E* gene) from 3 up to 72 hours the highest level of PKCεA/E mRNA was observed by 3 hours of induction. The highest level of PKCεA/E protein detected by Western blot analysis was indicated in 24 hours induction of *PKCεA/E* gene expression.

The anti-autophagic effect was observed in tested cells and manifested by significantly lower levels of *BECN1, ATG-5, PI3K, LC3* transcript and higher level of *mTOR* in cells overexpressed PKCεA/E compared to cells without doxycycline-induced PKCεA/E expression.

Interestingly, in cells overexpressed PKCεA/E inhibition of mTOR by rapamycin caused autophagy induction. We have shown increased levels of Beclin-1, Atg-5, PI3K and LC3 protein and decreased level of mTOR.

Conclusions: Our results suggest *PKCε* as a possible target in anticancer therapy.

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Keywords: autophagy, cancer, PKC epsilon.

WED-253

Functional study of the IRE1 interactome: fine tunning of UPR signaling

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Adaptation to Endoplasmic Reticulum (ER) stress depends on the activation of the unfolded protein response (UPR) stress sensor inositol-requiring enzyme 1 (IRE1), which functions as an endoribonuclease that splices the mRNA of the transcriptional factor X-box-binding protein 1 (XBP1). XBP1 mediates the upregulation of crucial UPR-related genes involved in folding, secretion, protein quality control, and many other functions. Besides, active IRE1 degrades mRNAs encoding certain proteins through a process known as regulated Ire1-dependent decay of messenger RNAs (RIDD). Under prolonged ER stress IRE1 is turned off, which may sensitize irreversible damaged cells to undergo apoptosis. The mechanisms underlying IRE1 activation/inactivation are not well defined, but we have recently described a cluster of new function for major apoptosis-related proteins in the control of the kinetic and amplitude IRE1 signaling through a regulatory protein platform termed the *UPRosome*. Using several proteomic approaches here we have characterized the *UPRosome* and identified novel regulators of IRE1 that modulate both activation and inactivation phase. Some of these interactores connect IRE1 with unexpected biological function related to cytoskeleton, mitochondria and ER-resident chaperones, which have the capability to activate or repress the amplitude and kinetics of IRE1. Thus, we used *Drosophila melanogaster* in order to validate the effect of these new IRE1 regulators in both xbp-1 splicing and RIDD processes. All this evidence suggests that IRE1 is finely regulated and together with its regulators constitute a functional platform, the *UPRosome*, which is functional *in vitro* and *in vivo*.

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Keywords: IRE1, UPRsome, XBP1.

WED-254

Mechanism of autophagy induction in breast cancer cells treated with synthetic triterpenoids

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Introduction: Autophagy, a process of self-degradation and turnover of cellular components, is engaged not only in tumor growth and progression but also functions as a cell death and tumor suppression mechanisms. Thus, the ability of autophagy modulation became a promising potential anticancer strategy. Especially natural-derived compounds and their derivatives seem to constitute an abundant source of autophagy modulators. Here we reveal the mechanism of autophagy induction in breast cancer cells after treatment with two synthetic derivatives of oleanolic acid (OA), HIMOXOL and Br-HIMOLID, showing a higher cytotoxicity in breast cancer cells than the maternal compound, OA.

Materials and methods: MCF7 (ER+), MDA-MB-231 (ER-) and MDA-MB-468 (ER-) breast cancer cells and non-tumorigenic MCF-12A (ER+) breast cells were treated by methyl 3-hydroxyimino-11-oxoolean-12-en-28-oate (HIMOXOL) and 12α-bromo-3-hydroxyimonoolean-28→13-olide (Br-HIMOLID) in different concentrations, corresponding to 0.5–1.5 IC_{50} values. On the basis of preliminary autophagy screening tests with monodansylcadaverine (MDC) staining and LC3 assessment (a specific-autophagy marker) we obtained information about selective, pro-autophagic effect of OA's derivatives in breast cancer cells. To evaluate the mechanism of action of studied compounds, expression of the crucial autophagy regulators: *LC3, Beclin 1 (BECN1), Atg5, PI3K* and *mTOR* was assessed in breast cancer cells by qPCR.

Results and discussion: We demonstrated that pro-autophagic effect, observed only in studied breast cancer cells, depended on a type and concentration of applied oleanolic acid derivatives. In MCF7 cells treated with HIMOXOL autophagy induction was mediated by overexpression of *BECN1*, *PI3K*, *Atg 5* and downregulation of *mTOR*. However, after treatment of these cells with Br-HIMOLID, we observed *LC3* and *PI3K* upregulation accompanied by a decrease in *mTOR* expression. In MDA-MB-231 cells, the autophagy process provoked by treatment with HIMOXOL was associated with *BECN1* and *PI3K* upregulation. However, treatment of this cells with Br-HIMOLID caused *BECN1* and *Atg5* overexpression accompanied by *mTOR* downregulation. Moreover, autophagy induced by HIMOXOL and Br-HIMOLID in MDA-MB-468 cells was mediated by *BECN1* and *mTOR* genes.

Conclusions: Autophagy in breast cancer cells provoked by synthetic triterpenoids is mediated by *LC3*, *BECN1*, *PI3K*, *Atg5* and *mTOR*. The effect of OA derivatives depends on a type of chemical modification, concentration and characteristics of target, breast cancer cells.

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Keywords: autophagy, breast cancer.

WED-255
Mechanisms involved in the induction of cell death by desacetylnemorone in colorectal cancer cells

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Introduction: The desacetylnemorone (HCRH), previously isolated from *Salvia* species, is an abietane diterpene with para-quinone ring in its structure, and then classified as a tanshinone that are often described by their broad spectrum of biological activities. The aim of this study was to evaluate the mechanisms underlying the anticancer activity of HCRH isolated from *Hyptis carvalhoi* roots.
Methods: The cytotoxicity of HCRH was tested against several human cancer and normal cell lines by MTT assay, after 72 h of incubation. The antiproliferative effect was also determined by reduction of BrdU incorporation in HCT-116 cells. To further investigate the mechanisms involved in the cytotoxic activity, the effects of HCRH were determined by flow cytometry, confocal microscopy and western blot test at different times in HCT-116 cell line.
Results and Discussion: After 72 h of incubation, HCRH showed IC_{50} values ranging from 3.9 to 32 μM in HCT-116 and HL-60 cancer cells, respectively. While for normal cells IC_{50} values ranged from 35.7 μM in V-79 to higher than 72 μM in 3T3-L1 and PBMC cells. In HCT-116 cells HCRH showed antiproliferative potential of time-dependent manner, leading to increased number of cells in the G_0/G_1 phase of the cell cycle and a substantial decrease in DNA synthesis. These effects were accompanied by changes in the levels of cyclins and CDKs, in addition to the increase in the levels of proteins $p21^{waf1/cip1}$ and $p27^{kip1}$, independent of p53 activation. The study of cell death pathway revealed that treatment with HCRH induces apoptosis in a small population of cells in time-dependent manner, suggesting that apoptosis is not the main death pathway. Autophagy was detected by the formation of acid compartments within the cell visualized by staining with AO and MDC. Autophagy was confirmed by analysis of the expression of related proteins such as LC-3, beclin-1 and Bcl-2. Among the initial events induced by HCRH are the generation of ROS that can lead to DNA damage detected by increased phosphorylation of histone γH2AX, since the first 6 h of treatment. These effects may induce cell death primarily by autophagy then the secondary activation of apoptotic pathways.
Conclusion: The results suggest that HCRH has potent antiproliferative activity associated with ROS generation leading to DNA damage, which prevents cell cycle progression and drive cells to the process of death by autophagy and apoptosis.
Financial Support: CNPq, CAPES, PRONEX.
Keywords: Apoptosis, Autophagy, Desacetylnemorone.

WED-256
Mutation in VPS35 associated with Parkinson's disease impairs WASH complex association and inhibits autophagy

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The retromer complex is important in endosomal protein sorting, including the endosome-to-Golgi retrieval of membrane proteins such as the cation-independent mannose 6-phosphate receptor. In addition, retromer recruits the actin nucleation-promoting WASH complex to endosomes. Together, these complexes are responsible for the sorting and localization of many physiologically important proteins and are linked to several neurodegenerative diseases. In fact, the D620N mutation in VPS35 results in a rare form of autosomal dominant Parkinson's disease (PD). This mutant is impaired in binding the WASH complex and in recruiting it to endosomes. Furthermore, PD mutant VPS35 as well as WASH1 depletion lead to autophagy defects by impairing the trafficking of the autophagy protein ATG9A. Thus, the Parkinson's disease-causing D620N mutation in VPS35 reduces WASH complex recruitment to endosomes, and reveals a novel role for the WASH complex in autophagosome formation.
Keywords: autophagy, neurodegeneration, retromer.

WED-257
New autophagy inducer from phenotypic screening induces autophagy in vitro and in vivo

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Autophagy is the process of mass degradation of unnecessary elements within the cell. It is related to many diseases such as cancer, atherosclerosis, and neurodegenerative diseases, but the detailed mechanism mostly remains to be uncovered. Therefore, autophagy inducers are useful chemical probes for exploring autophagy biogenesis and potential candidates as new drug entity. In the present study, a novel autophagy inducer, inda, was discovered from the cell based screening with John's Hopkins Drug Library (JHDL). Inda is an antidepressant as a no-specific monoamine reuptake inhibitor, but its effect on autophagy was not examined before. Inda-induced autophagy was validated by Lysotracker and MDC staining. EGFP-LC3 stable cell line and TEM images were examined for autophagosome formation. Molecular validation resulted in LC3 conversion in inda-treated cells. Inda suppressed mTOR/S6K activity by inducing AMPK. Furthermore, inda exhibited antidepressant effects in mouse behavior test and molecular validations revealed LC3 conversion and mTOR suppression in the mouse cortex. In conclusion, inda induces autophagy *in vitro* and *in vivo*, and its effects on autophagy results from AMPK induction and mTOR suppression. These results provide the possible relationship between antidepressant and autophagy signaling, and the mechanism behind inda-induced effects *in vivo*.
Keywords: autophagy, cell-based screening, chemical genomics.

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WED-258
Rapamycin-loaded solid lipid nanoparticles for brain targeting

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Neurodegenerative diseases are very debilitating conditions with an increasing impact on the world population and as yet without an effective remedy. Therefore, the development of new strategies for early diagnosis and treatment of these diseases is becoming a priority. The selectivity of the blood-brain barrier (BBB) strictly limits the number of therapeutic substances able to reach the brain and therefore, in recent years, many studies have been directed to the development of systems that facilitate the passage of drugs to the central nervous system (CNS). One of the most crucial protein complexes, that regulates vital processes within signal transduction, proliferation and autophagy pathways, is the mammalian target of rapamycin (Rp) complex (mTOR). Specific inhibition of mTOR by Rp in-feed administration has been recently demonstrated to ameliorate the cognitive behavior in an Alzheimer's mice model [1]. Rp is an immunosuppressive drug that can pass the BBB, but its systemic administration produces a number of side effects that may impair its therapeutic efficacy [2].

Nanoparticles represent one of the most innovative and non-invasive approaches for drug targeting. In particular, due to an average size below 100 nm and lipophilic properties, the use of P80-coated solid lipid nanoparticles (SLNs) can overcome the BBB and allow accumulation of drugs directly into the brain tissue, through the apolipoprotein blood-to-brain pathway. Here, we report the development of a novel formulation of Rp loaded SLNs and the investigation of the effect upon treatment of SH-SY5Y neuroblastoma cells. Our results show that Rp loaded SLNs are able to inhibit mTOR activity over a longer period of time compared to free Rp. This prolonged action is rather evident even at concentrations as low as 2 nM and it is consistent with a longer retention of Rp due to a slower release from the SLNs. On the basis of the data obtained, we will perform experiments to assay *in vivo* the ability of Rp-SLNs to deliver Rp therapeutic doses to the CNS, limiting systemic exposure to the drug side effects.

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Keywords: Rapamycin Brain Drug Delivery, Solid Lipid Nanoparticles.

WED-259
Seeing is believing – visualizing the autophagic membrane-scaffold

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Autophagy is a catabolic pathway that delivers cytoplasmic components to the lysosome for degradation. During macroautophagy, a *de novo* generated membrane sack progressively encloses cytoplasm, giving rise to a double-membrane autophagosome after sealing. Numerous proteins orchestrate the formation, closure, and fusion of autophagosomes with lysosomes. Two Ubiquitin-like conjugation systems are involved in these processes: one involves the conjugation of ubiquitin-like Atg12 to Atg5, which forms a constitutive complex with the coiled-coil protein Atg16. The second conjugation system covalently attaches Atg8 to the lipid phosphatidylethanolamine (Atg8-PE) in autophagic membranes. We reconstituted the *S. cerevisiae* Atg8 conjugation reaction on giant unilammelar vesicles to investigate the interactions of the proteins involved during autophagosome formation. These experiments indicated that upon association of Atg12–Atg5-Atg16 with Atg8-PE, immobile multimeric complexes are formed, which might represent a new kind of membrane scaffold. In order to visualize the scaffold, we reconstituted the conjugation-reaction on supported lipid bilayers and analyzed them by Atomic Force Microscopy (AFM). Our investigations demonstrated that membrane-anchored Atg8-PE is cross-linked by the Atg12–Atg5-Atg16 complex to form a two dimensional membrane scaffold with meshwork-like architecture. A quantitative analysis of our AFM-data revealed important insights into the molecular structure of the scaffold. Yeast cells, expressing Atg16-mutants which do not support scaffold formation *in vitro*, exhibit strongly decreased autophagic activity, suggesting scaffold formation to be required for autophagosome generation *in vivo*.

Keywords: Atomic Force Microscopy (AFM), autophagy, membrane-scaffold.

WED-260
The role of polyamines in the stress response

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P { margin-bottom: 0.21 cm; }Polyamines – putrescine, spermidine and spermine – are essential organic cations for cell growth that are present in all living organisms. Dysregulation of the polyamine metabolism is associated with ageing and several pathological conditions such as cancer and neurodegenerative diseases. These conditions are intimately associated with an imbalanced stress response. Recently, we found that the yeast Tpo1, a polyamine transporter not only regulates the levels of these metabolites but also controls the expression of proteins associated with stress, such as Hsp90 an Hsp70. We are currently studying the role of Tpo1 and other players of polyamine metabolism to further dissect their role in the stress response.

Keywords: ageing, Oxidative stress, polyamines.

WED-261
WAC is a positive regulator of autophagy that interacts with GM130 on the cis Golgi

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Background: Autophagy is a conserved cell-survival membrane trafficking pathway implicated in pathologies such as cancer. Much is still unknown about how autophagy is regulated.

WAC was discovered in our genome-wide screen to be an effector of autophagy (McKnight et al., 2012). WAC is in the nucleus and on the Golgi, where it affects transcription and Golgi biogenesis respectively (Totsukawa et al., 2011; Zhang and Yu, 2011).

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Aims & Methods: We aim to understand the mechanism behind WAC's regulation of autophagy and how WAC itself is regulated. We hypothesise WAC is affecting autophagy through its nuclear and cytoplasmic functions. WAC's effect on genome-wide transcription has been analysed. In conjunction, cytoplasmic binding partners of WAC have been investigated by mass spectrometry. Furthermore, we are employing a high-throughput imaging approach with a chemical library to investigate how WAC's subcellular localisation is controlled.

Results: We have investigated: Atg9 localisation, ULK1 signalling, LC3 lipidation, p62 degradation, LC3B and WIPI2 puncta formation. This has confirmed that WAC is a positive regulator of autophagy that acts early on in the pathway. Moreover, reintroduction of WAC into WAC-depleted cells rescues WIPI2 and LC3B puncta formation, two markers for autophagosomal structures. BeadArray analysis and qPCR validation shows that depletion of WAC reduces the mRNA expression of genes involved in autophagy. Additionally, through its coiled-coil domain WAC interacts with GM130 on the cis Golgi, whereas WAC is excluded from the trans Golgi. Knockdown and rescue experiments suggest that GM130 is the receptor for WAC on the Golgi. Autophagy assays reveal that GM130 is a negative regulator of autophagy. Finally, the WAC:GM130 interaction is enhanced upon starvation.

Conclusion: WAC is a novel positive regulator of autophagy that acts early on in the pathway. In the nucleus, WAC regulates the mRNA expression of genes involved in autophagy, whereas on the Golgi WAC interacts with the negative autophagic regulator GM130. We propose a model whereby upon amino acid starvation the WAC:GM130 interaction is enhanced, in order to supress the negative impact of GM130 on autophagy.

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Keywords: amino acid starvation, Autophagy, Golgi.

WED-262

Xanthohumol from *Humulus lupulus* L. induces glioma cell autophagy via inhibiting Akt/mTOR/S6K pathway

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Xanthohumol is the principal prenylated flavonoid in the hop plant (*Humulus lupulus* L.). Xanthohumol has potent cancer chemopreventive properties that occur through the regulation of various mechanisms. Although, xanthohumol-induced apoptosis of glioma cells has been demonstrated, the details of the effects of xanthohumol-induced autophagy on glioma cells remain unclear. The aim of our study was to determine the effects of xanthohumol on the C6 and U87 glioma cell lines. Using a tetrazolium-based colorimetric assay, we showed that xanthohumol (5~50 microM) possesses anti-proliferative activities. Xanthohumol also induced autophagy in C6 and U87 glioma cells, as evidenced by acridine orange staining. Moreover, we found that xanthohumol inhibited phosphorylation of Akt, the mammalian target of rapamycin (mTOR), and p70S6 kinase (S6K), a downstream effector of mTOR. The formation of LC3-II, an autophagic maker, and p62 degradation also occurred in xanthohumol-treated glioma cells. In addition, xanthohumol treatment resulted in the activation of mitogen-activated protein kinases (MAPKs), including p38, Erk, and JNK. The xanthohumol-mediated formation of LC3-II was partially reversed by the Erk inhibitor PD98059 and the JNK inhibitor SP600125, but not by the p38 inhibitor SB203580, indicating that Erk and JNK may be involved in xanthohumol-induced glioma cell autophagy. This study demonstrates for the first time that xanthohumol induces glioma cell autophagy at least in part through inhibition of the Akt/mTOR/S6K pathway, the activation of MAPK cascade, and subsequent LC3-II formation and p62 degradation, which lead to inhibition of glioma cell growth and migration. Therefore, the multiple biologic activities of xanthohumol may represent a high therapeutic potential for treatment or prevention of glioblastoma multiforme.

Keywords: Autophagy, Glioblastoma multiforme, Xanthohumol.

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CSV-02 – Chromatin organisation and the nucleus

WED-264

"Rods and Rings" inclusions formed by Inosine-5′-monophosphate dehydrogenase 2

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Inosine-5′-monophosphate dehydrogenase catalyzes the critical step in the de novo synthesis of guanosine nucleotides, the oxidation of inosine monophosphate to xanthosine monophosphate. This reaction can be inhibited by specific inhibitors like ribavirin or mycophenolic acid that are widely used in clinical treatment when required to inhibit the proliferation of viruses or cells. However, it was recently found that such an inhibition affects the cells, leading to redistribution of IMPDH2 and appearance of IMPDH2 inclusions in cytoplasm. According to their shape, these inclusions have been termed "Rods and Rings" (R&R). In this work we focused on subcellular localization of IMPDH2 protein and ultrastructure of R&R inclusions. Using microscopy and Western blot analysis, we showed the presence of nuclear IMPDH2 in human cells. We found that the nuclear pool has an ability to form Rod structures after inhibition by ribavirin. Concerning the ultrastructure, we observed that R&R inclusions in cellulo correspond to accumulation of fibrous material that is not surrounded by a biological membrane. The individual fibers were made up of regularly repeated subunits with length about 11 nm. Together, we showed the localization of IMPDH2 also inside the nucleus of human cells and described the ultrastructure of R&R inclusions.

Keywords: Inclusions, Inosine-5′-monophosphate Dehydrogenase, Rods and Rings.

WED-265

An essential role for an RNA polymerase III subunit in determining sites of retrotransposon integration

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Mobile genetic elements are ubiquitous and dynamic components of genomes, and their integration site choice influences genome stability and gene expression. The Ty1 retrotransposon of the yeast *Saccharomyces cerevisiae* is closely related to retroviruses and replicates by reverse transcribing its genomic RNA into cDNA, which is then integrated into the host cell genome. Ty1 integrates upstream of RNA polymerase III (Pol III)-transcribed genes, yet, the primary determinant of target specificity has remained elusive. Here, we describe a functional interaction between Ty1 integrase and a subunit of Pol III. Whereas the absence of interaction does not significantly affect Ty1 integration frequency, it dramatically alters target site choice, leading to a redistribution of Ty1 insertions in the genome. Therefore, this Pol III subunit through its interaction with Ty1 integrase is the major determinant of Ty1 integration targeting upstream of genes transcribed by Pol III.

Keywords: Integration site determination, Retrotransposon, RNA polymerase III.

WED-267

Comparison of oxaliplatin and carboplatin binding affinity to chromatin

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Platinum anticancer drugs are potent chemotherapeutic agents currently used to treat a number of cancers. Carboplatin and oxaliplatin are second and third generation platinum drugs. They exert their cytotoxic effects mostly through DNA damage and form intra and interstrand crosslinks in cellular DNA and induce apoptosis. In the cell nucleus, DNA is compacted with histone proteins into a nucleoprotein complex known as nucleosome that is repeating unit of chromatin.

Thus chromatin not DNA, is the major target for platinum anticancer drugs *invivo*. How chromatin structure is affected by the binding of this drug is poorly understood.

In the present study, we have investigated the binding affinity of oxaliplatin and carboplatin to chromatin by equilibrium dialysis and fluorescence spectroscopy techniques.

Fluorescence quenching data were analyzed in term of Stern–Volmer constant, Ksv. The association constant (K_a) and number of binding sites (n) were determined using modified Stern-Volmer equation and Scatrchard plot obtained from equilibrium dialysis.

The results of fluorescence spectroscopy indicated that upon addition of oxaliplatin, emission intensity of chromatin was significantly decreased, but in the case of carboplatin this reduction was negligible. Stern-Volmer curves showed positive and linear relationship and the higher temperature exhibited the higher Stern–Volmer constant for both drugs.

The calculated binding constants for oxaliplatin and carboplatin from modified Stern-Volmer plots indicated that the value of K_a for oxaliplatin in both 25 and 37 °C was higher than for carboplatin. In both cases, Scatchard plot exhibited a cooperative binding pattern and K_a of oxaliplatin (58.1×10^3 M^{-1}) was higher than carboplatin (0.332×10^3). Hill coefficient for both drugs was higher than 1 that confirmed positive cooperative binding pattern of the drugs.

Based on the results, it is calculated that oxaliplatin exhibits higher quenching effect compared to carboplatin and in both cases mechanism of chromatin fluorescence quenching is dynamic which indicates that binding forces are mainly hydrophobic. The results suggest higher binding affinity of oxaliplatin to chromatin compared to carboplatin, which may help in combination therapy and drug design.

Keywords: carboplatin, equilibrium dialysis, oxaliplatin.

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WED-268
ERH structurally differentiates between the highly homologous SAFB1 and SAFB2 proteins

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SAFB1(Scaffold Attachment Factor) is a nuclear matrix protein that is implicated in a multitude of cellular processes including chromatin organization, transcriptional regulation, RNA processing, as well as stress response. SAFB2, a protein highly homologous to SAFB1 (70% in its whole length) shares with it numerous highly conserved functional domains. Their genes are localized head to head on the same chromosome and they share a common promoter. The function the best established of SAFB1 is as transcriptional co-repressor of nuclear receptors and particularly of the Estrogen Receptor a (ERa), a property SAFB2 harbors too. Although indirect evidence suggests that SAFB1 and SAFB2 might have unique properties, their biologic and functional differences are still obscure.

We have examined the interaction of SAFB2 with already known SAFB1 molecular partners in a directed two hybrid system. Among the clones tested, one clearly distinguishes between the two SAFB proteins and was chosen for further examination of its structural and functional relation to SAFB1 and SAFB2. ERH (Enhancer of Rudimentary Homolog), a small protein (104 aa) of strikingly high inter-species conservation (100% amino acid sequence identity between human and *xenopus*, 80% similarity between human and *dyctyostelium*), was bacterially expressed and shown by pull down assays to bind *in vitro* to SAFB1 but not to SAFB2. Studies on the effect of ERH on the ERa corepressor activity of SAFB1/2 are currently under way.

The significance of the SAFB1/ERH interaction and SAFB1-SAFB2 discrimination by ERH will improve our knowledge on both ERH and SAFB protein function.

Co-financed by the European Union (ESF) and Greek national funds (Education and Lifelong Learning-NSRF, Program THALIS)
Keywords: ERH, SAFB1, SAFB2.

WED-269
Genotoxicity on essential oils of *Origanum onites* L., *Laurus nobilis* L. and *Salvia fraticosa* in cultured human lymphocytes

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Essential oils extracted from several plants have a wide spectrum of use ranging from aromatherapy, cosmetics and food production to pharmaceutical industry and production of pesticides and household products such as detergent and soap due to their different biological activities. These biological activities include mainly antibacterial, antifungal and antioxidant effects. The potential toxic and genotoxic effects of these compounds with uncontrolled and unconscious consumption are important for humans. In the present study, genotoxic effects of essential oils extracted from *Origanum onites L.*, *Laurus nobilis L.* and *Salvia fraticosa* were investigated after 24 and 48 hours treatment with cultured human lymphocytes at doses of 0.05, 0.025, 0.01, 0.006, 0.003, 0.001 μl/ml by using Cytokinesis Blocked Micronucleus (CBMN) and Hypoxanthine Guanine Phosphoribosyltransferase (HGPRT) methods. The findings of the experiment supported that *Origanum onites L.* essential oil has clastogenic effect on cultured human lymphocytes through significant increase in micronucleus formation at doses of 0.05, 0.025, 0.01 and 0.006 μl/ml when compared to control experiment. In addition, *Laurus nobilis L.* essential oil displayed significant activity only at dose of 0.025 μl/ml, while *Salvia fraticosa* essential oil did not display significant activity at any doses after 48 hours treatment. No mutagenic effect was detected when cells pretreated with a positive mutagen were treated with *Laurus nobilis L.* and *Salvia fraticosa* essential oils which were found to be non-mutagenic in terms of MN formation. On the other hand, by HGPRT gene mutation test that investigate genotoxic effects able to cause gene mutations, it was seen that *Origanum onites L.* essential oil was effective on HGPRT gene mutation by causing considerable increase in variance frequency at all doses applied. However, *Laurus nobilis L.* and *Salvia fraticosa* essential oils caused HGPRT gene mutation in a dose-dependent manner by increasing variance frequency compared to control solvent at doses of 0.05, 0.025, 0.01 μl/ml. Together with these findings, it was concluded that *Origanum onites L.* essential oil have significant genotoxic activity on human cells at chromosomal and gene levels, while *Laurus nobilis L.* and *Salvia fraticosa* essential oils have more limited and weak mutagenic activity in terms of gene mutation.

Keywords: essential oil, genotoxicity, human lymphocytes.

WED-270
HP1 dynamics reports a rolling continuum of chromatin states in pluripotent and terminally differentiated cells

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Differentiation of embryonic stem cells (ESCs) is associated with progressive immobilization of chromatin proteins, decrease of bulk transcription and selective induction of lineage-specific genes. Employing mouse ESCs and primary fibroblasts (MEFs) isolated from either normal or developmentally compromised (ichthyosic) embryos, we show here that the average mobility of heterochromatin protein-1 (HP1) correlates closely with the transcriptional status and the expression profile of a cell population, but does not predict exit from the pluripotent state or change of cellular phenotype. In fact, large differences in HP1 mobility can be detected even among the cells of a clonally derived, synchronous population, in parallel to packing and fractal geometry differences that occur naturally across the same chromatin landscape. These observations are consistent with the concept of a rolling chromatin state "continuum" that shifts subtly and in a stochastic fashion when embryonic cells attempt to differentiate or functionally compensate in the course of disease.

Keywords: chromatin, heterochromatin protein 1, protein dynamics.

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WED-271
Inner nuclear envelope architecture and chromatin recognition

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Top preferred session: Chromatin organisation and the nucleus

The complex formed by lamin A/C and emerin is an essential component of the inner nuclear envelope, located at the interface between the nuclear membrane and chromatin. It is at the center of the nuclear force response pathway and it is mutated in patients with Emery-Dreifuss Muscular Dystrophy (EDMD), which suggests that its biological function is particularly important in skeletal and cardiac muscle cells. Emerin role in nuclear mechanics was recently enlightened. It was shown that emerin depletion increases nuclear rigidity and prevents nuclear adaptation to force (Guilluy et al., *Nat Cell Biol* 2014). At the molecular level, mechanical force strongly induces tyrosine phosphorylation of emerin by the kinase Src. Emerin also regulates signaling and related transcription events. It controls transcription factor localization by influencing polymerization of nuclear actin (Ho et al., *Nature* 2013). Moreover lamin and emerin binds chromatin. Here we present a structural characterization of the emerin nucleoplasmic region. We had already determined the 3D structure of the emerin N-terminal LEM domain (Wolff et al., *FEBS Letter* 2001). We now show that a larger emerin fragment can be produced that dimerizes *in vitro*. We identify the impact of EDMD-causing mutations on this emerin fragment structure and oligomerisation properties. We analyze the impact of emerin structural characteristics and phosphorylation states on its capacity to bind lamin A/C tail, actin and chromatin, and we discuss the impact of EDMD-causing mutations in emerin and lamin A/C on these interactions.

Keywords: emerin, lamin, structural biology.

WED-272
Muscle-specific laminopathies – developing regulatory elements for high-level lamin A expression in muscle tissue

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Muscle-specific laminopathies are rare, currently not curable diseases, caused by mutations in gene *LMNA* coding for nuclear proteins – A type lamins. Lamins are responsible for mechanical functions, organization of chromatin, DNA replication, regulation of transcription factors, epigenetics, DNA repair, transcription, cell cycle regulation, cell development and differentiation, nuclear positioning and apoptosis. We showed that *in vitro* overexpression of lamin A mutants causes nuclear lamina disruptions, translocation of lamin C to the cytoplasm, changes in proliferation rate and broad spectrum of nuclear phenotypes, depending on mutation.

Probably the only possible method for muscle-specific laminopathies treatment is a gene therapy, but two major problems need to be solved first: development of muscle-directed virus vector providing long term expression of the genetic drug and muscle specific, highly efficient expression cassette. We designed a muscle-specific, highly active expression cassette containing hybrid promoter for therapeutic virus.

The hybrid promoter is composed of various, mammalian, muscle specific regulatory elements, that provides a high level of a gene expression in myoblasts, myotubes and cardiomyocytes. It consists of elements such as enhancers, core promoter and intron with small intronic enhancer. They originate from murine, muscle specific genes chosen and modified basing on their expression profiles, previous research data and *in silico* analysis.

The studies on the activity of the hybrid promoter's variants were performed by analyses of expression of a reporter genes – secretory luciferase and EGFP. Our data shows, that developed promoter provides several folds higher level of the expression in C2C12 cell line that promoter of desmin, currently used in gene therapy of muscular dystrophies, but providing too low level of expression. The activity and specificity of the promoter was also examined using different cell lines and primary cells. The influence of the particular components of the hybrid promoter on the expression level in muscular and non-muscular cell lines was also investigated.

We also analyzed efficiency and effects of prelamin A expression under control of hybrid promoter, delivered by pseudotyped lentiviral vector in myoblasts and cardiomyocytes cell lines *in vitro*.

Keywords: gene therapy, laminopathies, muscle-specific promoter.

WED-273
Nucleosomal intermediate structures characterized by molecular dynamics simulations and atomic force microscopy

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Posttranslational modifications of the nucleosome can affect stability of chromatin both on local and large-scale levels as well as accessibility of DNA to chromatin remodeling factors. Mounting evidence supports the hypothesis that the nucleosome is in dynamic equilibrium with its different intermediate states; these states are characterized by varying degrees of wrapping of the DNA around the histone core and the composition or stoichiometry of the core. Existence of several intermediate structures, lacking one or more histone heterodimers, is assumed. However, these structures are still not well characterized, either experimentally or theoretically; in particular, no atomistic models of them exist.

We have constructed all-atom models of the nucleosome intermediate states including two variants of the hexasome and the tetrasome. The complete nucleosome was represented as the octasome structure, with all the histones present. Dynamic characteristics of these structures were obtained from a series of 75 ns long molecular dynamics simulations in implicit solvent. During the simulations the octasome, hexasome and tetrasome retain the over-all o, σ and υ-shape respectively, while the "tails" of unbound DNA fluctuate with varying amplitudes. Dynamic

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characteristics of structures were described in terms of following parameters: RMSD of individual histone subunits, distances between centers of mass of histone dimeres, distances from each pair of nucleotide to the closest amino acid of histones, angle of entry of two DNA arms to histone core, and distances between two nucleotides marked with FRET labels, as described in literature[1]. Based on the latter distances, ensembles of possible conformations were divided into low-, middle- and high-FRET intensity subpopulations and frequencies of their occurrence were calculated and compared with experimentally observed values[1].

The existence of the modeled structures was further corroborated by an AFM-based analysis of reconstituted nucleosomes in different concentrations and NaCl salt conditions. Three main parameters, namely – volume of core particle, number of turns of DNA wrapped around the core and angle of entry of DNA arms to the core, allowed us to divide the observed nucleosome images into three partially intersecting arrays that were interpreted as the octasome, hexasome and tetrasome moieties respectively.

Reference

1. Gansen et al. PNAS, 106(36), 15308.

Keywords: nuclesome stability, transient nucleosomal structures.

WED-274
Post-translational modifications of nuclear proteins of placenta in complicated pregnancy

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Background: Metabolic sufficiency of placenta is largely determined by the state of its protein component. An important contribution to the maintenance of protein properties and functions is made by post-translational modification reactions, the disturbance of which is accompanied with the imbalance of cellular regulation processes in placenta. Particularly negative consequences for the functional metabolic state of placenta might be brought by post-translational damages of nuclear proteins involved in the formation of chromatin structure and regulation of gene expression.

The aim of this research is the study of intensity of cyclic nucleotide-dependent phosphorylation, carbonylation and amidation of nuclear proteins in placenta in placental insufficiency (PI).

Methods: Full-term placentas were obtained after delivery (39–40 weeks of gestation) from women with physiological pregnancy (n = 21) and with PI (n = 26). Histones and non-histone proteins of chromatin were separated from placental tissue after differential ultracentrifugation, isoosmotic lysis and multistage extraction. The intensity of post-translational modifications of those proteins was determined using kits.

Results: The activity of cAMP- and cGMP-dependent phosphorylation of histones in PI is lower as compared with physiological pregnancy by 24% and 18%, correspondingly. The phosphorylation degree of non-histone proteins of chromatin, especially of those which are labile associated with DNA, is more expressed and equals to 32% and 24%. The intensity of carbonylation of non-histone proteins and histones in PI increases by 30% and 23%, which indicates an increase of their oxidative modification under the conditions of intrauterine hypoxia and may be accompanied with the disturbance of different levels of protein structure. A reversed situation is characteristic of dynamics of amidation of placental nuclear proteins: the content of amide groups in histones is reduced by 27%, in non-histone proteins – by 34% that results in an increase of the negative charge of protein molecules. The detected changes of post-translational modifications of placental nuclear proteins will affect their regulatory activity.

Conclusion: Changes in the intensity of post-translational reactions may be important causes of PI development and may serve as markers of this obstetrical pathology.

Keywords: post-translational modifications, proteins, placenta.

WED-275
Prediction of the sequence effect on B-DNA shape from NMR

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P { margin-bottom: 0.21 cm; direction: ltr; color: rgb(0, 0, 0); } P.western { font-family: "Times New Roman",serif; font-size: 12pt; }P.cjk { font-family: "DejaVu Sans","MS Mincho"; font-size: 12pt; }P.ctl { font-family: "Lohit Hindi","Times New Roman",serif; font-size: 12pt; }A:link { }

Solution NMR studies of four B-DNA dodecamers were used to investigate the intrinsically preferred shape of free DNA, sequence-dependent, *via* the phosphate linkage behavior (BI ↔ BII equilibrium).

The study provided extensive evidence of the DNA intrinsic mechanics through a consistent series of correlations between NMR observables. The differences between two successive Residual Dipolar Couplings (ΔRDCs) on one hand and internucleotide distances on the other hand are both correlated to ^{31}P chemical shifts (δP), which reflect the populations of the BI and BII backbone states. Using high resolution X-ray structures as templates showed that ΔRDCs depend mostly on roll while internucleotide distances are sensitive to both roll and twist. In addition, NMR data showed that the backbone behavior is intimately coupled to the minor groove dimension. Therefore δP measurements probe not only the BI ↔ BII backbone equilibrium but, crucially, also inform on helicoidal parameters of B-DNA.

Importantly, the δP collected here confirmed that specific δP values and, hence, specific BI/BII ratios characterize the 10 complementary dinucleotides composing DNA. Combining this sequence effect and the structural couplings allowed the generalization of an NMR-based quantitative TRX scale previously proposed, which now predicts the sequence dependent intrinsic structural properties of each of the 10 complementary dinucleotides in any B-DNA in solution.

Finally, our NMR data highlight the significant progress achieved with the recent force field CHARMM36 towards a realistic description of phosphate group motions modulated by the sequence, resulting in a credible representation of B-DNA by molecular dynamics.

In sum, the prediction of the intrinsic structural DNA behavior by TRX, supplemented by molecular dynamics, offers a powerful framework to illuminate the molecular basis of the reading of DNA by proteins.

Keywords: BI ↔ BII backbone equilibrium, DNA sequence effect, DNA structure and dynamics.

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WED-276
Resveratrol activates SIRT1 in a Lamin A-dependent manner

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Human sirtuin1 (SIRT1), the closest homolog of the yeast sir2 protein, functions as an NAD+-dependent histone and non-histone protein deacetylase in several cellular processes, like energy metabolism, stress responses, aging, etc. We found that lamin A (a major nuclear matrix protein) directly binds with and activates SIRT1. Resveratrol, a natural phenol, has long been known as an activator of SIRT1. However, resveratrol's direct activation of SIRT1 has been refuted several times. We identified a novel mechanism underlying the activation of SIRT1 deacetylase by resveratrol. Resveratrol activates SIRT1 by increasing its binding with lamin A, thus aiding in the nuclear matrix (NM) localization of SIRT1. We found that rescue of adult stem cell (ASC) decline in laminopathy-based premature aging mice by resveratrol is SIRT1-dependent. Further, resveratrol's ameliorating effects on progeria and its capacity to extend lifespan in progeria mice has been established. Our data suggested that Lamin A is an endougenous activator of SIRT1 deacetylase and resveratrol can enhance the binding between SIRT1 and lamin A, therefore promote its activity. Treatment of progeroid mice with resveratrol significantly ameliorates premature aging and extends lifespan in progeria mice. Our finding suggests a new therapeutic strategy for Hutchinson-Gilford Progeria Syndrome and aging associated disorders.

Keywords: Resveratrol, Aging, Sirt1, Lamin A, HGPS.

WED-277
Sequence-dependent conformations and microsecond-scale dynamics of B-DNA and bound cations

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It is well recognised that DNA is more than just a passive vector of genetic information. Instead, its mechanical properties play a significant role in protein-DNA interactions, which are important in all cellular processes that involve DNA, including genome organization and transcription regulation. As these properties depend strongly on the local base sequence, their variations along the genome effectively represent a second genetic code. Understanding how these two layers of genetic information interact, and how their interplay contributes to the regulatory network of the cell, requires extensive structural and dynamical knowledge of B-DNA, which is currently unavailable from experiment.

The Ascona B-DNA Consortium (ABC) was formed in order to obtain such information using molecular simulations. The collaboration and joint computational effort of thirteen laboratories around the world allowed the collection of more than 50 microseconds of state-of-the-art molecular dynamics (MD) simulations of a wide array of DNA sequences in water at physiological salt concentration. The ABC dataset is, to date, the most complete collection of simulation trajectories for the systematic study of the molecular-detail mechanical properties of B-DNA.

By performing detailed statistical analysis of this large database, we were able to identify the mechanical couplings at the basis of the correlated motions that characterise the nanosecond time-scale interconversion between few well-defined local B-DNA conformational sub-states. The unique features of the ABC dataset allowed us, on one hand, to define the relationship between these collective movements and specific interactions within the double helix, and on the other hand to exhaustively establish sequence-dependent variations in the relative population of the conformational sub-states. Furthermore, the analysis of the distribution of positive ions around DNA using helicoidal coordinates revealed sequence-dependent variability of the cation densities that is coupled to the conformational fluctuations of the double helix.

These results represent an important step towards a detailed mechanical understanding of the sequence dependence of B-DNA fluctuations and recognition by proteins, and are of great relevance in guiding future endeavours aimed at modeling the physics of B-DNA.

** The Ascona B-DNA Consortium is composed of the Authors and David Beveridge, Thomas C. Bishop, David A. Case, Thomas Cheatham III, Surjit Dixit, B. Jayaram, Filip Lankas, Charles Laughton, Roman Osman, Modesto Orozco, Jiri Sponer and Krystyna Zakrzewska.

Keywords: DNA, Molecular Dynamics simulations, Protein-DNA interactions.

WED-278
Structural basis of a Polycomb body

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A Polycomb (PcG) body is a microscopically visible nuclear subcompartment initially characterized by accumulations of Polycomb repressive complex 1 (PRC1) proteins. Even though the PcG bodies were described 15 years ago, the structural basis of the PcG body is still under debate. To directly visualize the fine structure of the PcG body in human U-2 OS cells stably expressing recombinant polycomb BMI1-GFP protein, we used correlative light electron microscopy combining live cell imaging and high-pressure freezing. The immunolabeled BMI1 polycomb protein was detected to be specifically enriched within condensed chromatin fascicles, large scale heterochromatin fibers, throughout the nucleus. The accumulation of label in PcG foci was shown to be generated by the local accumulation of condensed chromatin fascicles in space. However, seemingly contradictory reports have appeared that describe the PcG bodies either as protein-based bodies in the interchromatin compartment or chromatin domains. In order to settle this contradiction, crowding experiments, that represent a convenient model distinguishing between interchromatin and chromatin compartments, were carried out. By crowding approach, we observed that the behaviour of the fluorescent PcG foci in cells grown under changed molecular crowding conditions vastly differs from the behaviour of nucleoplasmic bodies. We detected that, in cells grown in hypertonic medium, PcG foci disappeared, whereas typical nuclear bodies were maintained. Importantly, "PcG bodies" as nuclear domains characterized by accumulations of DNA persisted. Our findings clearly indicate that the PcG body in U-2 OS cells is a chromosomal domain, rather than a nucleoplasmic body.

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Keywords: Nuclear subcompartments, Polycomb body, Polycomb proteins.

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WED-279
Structural features and molecular interactions of Lamin B Receptor: an interplay between order and disorder

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Lamin B Receptor (LBR) is a ubiquitous protein of the nuclear envelope thought to connect the inner nuclear membrane with the underlying nuclear lamina and peripheral heterochromatin. The nucleoplasm-facing amino-terminal part of the protein has been shown to mediate most of LBR interactions and harbors a 40-residue region rich in Arg-Ser repeats (RS region) that has the typical features of an IDP protein and is flanked between two globular domains (a well-folded Tudor domain and a 110-amino acid segment with no apparent homologues). LBR-Tudor exhibits tight and stoichiometric binding to the “histone fold” region of free histone H3, suggesting an interesting role in histone assembly. In addition, robust binding to native nucleosomes, both unassembled and assembled H3/H4 histones and several other LBR partners, such as nuclear lamins and heterochromatin proteins, is observed, when LBR-TD is extended towards its carboxy-terminus to include the RS region. The RS region is also responsible for LBR homopolymerization. However, its conformational and functional properties are most likely modulated by the type and the extent of post-translational modifications, mainly phosphorylation and methylation. Here, we present the results of a biochemical, biophysical and computational study of LBR regions addressing the effect of physiologically relevant PTMs and interdomain interactions on LBR structure and function.

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Keywords: intrinsically disordered protein, Lamin B Receptor, Tudor domain.

WED-280
The pattern of reorganization of nucleosome structure chromatin in coding region under gene activation

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The analysis of nuclease DNA fragmentation within cell nuclei that reveals the changes in DNA-histone interactions is a helpful approach to elucidate pattern of nucleosome remodeling under gene activation. We studied fragmentation with micrococcal nuclease (MNase) and DNase I of coding region chromatin of trp-dioxygenase gene (*tdo*) in rat liver nuclei where its copies are presented in two states, i.e., transcriptionally competent and transcriptionally active. DNase I introduces nicks (single-stranded breaks) in free DNA. Coincidence of nick positions on complementary chains produce double-stranded breaks. MNase generates symmetric double-stranded breaks. It was found that under short-term action of MNase at saturating concentration (at limit of MNase digestion of total chromatin DNA), two kinds of *tdo* gene fragments are mainly are produced: about full-size *tdo*-transcription units (19000 bp) and their large (from 1500 bp) segments heterogeneous in length. This means that these fragments originate from two distinct varieties of *tdo*-transcription units coexisted in liver nuclei. Some of them do not contain MNase accessible sites across their length, whereas in others resistant regions alternate with rare irregular located MNase-sensitive regions. Under similar conditions the whole of *tdo*-DNA converts into acid-soluble products in rat liver nuclei treated with DNase I. We presume that the inaccessibility of DNA to MNase over entire length of coding region is inherent transcriptionally competent *tdo*-copies. Transcription generates MNase sensitive areas that it is possible flank elongating RNA polymerases.

After the gentle ultrasound treatment of liver nuclei, three subfractions of chromatin (DNP) fragments were isolated by selective extraction. By dot blot analysis, we showed that *tdo*-DNA is 20 – 30-fold enriched in these fractions. Minor fraction (nearly 1% of total DNA) in 0,005 M MgCl2 solution releasing (DNP-AI), consisted of long fragments (6000–20000 bp on DNA scale); the fraction extracted by 0,2 M NaCl solution (DNP-AII) is represented by single peak of short DNP fragments (200–1000 bp). The dissoluble in the 0,05M Na-pyrophosphate with 0,005 M MgCl2 solution fraction (DNP-AIII) included both DNA-peaks. Fragments of 5′-flanking *tdo* gene region predominated in low-molecular DNAs of DNP-AII and DNP-AIII. Transcription units of *tdo* were represented in DNP-AI and DNP-AIII by DNA fragments more than 10000 bp long. Non-equimolar ratio of four core histones is a distinctive feature of the fractions under study: content of H2A and H2B is lower in all the fractions; only alone H4 is revealed in DNP-AII.

Possibility of coupled linearization of DNA and histone octamers in coding regions of chromatin at gene activation is discussed.

Keywords: chromatin of active genes.

WED-281
Transcription shutdown induced by heat shock in Drosophila induces reorganization of chromatin and increases the binding of lamin Dm and topoisomerase II to chromatin and DNA

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Drosophila lamins and topo II are major karyoskeletal proteins important in chromatin organization, remodeling and regulation of gene expression. Both lamin Dm and topo II proteins interact with and bind DNA and chromatin components in vivo. Some of these in vivo interactions occur through AT rich DNA sequences (SAR/MAR DNA). In flies, the induction of heat shock induces genome wide epigenetic changes in chromatin which results in total transcription shutdown except the induction of six heat shock loci. This offers the unique opportunity to induce the genome wide epigenetic changes in chromatin and research the role of lamin Dm and topo II and other proteins in transcription regulation and chromatin organization on genome wide scale as well as ability to visualize this processes on particular loci using salivary glands polytenic chromosmes. With transcription activation (or shutdown) within several minutes time of heat shock induction this method allows for detection of precise correlation between transcriptional activity of locus and its chromatin modification.

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We analyzed the in vivo DNA and chromatin binding ability of lamin Dm, lamin C and topo II binding and showed that lamin Dm binds about 3–4 fold weaker with chromatin than topo II in vivo in normal conditions. The change in chromatin organization induced by heat shock increased overall lamin Dm binding with chromatin by 4 - folds while topo II binding was increased about 15 – fold. Specificity of binding to target chromatin elements (DNA, RNA, histones) was also detected for both proteins. Soluble (low salt extractable) fraction of lamin Dm and topo II was the most efficient in chromatin binding. Upon heat shock induction/transcription shutdown several fold increase in chromatin binding was detected in insoluble (nuclear matrix) fraction of lamin Dm and topo II. This correlated with redistribution of karyoskeletal proteins and changed solubility of lamins, topo II and F/G repeat nucleoporins. Changes in distribution of modified histones as well different staining pattern for lamin Dm and topo II have been detected as well. Our data indicate that lamin Dm (similarly to topo II) may be involved in chromatin opening and closing in vivo.

This work has been partially supported by the Polish Ministry of Science and Higher Education "Statutory Grant (1013/S/WB/2011-2013) and COST Action BM1002 Nanonet: Nanomechanics of Intermediate Filament Network (R.R.).

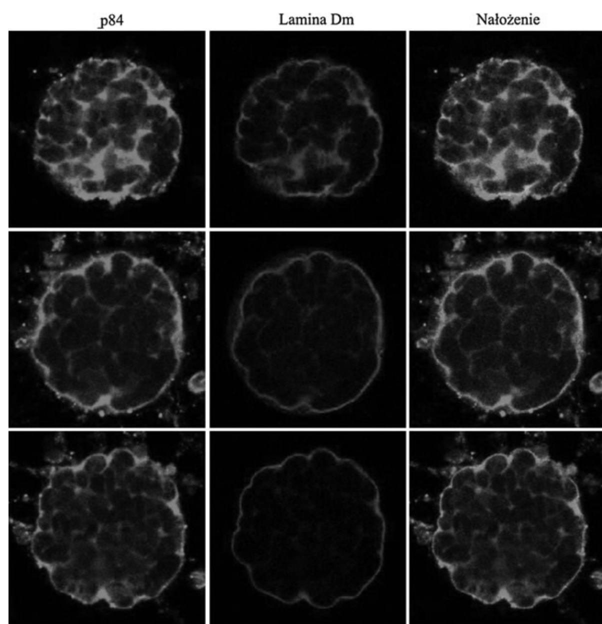

Fig. 1. xxxxxxx.

Keywords: lamins, topoisomerase II, chromatin binding.

WED-282

Two new insulator proteins, Pita and ZFPIC, target CP190 to chromatin

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Insulators are multi-protein–DNA complexes that regulate the nuclear architecture. The Drosophila CP190 protein is a cofactor for the DNA-binding insulator proteins Su(Hw), dCTCF, and BEAF-32. The fact that CP190 has been found at genomic sites devoid of either of the known insulator factors has until now been unexplained. We have identified two DNA-binding zinc-finger proteins, Pita and a new factor named ZFPIC, that interact with CP190 in vivo and in vitro at specific interaction domains. Genomic binding sites for these proteins are clustered with CP190 as well as with dCTCF and BEAF-32. Model binding sites for Pita or ZFPIC demonstrate a partial enhancer-blocking activity and protect gene expression from PRE-mediated silencing. The function of the dCTCF-bound Mcp insulator sequence requires binding of Pita. These results identify two new insulator proteins and emphasize the unifying function of CP190, which can be recruited by many DNA-binding insulator proteins.

Keywords: chromatin organization, insulator.

WED-283

Unique mechanical properties of isolated nuclei regulated by chromatin compaction

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Nuclear mechanics and structure could affect gene regulation and gene expression. Chromatin, a major component of cell nuclei, could play an important role in maintaining nuclear integrity and their mechanical properties. Previous studies on isolated nuclear mechanical properties have focused largely on the role of the nuclear lamina, using techniques such as AFM and micropipette aspiration. In this work, we explicitly address the contributions of chromatin to nuclear rheology using a microfluidic optical stretcher, which probes the mechanical response of isolated nuclei to an optically induced stress applied in their *suspended* state. Intriguingly, we find that isolated nuclei expand in volume when stretched in one direction, and exhibit significant softening with increased nuclear size. In addition, changes to the state of chromatin condensation using enzymatic perturbations or ionic conditions dramatically alter the nuclear morphology and compliance. Specifically, isolated nuclei stiffen significantly in the presence of high salt concentration, and exhibit contraction in the presence of multivalent ions due to hyper-condensation of chromatin. The presented work suggests that the unique nuclear mechanical properties may be linked to the compaction state of chromatin, which can be modulated by a change in nuclear volume or electrochemical environment.

Keywords: None.

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CSV-03 – DNA Repair

WED-285
Association of DNA damage response genes polymorphism with individual radiosensitivity in Kazakhstan population

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It is suggested that inherited variations in radiosensitivity genes may contribute to formation of response to cancerogenic agents of anthropogenic etiology. Considering the fact that induction of DNA damage as number one marker of radiation influence, variations in genes involved into DNA repair and cell cycle regulation maybe implied as strong markers of individual radiosensitivity.

The ATM gene plays crucial role in responding to DNA damage, in particular to double strand breaks. In order to maintain the genome integrity it is believed that ATM tightly cooperates with TP53. Therefore alteration in genes encoding these proteins may result in accumulation of genomic mutations including deletions and translocations.

The aim of the study was to investigate the association between variants of DNA damage response genes and radiosensitivity in population from Kazakhstan. DNA and blood samples were obtained from the unique biobank, representing people living in close proximity to former Semipalatinsk nuclear testing site as exposed cohort and residents of "radiation free" districts of Almaty region as control group. PCR and PCR-RFLP methods were used to study *ATM* G5557A and *TP53* Arg72Pro polymorphisms.

According to cytogenetic study results, total frequency of chromatid and chromosome aberrations in exposed cohort is more that 2.5 times higher in contrast to control group. The high frequency of chromosomal aberrations (more than 3%) was considered as a criterion of radiation exposure for the analysis of the association with individual radiosensitivity.

Obtained results demonstrate association between high frequency of chromocomal aberrations and studied genotypes: *TP53* Arg72Pro (OR = 1.36, p = 0.009) and *ATM* Asn1853Asn (OR = 1.41, p = 0.98).

We believe our results can be useful in early identification individuals under greater genetic risk in order to improve health issues.

Keywords: None.

WED-286
BRCA1/BARD1 retention at sites of DNA damage through HP1gamma interaction with BRCT domain of BARD1

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Stable retention of the BRCA1/BARD1 complex at sites of DNA damage, which is mediated by the BRCT domains of BRCA1, is required for the proper response to DNA double-strand breaks (DSBs). Here, we demonstrate that the BRCT domain of BARD1 is also required for the retention of the complex at damaged sites through its interaction with HP1gamma. In response to DNA damage, BARD1 interacts with Lys9-dimethylated histone H3 (H3K9me2) in an ATM-dependent but RNF168-independent manner. This interaction is mediated by HP1gamma. A conserved HP1-binding motif in the BARD1 BRCT domain directly interacts with the chromoshadow domain of all three isoforms of HP1 *in vitro*; mutations in this motif disrupt the retention of BRCA1/BARD1 at DSB sites and allow ectopic accumulation of RIF1, an effector of non-homologous end joining, at the damaged loci in S-phase. The histone lysine methyltransferase (HKMT) inhibitors chaetocin and UNC0638 abolish the retention and exhibit synergistic inhibition of clonogenic cell growth with the poly (ADP-ribose) polymerase (PARP) inhibitor olaparib. We reveal that the BRCT domain of BARD1 promotes retention of a non-BRCA1-Abraxas BRCA1/BARD1 complex at damaged DNA sites and demonstrate the therapeutic potency of HKMT inhibitors that disrupt the response of BRCA1/BARD1 to DNA damage.

Keywords: BRCA1/BARD1, HP1gamma, DNA damage.

WED-287
Cdc7, Rad53 and Asf1 stabilize Rad52 binding to replicative DNA damage

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The response to DNA lesions that impair the advance of replication forks relies on DNA damage tolerance (DDT) mechanisms, which facilitate fork bypass across the lesions and repair of the ssDNA fragments generated during this process. One of these mechanisms is Homologous Recombination (HR), which uses the information of an intact template to repair DNA breaks. We have recently shown that the recombination proteins Rad52 and Rad51 contribute to tolerate replicative DNA damage through replicative and repair activities, which are regulated during the cell cycle. In S phase, Rad52 and Rad51 travel with the fork and facilitate replication bypass across the lesion by unknown mechanisms. Repriming of the DNA synthesis downstream of the lesion leaves Rad52 and Rad51 loaded at the ssDNA lesions left behind the fork, which are repaired at HR centers formed in G2/M (González-Prieto et al. 2013, EMBO J 32:1307). Here, we report a role for the kinase Cdc7 in promoting the loading/stabilization of Rad52 to the ssDNA lesions generated upon treatment with the alkylating agent methyl-methane sulfonate (MMS). This effect is partially mediated by phosphorylation of Mcm2. In addition, we show that Rad53, which requires the kinase activity of Cdc7 for full activation, is also required for Rad52 binding to DNA damage. Notably, Mec1 does not share this function. In addition, we have observed that the histone chaperone Asf1, which forms a complex with Rad53, is also required for Rad52 binding to replicative DNA damage. This role is independent of histone H3K56 acetylation and chromatin assembly, but also of the interactions between Asf1 with Rad53 and histones. We are currently exploring the mechanisms by which Cdc7, Rad53 and Asf1 stabilize Rad52 at replicative lesions during S phase.

Keywords: DNA damage, DNA repair.

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WED-288

Combined Wee1 and Rad51 inhibitor treatment decrease repair of double-strand breaks, survival and alters cells cycle of irradiated T-leukemia cells

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Aim: To evaluate cytotoxic and apoptosis-inducing effects of ionizing radiation, specific inhibitor of Wee1 kinase 681641, novel inhibitor of Rad51 recombinase RI-1 and its combination on Jurkat (p53 deficient) and MOLT-4 (p53 wild-type) leukemic cell lines. We also aim to clarify basic cellular and molecular mechanisms involved in ionizing radiation induced double-strand breaks (DSBs) repair in presence or absence of examined inhibitors.

Methods: In this work, we measured the mitochondrial dehydrogenase activity to quantify survival of Jurkat and MOLT-4 cells. Flow cytometry based methods were used to assess the effect of the two inhibitors on cell cycle and apoptosis quantified by means of Annexin V-FITC and propidium iodide dual staining. Immunofluorescence stained gammaH2AX positive cells were detected 24 h after irradiation to semiquantitatively analyse delayed DSBs repair by epi-fluorescence microscopy under low magnification. Visual aspects of persistent gammaH2AX foci with 53BP1 colocalization were captured by high-resolution imaging using confocal microscope. Expressions of selected proteins involved in DNA repair and cell cycle arrest were detected by Western blot.

Results: Combining Wee1 681641 and Rad51 RI-1 with ionizing radiation significantly reduced the cell survival for both Jurkat and MOLT-4 cells. Pre-treatment with Wee1 681641 or Rad51 RI-1 inhibitor alone increased the sensitivity of Jurkat cells to the irradiation, however combining both inhibitors together resulted in further enhancement of apoptosis. Jurkat cells pretreated with inhibitors were positive for gammaH2AX foci 24 h upon irradiation. MOLT-4 cells were less affected by inhibitors application followed by ionizing radiation exposure. Pre-treatment with Rad51 RI-1 had minimal effect on increased apoptosis induction; however Wee1 681641 increased ionizing radiation-induced cell death in MOLT-4. When dosed together, the combination had additive effect on MOLT-4 cell death.

Conclusion: This work suggests that further investigation of Rad51 inhibitors in combination with Wee1 inhibitors as potential anticancer therapy sensitizers preferentially for p53-deficinet cancer cells is warranted.

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Keywords: Apoptosis, Rad51 inhibitor II RI-1, Wee1 kinase inhibitor II 681641.

WED-289

Comparison of metabolome and proteome profiles of *E. coli* lacking HU protein

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HU is a nucleoid associated protein (NAP) that is present in every bacterial species. HU binds non-specifically to duplex DNA with a particular preference for targeting nicked or bent DNA. HU deletion is lethal for the majority of bacteria. Although *E.coli* lacking HU is viable, it is sensitive to a variety of stresses.

The effects of the HU protein on gene expression are known from microarray analysis. Absence of this DNA architectural protein causes a disorder in *E.coli* gene regulation. On the other hand, *E.coli* growth at standard conditions is almost unaltered in the absence of HU. To understand how bacteria withstand the chromosomal disorder we performed a proteomic analysis to compare *E.coli* lacking HU with wild-type *E.coli*. Comparison of the proteomic profiles was performed using SWATH acquisition on QqQ-TOF 5600 AB-Sciex mass-spectrometer. Among the proteins up-regulated in *E.coli* lacking HU are the proteins responsible for amino acid biosynthesis aminoacid-tRNA ligases as well as proteins, whose expression is altered by aerobic/anaerobic conditions. Proteins responsible for the stresses are usually down-regulated in the absence of HU.

In this study we also compared metabolomes of wild-type *E. coli* and *E. coli* lacking HU protein using QTOF mass spectrometry. Here we present reliable measurement of a hundred of metabolites. Detected changes in the E. *coli* metabolome profile caused by the lack of HU correlate well with the changes in protein content observed in proteomic studies.

Combined results of comparative metabolome and proteome studies show that oxygen consumption, protein and amino acid biosynthesis, and stress adaptation are the most altered functions in *E. coli* lacking HU protein.

The work was supported by the RFBR (grant 13-04-12084).

Keywords: HU protein, metabolome, proteome.

WED-290

Conformational changes and unzipping of DNA by surface modified gold nanoparticle

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Damage to DNA in biological environment has been associated with alterations in DNA sequence. In the repairing process of this kind of damage, DNA unzipping or DNA unwinding is an important step. In biological system this type of unzipping is carried out by specific enzymes or heliceses. Besides that, some of non-biological complexes also show this kind of unzipping properties.

Adsorption of DNA on the surface of gold nanoparticle is a common phenomenon. This was a remarkable observation as in this case DNA and goldnanoparticle both were negatively charged. So by virtue of the electrostatic charge both the particle should repel each other that can be overcome by the use of salts. Here we have shown the interaction between dsDNA with gold nanoparticle, surface modified with L-cysteine, in biological condition. DNA-modified AuNP interaction causes a conformational change as well as unzipping of the DNA double strands. This unique observation was studied by the help of circular dichroism (CD) spectrophotometer, UV-Vis and Fluorescence techniques. It

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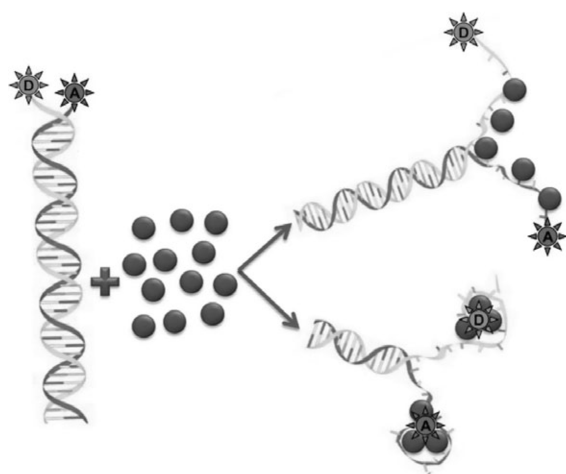

Fig. 6: Possible Mechanism of interaction of AuNPs to dsDNA.

was also supported by time resolved fluorescence and single-molecule fluorescence microscopic techniques.

The above results showed that in presence of AuNPs, surface modified with L-cysteine, DNA unzipping happening in case of both long as well as short DNAs. These AuNPs, having surface with positively charged, interacts with the phosphate group of the dsDNA and breaks the H-bonding responsible for DNA double strand formation. The actual mode of interaction still under investigation, but the possible way of interaction was proposed in the Figure.

Keywords: Dna unzipping, Goldnanoparticle.

WED-291
Contribution of multifunctional protein YB-1 in the BER pathway during oxidative DNA lesions repair

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Aim: Single oxidative DNA lesions (such as abasic sites, single-stranded DNA breaks, oxidized bases) and their combinations that are referred to as oxidative clustered DNA lesions – OCDLs are generally corrected by base excision repair pathway (BER). During BER, the damaged DNA strand is cleaved by AP endonuclease APE1 or bifunctional DNA glycosylases like NEIL1 that results in nicked intermediate. Due to this fact, repair of abasic sites located within bistranded OCDLs or single-stranded DNA regions is closely connected with the risk of high cytotoxic DNA double strand breaks formation. Pleiotropic protein YB-1 is extensively studied in the context of cellular response to genotoxic stress, but its unmediated role in DNA repair is still open to question. The aim of present research was to investigate multifunctional protein YB-1 influence on repair of abasic sites located within oxidative clustered DNA lesions (OCDLs) or single-stranded DNA fragments.

Methods: In present work bistranded OCDLs in DNA were imitated by combination of abasic site and 5-formyluracil (potential product of thymine oxidation) in opposite DNA strand. YB-1 interaction with oxidative DNA lesions was determined by $NaBH_4$-mediated crosslinking. YB-1 influence on major components of BER machinery was performed by functional studies and gel retardation assays. Posttranslational modification of YB-1 was analyzed by Western-blot analysis and Coomassie staining. Results

The data obtained demonstrated that YB-1 directly interacts with abasic site and 5-formyluracil in DNA via intermediate Schiff base formation. YB-1 was shown to promote APE1- and NEIL1-depending cleavage of either single abasic sites or AP sites within OCDLs. YB-1 influence on APE1 occurs during substrate-binding step. Presence of high YB-1 concentrations resulted in blocking APE1-depending pathway via inhibition of APE1 activity as well as polβ dRP lyase activity. With AP sites being in the single-stranded DNA regions, YB-1 inhibited both APE1 and NEIL1 activities. In vitro studies revealed a possibility of a novel posttranslational modification of YB-1 that could influence on its interaction with DNA.

Conclusions: Pleiotropic protein YB-1 directly interacts with AP sites and 5-formyluracil within DNA and possesses the capability of modulating of APE1- and NEIL1-depending AP site cleavage. YB-1 is able to go through posttranslational modification that can affect the role of YB-1 in DNA repair.

This work was supported by grants from Russian Foundation for Basic Research (14-04-00268, 12-04-00337), MCB program, grant from Ministry of Science and Education for Scientific School – 420.2014.4.

Keywords: BER, OCDLs, YB-1.

WED-292
Crosstalk of repair mechanisms and replication in pluripotent stem cells

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Human embryonic stem cells (hESC) may present a breakthrough in regenerative medicine. In order to obtain enough cells for possible cell replacement therapy or research it is necessary to maintain hESC in *in vitro* cultivation. Long term propagation of hESC is associated with genome mutagenesis. To defend their genome hESC possess elevated mechanisms that repair DNA damage and errors. Some of the repair mechanisms such as base excision repair (BER), however elevated in early passage, are decreasing with prolonged cultivation. The BER seems to be involved in double strand break (DSB) release when dealing with clustered damage. DSBs can further be repaired either by homologous recombination (HR) or non homologous end joining (NHEJ). HR, being considered error-proof, is usually active when sister chromatid is present in the cell while NHEJ, the believed error-prone mechanism, ligates DSBs′ ends throughout the whole cell cycle. Thus the pathway choice seems to be one of the crucial moments in genome stability maintenance. Our results suggest that BER plays an important role in DSBs′ repair pathway choice due to DSBs released upon BER repair of clustered oxidative damage. Subsequently the BER-mediated DSB-damage released after ionizing radiation is repaired by the cells' repair machinery within 45 minutes. That suggests that NHEJ is involved in amelioration of the damage. Moreover, inhibition of BER results in replication stress-coupled HR shown by elevated Rad51 foci and sister chromatid exchange (SCE). Interestingly, the mutation frequency (MF) is significantly elevated in the absence of the BER-mediated DSB release, when cells activate their replication stress coupled HR. Contrary to generally accepted model, we propose that hESC employ NHEJ or its subpathways to ameliorate the BER mediated DSB damage in order to minimize the mutagenesis of their genome.

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Keywords: DNA repair, stem cells.

WED-293
Dissecting NUP98 function in human leukaemogenesis

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Acute myeloid leukaemia (AML) is a heterogeneous disease characterized by uncontrolled proliferation of neoplastic haematopoietic precursor cells, which leads to the disruption of normal haematopoiesis and bone marrow failure. Impaired haematopoiesis is often associated with balanced chromosomal translocations that involve the nucleoporin Nup98 fused to more than 30 different partner genes, such as the homeobox genes, HoxA9 and PMX1. Nup98-associated AML is characterized by the poor prognosis and poor treatment outcome for the patients. Our aim is to better understand the underlying molecular mechanism that leads to chemotherapy-resistance and we found that the expression of Nup98-fusion proteins cause versatile alterations in nuclear organization, including changes in chromatin organization. As changes in DNA topology might affect DNA damage response and as chemotherapeutic drugs are typically DNA double-strand break (DSB) causing agents, we investigated whether the Nup98-fusions alter DSB break repair pathways. To do so, we studied the impact of the Nup98-fusions on exogenously induced DNA DSB repair by treating cells with the topoisomerase II inhibitor etoposide (ETO) or the γ-radiation mimicking agent neocarzinostatin (NCS). We found that cells expressing Nup98-fusion proteins show less DSB foci as compared to control cells. This suggests that the Nup98-fusions render the cells less sensitive towards ETO- and NCS-induced DSB, or that early events of the DSB repair pathways that allow foci formation are inhibited in presence of Nup98-fusion proteins. Experiments to further distinguish these two possibilities are under way, as well as the analysis, which part or specific domain of the fusion proteins is contributing to the altered DSB repair mechanism.
Keywords: Acute Myeloid Leukaemia, DNA repair, Nuclear pore complex.

WED-294
DNA mismatch repair in *Neisseria gonorrhoeae*: beyond the *E.coli* paradigm

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Neisseria gonorrhoeae causes gonorrhoeae, a debilitating disease and the continuous appearance of new drug-resistant strains is fast rendering standard treatments ineffective. The development of an effective prophylactic vaccine has been hindered by the fact that this organism exhibits phase variation. Successful phase variation is dependent on regulating mismatch repair (MMR) pathway in appropriate manner. In *E. coli*, the specific components of MMR are MutS, MutL and MutH. Of these proteins, MutH is a site specific endonuclease and plays a vital role in strand discrimination and creation of a nick in the daughter strand. *Neisseria gonorrhoeae* lacks a homolog of MutH and therefore, it is expected that it will show significant differences in MMR pathway especially in the strategies utilized for strand discrimination and nick-creation. The key question is how recognition of a mismatch on duplex DNA by NgoS is coupled to nicking activity by homodimeric NgoL directed towards the newly synthesized strand. Our overall strategy towards this end includes detailed biochemical charaterization of the various proteins involved in MMR (MutS, MutL, β-clamp, RecJ, UvrD helicase) and determining structures of the components of MMR in their apo- and functional states.

All the components of MMR have been purified to high homogeneity and a rigorous structural and biochemical characterization of these proteins is currently ongoing. Single crystals of MutS in its apo-state shows that MutS exists in the form of a dimer but there are significant differences(as compared to the functional complexes of MutS from *E.coli* and *T.aquaticus*) in the manner in which the two monomers associate to form a dimer. Overall, the structure of NgoS in the apo-state reveals how a functional MutS-DNA complex is assembled. In addition, we see that MutL interacts with β -clamp through its C-terminal domain (CTD). The nicking endonuclease activity is resident in this CTD of MutL and is dependent on the integrity of a conserved metal binding motif. We had earlier shown that the CTD *Neisseria gonorrhoeae* MutL acts as a nicking endonuclease and exists in the form of an elongated inverted homodimer placing the two composite active sites in each subunit on opposite lateral sides of the homodimer. Due to this arrangement, only of the active sites is presented to DNA, thereby preventing the creation of a double stranded break. Overall, our efforts have shed more light on how the functional mismatch repairosome is assembled and the repair reaction initiated.
Keywords: mismatch repair.

WED-295
Formamidopyrimidine-DNA glycosylase from *E.coli*: new mechanistic details of the enzyme activity

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Formamidopyrimidine DNA glycosylase (Fpg) from Escherichia coli is one of the main tools protecting the bacterial genome from lesions caused by environmental and internal insults. Fpg initiates base excision repair in DNA by removing oxidized nucleobases. A major DNA oxidation product is 8-oxoguanine (8-oxoG), a base with a high mutagenic potential. We have used kinetic and thermodynamic approaches to analyze 8-oxoG recognition and excision by Fpg. First, we have found that the energy of stacking interactions of 8-oxoG was in strict dependence on nucleotide environment, which may affect lesion recognition and the efficiency of eversion of 8-oxoG from the double helix by DNA glycosylases. In addition, we observed that the presence of the lesion affects the kinetics of association of oligonucleotides into a duplex. Using site-directed mutagenesis, we have addressed the functions of many previously unstudied amino acid residues that were predicted to be important for Fpg activity and structure maintenance. Of note, many substitutions abolished the excision of 8-oxoG but did not affect the cleavage efficiency of abasic substrates. Finally, we investigated the contribution of separated structural domains of Fpg to specific enzyme-substrate interactions. Surprisingly, despite the absence of the catalytic domain, C-terminal domain of Fpg possessed a low residual ability to recognize and cleave abasic substrates. This is can shed light on unknown functions of truncated Fpg homologs from Mycobacte-

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rium tuberculosis. Our study uncovers new mechanistic details of Fpg activity, allowing us to consider Fpg as a promising scaffold for applied nanotechnology and molecular medicine.

Keywords: 8-oxoguanine, DNA glysosylase, DNA repair.

WED-296

Functions of WT Apn1 from *Saccharomyces cerevisiae* and its H83A mutant involved in BER and NIR pathways

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Apurinic/apyrimidinic endonuclease from *Saccharomyces cerevisiae* Apn1 is one of the key enzymes involved in base excision (BER) and nucleotide incision (NIR) DNA repair pathways. In BER Apn1 is mainly involved at the stage of cleavage of abasic sites, which are abundant and very genotoxic DNA damages. In NIR Apn1 is known to incise phosphodiester backbone 5′ to the damaged nucleotide (Ishchenko A. A., et al. 2003). In view of unresolved spatial structure of Apn1 from *S. cerevisiae*, based on the high aminoacid homology of yeast Apn1 to *E. coli* Endo IV, His-83 is believed to coordinate one of three Zn atoms in the active site of Apn1 like His-69 in Endo IV (Hosfield D. J., et al. 1999; Garcin E. D., et al. 2008). Substitution of His-83 for Ala is proposed to decrease AP endonuclease activity of the enzyme owing to weak coordination of Zn ion involved in catalysis. The kinetic analysis of the interactions of Apn1 and its mutant form with DNA should shed light on the key points of recognition and catalysis, namely, the role of conformational dynamics of the reactants, the participation of His-83 in metal ions coordination, etc.

In this study, kinetics of the interaction of WT and H83A Apn1 with DNA substrates was investigated. A stopped-flow method with the detection of fluorescence intensity of 2-aminopurine located adjacent to the damage allows conformational dynamics of DNA to be monitored. Substitution of His-83 for Ala was found to influence substrate binding and catalysis of the mutant Apn1. The location of 2-aPu relative to the damage was shown to have no influence on substrate cleavage by WT Apn1; but in the case of Apn1 H83A location of 2-aPu is essential. The experimental data analysis reveals that not only the catalysis is influenced by the location of fluorophor in DNA but also by the nature of damages. The initial conformation of double-stranded nucleic acid is of great importance for the formation of the proper enzyme-substrate complex in the case of H83A mutant because of weak coordination of active site Zn ion.

The work is supported by grants from the Program of the Russian Academy of Sciences "Molecular & Cell Biology" (6.11), the Russian Foundation for Basic Research (RFBR 13-04-00013, 14-04-31201, 14-04-31448 and 14-04-00806), the Russian Ministry of Education and Science (SS-1205.2014.4), the Russian Scientific Foundation (RSCF 14-14-00628).

Keywords: AP endonuclease, DNA repair, pre-steady-state kinetics.

WED-297

Genotoxic effects of different sizes of silica nanoparticules in rat lymphocyte cells

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Silicium dioxide nanoparticles (SiO_2NPs) are widely used in daily life. Toxic effects of these nanoparticules to living systems are gaining attention. In this study, we investigated the genotoxic effect of different sizes of SiO_2NPs in Wistar rat lymphocyte cells.

Thirty-five healthy male Wistar albino rats weighing 150–250 g (5-6 weeks of age) were used in this study. The experimental design of the study was conducted according to the international validation of the in vivo rodent alkaline comet assay for detection of genotoxic carcinogens for evaluating potential genotoxic effect of different size of SiO_2NPs (6, 20, 50 nm, 150 μg/mL) in case of chronic treatment (28 days). Rats were equally divided into five groups (n = 7 rats) which were treated with SiO_2NPs in physiological serum intraperitoneally in a volume of 1 mL. Group 1 was the vehicle (physiological serum) treated group. Group 2 was administered low particule size SiO_2NP (6 nm, 150 μg/mL/day). Group 3 was treated with medium particule size SiO_2NP (20 nm, 150 μg/mL/day). Group 4 received high particule size SiO_2NP (50 nm, 150 μg/mL/day) once a day throughout 28 days of the study for chronic treatment. Group 5 was positive control group (n = 7 rats), ethylmethane sulfonate was dissolved in physiological serum and administred intraperitonally 200 mg/kg BW, 24 h before sacrifice. Blood were sampled from the all groups for genotoxicity assessment. The alkaline comet assay was performed. After the staining process, a hundred cells were analysed using double slides. For quantification of DNA damage, comet tail length, tail intensity and tail moment were measured and calculated by the Comet Assay III image analysis system.

Tail length significantly decreased in 20 nm and 50 nm size groups and tail moment significantly decreased only 20 nm size group in comprasion to positive control group ($p < 0.05$). There was significant decrease between the 6 nm nanoparticule size group and 20 nm and 50 nm nanoparticule size groups ($p < 0.05$) for tail intensity and tail moment.

The results of DNA damage obtained in the comet assay indicated genotoxic potential of SiO_2NP in lymphocyte cells. This genotoxic potential is dependent on particle size.

Keywords: comet assay, genotoxicity, Silicium dioxide.

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WED-298
Human DNA polymerases catalyze lesion bypass across benzo[a]pyrene-derived DNA adduct in cluster of DNA lesions

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The genome is continuously affected by a variety of endogenous and exogenous factors that damage genomic DNA. The combined action of oxidative stress and genotoxic polycyclic aromatic hydrocarbons derivatives can lead to cluster-type DNA damage that includes both a modified nucleotide and a bulky lesion. As an example, we investigated the possibility of repair of an AP site located opposite a minor groove–positioned (+)-*trans*-BPDE-dG or a base-displaced intercalated (+)-*cis*-BPDE-dG adduct (BP lesion) by a BER system. Oligonucleotides with single uracil residue in certain position were annealed with complementary oligonucleotides bearing either a cis- or trans-BP adduct. Digestion with uracil DNA glycosylase was utilized to generate AP site which was then hydrolyzed by APE1, and the resulting gap was processed by X-family DNA polymerases β (Polβ) and λ (Polλ) or Y-family polymerase ι (Polι). By varying reaction conditions, namely, Mg^{2+}/Mn^{2+} replacement/combination and ionic strength decrease, we found that in the certain conditions both Polβ and Polι can perform lesion bypass across both *cis*- and *trans*-BP adducts in the presence of physiological dNTP concentrations. Polβ and Polι catalyzed gap filling trans-lesion synthesis in the error prone manner. In the contrast, Polλ introduced selectively correct dCTP opposite the modified dG in the case of *cis*-BP-dG adduct only and did not bypass the stereoisomeric *trans*-adduct in any conditions used in the study. The data obtained can suggest Polλ as a specialized polymerase in the process of repair of this kind of lesions.

This work was supported by RFBR (14-04-00268, 13-04-00538); grant of scientific school (420.2014.4).
Keywords: benz[a]pyrene, DNA repair, TLS.

WED-300
Influence of cytokines with anticancer activity on MGMT gene expression in human cell lines

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Repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT) eliminates O^6-methylguanin adducts in DNA and protects normal cells from damaging effects of alkylating agents. At the same time MGMT makes tumor cells resistant to alkylating drugs. Therefore in medicine MGMT is considered as a target which needs to be regulated. Interferon alfa 2b (IFN-α2b) and endothelial monocyte-activating polypeptide (EMAP) II were chosen by us for investigating their possible influence on *MGMT* gene expression.

The aim was to study the effect of IFN-α2b and EMAP II and their medical forms on *MGMT* gene expression in human cell lines.

Materials and Methods: Such cell lines were used: Hep-2 (laryngeal cancer); A102 (skin fibroblasts), provided by J.McCormick, USA; 4BL (fibroblast-like cells), obtained in our laboratory has MGMT-deficient phenotype. Such agents were used: recombinant protein IFN-α2b, IFN-α2b-containing preparations Laferon (Interpharmbiotech) and Laferobion (Biofarma), EMAP II and nanocomposite protein EMAP II (NCP EMAP II) provided by A.I.Kornelyuk, Ukraine. *MGMT* gene expression was analyzed at a protein level by Western blotting.

Results: *MGMT* gene expression was increased by EMAP II treatment at concentrations 0.2 and 0.02 μg/ml in Hep-2 cells. In MGMT-deficient 4BL cells EMAP II at a concentration 2 μg/ml induced *MGMT* expression. Increase of EMAP II dose to 2 μg/ml and 10 μg/ml for Hep-2 and A102 cells respectively resulted in complete inhibition of this expression. NCP EMAP II, unlike recombinant protein EMAP II, in a wide concentration range (0.002–2 μg/ml) did not induce *MGMT* gene expression in 4BL cells. We suppose that the cytokine activity may be affected by salt compounds of NCP EMAP II.

Recombinant protein IFN-α2b and Laferon with activity of 200 and 2000 IU/ml were shown to cause slight increase of *MGMT* gene expression in tumor Hep-2 cells. But Laferobion with the same activity on the contrary decreased the level of *MGMT* gene expression in 3 and 8 times respectively in Hep-2 cells. A102 cells were treated only with Laferon preparation with activity of 20 IU/ml and expression of *MGMT* gene was completely inhibited in such conditions. In 4BL cells we didn't observe any changes of *MGMT* gene expression after the treatment with IFN-α2b and IFN-α2b-containing preparations.

Conclusions: EMAP II influence on *MGMT* gene expression depending on the concentration while NCP EMAP II did not show any effects. The effect IFN-α2b and IFN-α2b-containing preparations depended on their compositions and type of cell lines.

Keywords: MGMT, IFN-α2b, EMAP II.

WED-301
Interaction of Ddc1 and RPA with single-stranded/double-stranded DNA junctions in *Saccharomyces cerevisiae* whole cell extracts

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DNA lesions and structural alterations induce binding of repair and checkpoint proteins to site of DNA damage to initiate DNA damage response. Checkpoint sensor proteins act at the early steps of the checkpoint response that involves primary detection of DNA damage. The proteins recognize DNA repair/replication intermediates containing single-stranded DNA (ssDNA), double-stranded/single-stranded (ds/ss) DNA junctions, gaps or specific DNA-protein complexes such as ssDNA coated by replication protein A (RPA). To characterize proteins that interact with single-stranded/double-stranded (ss/ds) DNA junctions in whole cell free extracts of Saccharomyces cerevisiae, we used [^{32}P]-labeled photoreactive partial DNA duplexes containing a 3′-ss/ds-junction (3′-junction) or a 5′-ss/ds-junction (5′-junction). Identification of labeled proteins was achieved by MALDI-TOF mass spectrometry peptide mass fingerprinting and genetic analysis. In wild-type extract, the checkpoint sensor protein, Ddc1, was found to be photocrosslinked at a 3′-junction independently of the two other components of the (Ddc1-Mec3-Rad17) checkpoint clamp. On the other hand, the large subunit of the replication protein A (RPAp70) was the predominant crosslinking product at a 5′-juction. Interestingly, ddc1Δ extracts did not display photocrosslinking of RPAp70 at 5′-junction. The results show

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that the RPAp70-DNA covalent product at a 5′-junction is subject to limited proteolysis in ddc1Δ extracts, whereas it is stable in WT, rad17Δ and mec3Δ extracts. The degradation of the RPAp70-DNA adduct in ddc1Δ extract is reduced in the presence of the proteasome inhibitor MG 132. We also addressed the question of the stability of RPA not covalently attached to DNA using anti-RPA antibodies. The data show that RPAp70 is also subject to proteolysis without photocrosslinking to DNA upon incubation in ddc1 extract. The data point to a novel property of Ddc1, modulating the degradation of DNA binding proteins such as RPA by the proteasome, which in turn could be used to regulate biological processes in yeast.

The work was supported by RFBR (13-04-40197, 14-04-00268), RAS Presidium Program "Molecular and Cell Biology", grant for Leading Scientific Schools (420.2014.4), CNRS and CEA.

Keywords: checkpoint proteins, replication protein A, DNA junctions.

WED-302
Investigation of the cytotoxic mechanicms induced by airborne particulate matter on human lung cells (MRC-5)

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The capacity of the airborne particulate matter to induce cytotoxic responses in human lung cells (MRC-5) cultures was investigated by testing aqueous and organic solvent extracts of different particle size fractions (d < 0.49, 0.49-0.95, 0.95-3, 3-7.2, and >7.2 μm) [1] for their biological impact in terms of DNA damage of cells and of levels of poly (ADP)ribosylation to elucidate the cytotoxic mechanism. Poly(ADP-ribose) polymerase 1 (PARP-1) is a zinc-finger DNA-binding enzyme that is activated by binding to DNA breaks. The enzyme is a global monitor of chromatin structure and DNA damage repair. Poly(ADP-ribosyl) ation of nuclear proteins by PARP-1 converts DNA damage into intracellular signals that activate either DNA repair by the base-excision pathway or cell death. In this study chromosomal DNA fragmentation was monitored in MRC-5 cells exposed to these particles by agarose gel electrophoresis of the isolated DNA and by single cell gel electrophoresis (Comet assay). The levels of PARP-1 were studied on MRC-5 cells extracts after 72 h of exposure to these particles to study a possible induced apoptosis mechanism using the universal colorimetric PARP assay kit. Most samples showed an increase of the PARP activity. Both Dot and Western blot analysis were performed to confirm PARP activity using anti-PARP monoclonal antibody (clone C2-10; Trevigen). During apoptosis, PARP activity (115 kDa) is increased triggering apoptotic mechanicms but later falls due to auto-modification and cleavage by caspases. Specific proteolytic cleavage of PARP constitutes a reliable marker for apoptosis in a wide variety of cell types, generating 85 kDa (including the catalytic and NAD-binding domain) and 26 kDa fragments, which corroborate with study.

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Fig. 1.

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Keywords: DNA damage, PARP1.

WED-303
Ku80 interaction with apurinic/apyrimidinic sites depends on the structure of DNA ends

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Apurinic/apyrimidinic (AP) sites are among the most frequent DNA damages. Of particular interest is the repair of AP sites in clustered DNA damages presented by combinations of AP sites, oxidized bases and single strand breaks within 1–2 turns of DNA helix. Such lesions arise in DNA under the action of ionizing radiation or radiomimetic drugs.

The aim of this study was to identify a human cell extract protein which specifically interacts with the AP site in the partial DNA duplex containing both 5′- and 3′-dangling ends of 8 nt and mimicking clustered DNA damage (DDE-AP DNA). The methods included the Schiff base-dependent cross-linking of proteins to AP DNA (borohydride trapping) in combination with gel electrophoresis and MALDI-TOF-MS.

The human cell extract protein which forms a major covalent adduct with an apparent molecular mass of 100 kDa with DDE-AP DNA was identified as Ku80 subunit of Ku antigen by peptide mass mapping based on MALDI-TOF-MS data. Ku antigen consisting of two subunits with molecular masses about 70 kDa (Ku70) and 83 kDa (Ku80) is a eukaryotic DNA-binding component of DNA-dependent protein kinase. The main function of Ku antigen is participation in the double-strand break repair by nonhomologous end joining. We have previously identified Ku antigen as a protein forming the predominant product of cross-linking with an apparent molecular mass of 90 kDa with the blunt-ended AP DNA duplex (Ilina et al. 2008).

The appearance of the Ku80 adduct with a lowered electrophoretic mobility characteristic for DDE-AP DNA can reflect the existence of two different modes of Ku80 binding with DNA or the highly efficient cross-linking of DNA with another Ku80 isoform. The Ku antigen purified from the HeLa cell extract was shown to form the covalent adducts with the same mobility as observed in cell extracts. Thus, Ku80 subunit of Ku antigen can specifically interact with AP DNA forming the Schiff base-mediated adducts, their electrophoretic mobility depending on the structure of DNA ends. The difference in electrophoretic mobility can be caused by the cross-linking of AP DNA to distinct target amino acids that appears to reflect unequal positioning of AP DNAs in the complex with Ku antigen.

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Keywords: Apurinic/apyrimidinic site, DNA repair, Ku antigen.

WED-304
Loss of SOD1 is connected to yeast selenite resistance

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Oxidative stress can lead to reactive oxygen species (ROS) leading to DNA damage and genomic instability. Selenium (Se) is a trace element that is essential for human health, but unbound Se can generate ROS. In addition, inorganic selenium (*sodium selenite*; Na_2SeO_3) may serve as an anticancer agent because of its toxic properties. Based on selenite toxicity, yeast has been used as a tool to study cellular mechanism(s) involved in selenite detoxification. E.g. yeast mutants devoid of DNA repair activities or antioxidant molecules like glutathione (GSH) have been shown to be hypersensitive to selenite. *CYS3* codes for a cystathionine gamma-lyase, which is essential for the synthesis of the GSH precursor cysteine. Surprisingly, yeast mutants lacking Cys3 are hypersensitive to selenite even if excess levels of cysteine are provided in the medium. In this work, we identified *sod1Δ* being a suppressor of *cys3Δ* selenity hypersensitivity. Interestingly, *SOD1* encodes for a superoxide dismutase needed for the tolerance to ROS by the conversion of ROS ion superoxide (O_2^-) into oxygen (O_2) and hydrogen peroxide (H_2O_2), and we therefore questioned, how *sod1Δ* mutants acquired selenite resistance. We will present a model how selenite suppression can be archived in *sod1Δ* mutants pointing to a complex interplay between mechanisms involved in oxidative stress response.

Keywords: ROS, Sod1, Sodium Selenite.

WED-305
Lys/Asp functionality in DNA glycosylases of HhH-GPD structural superfamily

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The common feature of DNA glycosylases of HhH-GPD structural superfamily is a helix-hairpin-helix (HhH) motif followed by a Gly/Pro-rich loop and terminating in an Asp residue, which is entirely conserved in these enzymes. The monofunctional glycosylases of this superfamily AlkA and MutY use the Asp residue as the catalytic nucleophile. In the cases of hOGG1 and EndoIII, which are bifunctional DNA glycosylases, a lysine is present in the active site. Presumably, the catalytic mechanism of bifunctional DNA glycosylases involves an attack on C1′ of the damage-containing nucleotide by the ε-amino group of lysine that leads to the cleavage of the N-glycosidic bond, producing an abasic site (apurinic/apyrimidinic site, AP-site). The incision of the DNA at the 3′-side of AP-sites proceeds via Schiff base formation and subsequent β-elimination reaction leaving a 3′-α,β-unsaturated aldehyde and a 5′-phosphate. The functional role of the invariant Asp residue in the bifunctional glycosylases is not fully understood. Recently it was shown (Dalhus B et al 2011) that Asp can be involved in base excision catalysis in the double mutant K249C C253K of hOGG1. Our previous data (Kuznetsova et al 2014) suggest that in hOGG1 the Lys-249 residue directly participates in the process of DNA distortion and the flipping out of the oxoG base.

Here we examined the role of Lys and Asp residues in mono- and bifunctional DNA glycosylases and described their involvement in the enzymatic pathway of N-glycosidic bond cleavage and incision of the DNA backbone. The conformational dynamics of mutant forms of hOGG1, EndoIII, AlkA and MutY, bearing mutations of Asp and Lys, as well as conformational dynamics of DNA-substrates, were investigated by the fluorescence stopped-flow method. The obtained data illustrate that the mechanisms of action of mono- and bifunctional DNA glycosylases are quite similar, so that a single amino acid change is enough to convert a monofunctional glycosylase to a bifunctional glycosylase/AP lyase and *vice versa*.

This work was supported by the Program of the Russian Academy of Sciences “Molecular & Cell Biology” [6.11]; the Grants from Russian Foundation for Basic Research [13-04-00013, 14-04-00531 and 14-04-31174]; the Grant from Russian Scientific Foundation [14-14-00063] and Ministry of Education and Science [SS-1205.2014.4, SP-4012.2013.4].
Keywords: Base excision repair, DNA glycosylase, catalytically important amino acids.

WED-306
Mechanisms of UVA-induced DNA single-strand breaks and alkali-labile sites formation

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The aim of this work was to examine mechanisms of single-strand breaks (SSB) and alkali-labile sites (ALS) DNA of human blood lymphocytes under exposure to 365-nm UV radiation in vitro. We used the blood of five healthy donors aged 21-26 years. For irradiation was used Bio-Link system BXL-365 giving the dose rate of 2.92 kJ/m^2 at 1 min at 4°C. The modified comet assay under alkali conditions was performed for analysis of SSD and ALS. Exposure of 365-nm UV-radiation at doses of 10-50 kJ/m^2 (nucleoids) or 5-20 kJ/m^2 (lymphocytes) induces a dose-dependent increase SSB and ALS DNA detected by comet-assay. Adding 10% dimethyl sulfoxide (DMSO, scavenger of HO·radicals) reduces the SSB and ALS yields ~ in 3 times. It is shown that the SSB and ALS yields in human blood lymphocytes under exposure to 365-nm UV radiation at dose of 10 kJ/m^2 correspond effect of X-ray at dose of ~ 1 Gy. Effect of incubation in NaCl hypertonic solutions (0.2, 0.35 and 0.5 M for 1 h at 4°C) on the SSB and ALS DNA yields induced by long-wave UV-radiation (365 ± 10 nm) in human blood peripheral lymphocytes in vitro was investigated. It was shown, that compared to the cell incubated in NaCl isotonic solution (0.14 M) statistically significant increases in the yields of both spontaneous (~ 2 times) and UV-A radiation induced (~ 1.7 times) DNA damages were observed only at a NaCl concentration of 0.5 M. It is assumed that at this concentration of NaCl dissociation of linker histone H1 occurs, is disrupted the structure of chromatin and dramatically increases the free radical-induced DNA damage output. Results have revealed significance of free-radical processes in formation of biological effects under UV-radiation influence.

Keywords: DNA damage, Lymphocytes, UVA.

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WED-307
Molecular dynamics and QM/MM free energy profiles of the reaction mechanisms in DNA N6 and C5 methyl transferases

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In this work we present a theoretical analysis of the mechanism for the reactions catalysed by M.TaqI and M.Hhai; enzymes that belong to the restriction-modification system of *Thermus Aquaticus* and *Haemophilus haemolyticus* respectively, which play and important role in the epigenetic regulation of gene function in vertebrates and carcinogenesis. We have studied the reaction consisting on the methyl transfer from S-adenosil-L-metionine (SAM) to N6 position of an adenine base generating N6-methyl-adenine, or to C5 position of a cytosine base of DNA leading to C5-mehtyl-cytosine.

The x-ray structure of the enzyme complexed with SAM and a DNA sequence was the starting point of our simulations. We performed all calculations considering the whole enzymatic and DNA environment, including the enzyme in an orthorhombic box of TIP3P water molecules with sodium counterions. Using the NAMD program we equilibrated the system by means of 10 ns of classical molecular dynamics (MD) with the AMBER force field, employing periodic boundary conditions, Ewald summations and a time step of 1 fs. The target temperatures were 333K for N6-MTase and 300K K for C5-Mtase, We then performed 100 ns of MD simulation in order to analyze the most important interactions formed between the enzyme and DNA, the interactions within the active site, how the unpaired base is stabilized and how the DNA helix accommodates the perturbation provoked by a flipped out base.

To analyse the chemical reaction we performed calculations using quantum mechanics/molecular mechanics (QM/MM) hybrid methodology using the fDynamo program. The quantum subsystem was treated using the AM1 semiempirical hamiltonian adding corrections at the M06-2x/6-311 + G** level. By means of the on-the-fly string method the minimum free energy path (MFEP) for each step of the reaction was obtained. Then, the path collective variable was defined along these paths, to obtain the potential of mean force (PMF) using umbrella sampling.

Our simulations show that for the N6-MTase the reaction takes place with methylation preceding N6-deprotonation through a water molecule and that the rate-limiting step is the methyl transfer. For the DNA-C5 MTase we unravelled the whole reaction mechanism which consists of five steps where the rate limiting step is the methylation. We have also performed free energy perturbation calculations to unravel which nucleophile is participating into the β-elimination step.

Our results agree with experimental data available and the experimental rate constants. The knowledge of these insights can lead to the design of new antibiotics and inhibitors for this family of enzymes.

Keywords: Enzyme catalysis, epigenetic modifications, QM/MM calculations.

WED-308
Nuclear basket nucleoporins ensure a correct accumulation of DNA damage response proteins at the site of DNA double strand breaks

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Nuclear pore complexes (NPCs) are large structure embedded in the nuclear enveloppe, which mediates the molecular exchanges between the cytoplasm and the nucleus of eukaryotic cells. Nucleoporins (Nups), proteins that are key components of the NPC, are reported to take part in a constantly growing number of functions that go beyond their role in nucleocytoplasmic transport. Along this line, Nup153, a component of the NPC's nuclear basket, has been shown to be involved in DNA double strand breaks (DSBs) repair. Here we show that not only Nup153, but also the two other components of the nuclear basket, Tpr and Nup50, have roles in DSB repair. We observed that human osteosarcoma U2OS cells lacking Nup153 and Tpr respectively, have a higher sensitivity to DSB-inducing agents, such as neocarzinostation (NCS), which coincided with alteration of both DSB repair pathways: non-homologous end joining (NHEJ) and homologous recombination (HR). Nup50 depletion, in contrast, leads to an inhibition of NHEJ, but not of HR. We furthermore found that the recruitment of 53BP1 (NHEJ specific) and BRCA1 (HR specific) to DSBs per se is not affected upon Nup153 and Tpr depletion, but that the kinetics of both repair pathways are altered. In particular, we observed an inefficient growth of the repair foci, indicating an inefficient accumulation of the repair factors at damage sites in the absence of Nup153 and Tpr, respectively. We provide evidence that this is due to SUMO-modification of 53BP1 and BRCA1.

Keywords: Double strand break foci, Nucleoporins, SUMOylation.

WED-309
Nuclear organization and chromatin status impact on homologous recombination efficiency and outcome

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The folding of eukaryotic genome in chromatin into the nucleus of cells can influence many genome functions, including genome stability. Genome integrity is insured by conserved DNA repair mechanisms among which Homologous Recombination (HR) that uses an intact homologous sequence to repair a broken chromosome. HR can be separated in two sub-pathways: Gene Conversion (GC) transfers genetic information from one molecule to its homologous and Break Induced Replication (BIR) establishes a replication fork than can proceed until the chromosome end. Using the budding yeast as a model, we characterized the contribution of chromatin status and 3D genome organization on DSB repair by homologous recombination.

Saccharomyces cerevisiae chromosomes are arranged in a Rabl-like configuration with their centromeres anchored to the spindle pole body and their telomeres grouped into 3 to 5 foci at the nuclear periphery. Telomeric foci form subnuclear compartments repressive for transcription in which the yeast heterochromatin factors (SIRs silent information regulators) concentrate. This organization can be modified through the overexpression of

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the Sir3 or sir3A2Q mutant proteins that both lead to the grouping of the majority of telomeres into a "hypercluster" localized into the interior of the nucleus. Whereas this clustering occurs in the absence of heterochromatin following sir3A2Q overexpression, Sir3 overexpression results in heterochromatin spreading along the subtelomeric regions. We used Sir3 and sir3A2Q overexpression to modify the physical distances between telomeres and/or the spreading of heterochromatin in subtelomeric regions and measured ISceI induced recombination rates between recombination cassettes positioned at various locations in the genome.

We observed that increasing spatial proximity between subtelomeres through sir3A2Q overexpression increased recombination rates supporting the former proposal that homology search is a limiting step for recombination. Whereas the sole viable repair event occurring at intrachromosomal DSB is GC, we observe that both GC and BIR account for the repair events stemming from subtelomeric DSB. The GC/BIR balance is modified following heterochromatin spreading either at the DSB site or on the recombination donor sequence. Heterochromatinization of the recipient locus favours GC by limiting resection whereas heterochromatin spreading at the donor site disfavours BIR.

These results highlight the contribution of spatial proximity to recombination efficiency and the importance of chromatin status onto repair events.

Keywords: heterochromatin, Homologous recombination, nuclear organisation.

WED-310
PARP1 as a mediator of the enzyme activities processing apurinic/apyrimidinic sites

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The apurinic/apyrimidinic (AP) sites are considered to be mutagenic and cytotoxic DNA lesions. The specific capacity of poly (ADP-ribose) polymerase 1 (PARP1) to interact with the AP sites has been demonstrated by us [1]. The identity of PARP1 cross-linked to AP sites in DNA in cell extracts was determined by mass spectrometry. PARP1 was weakly activated to conduct poly(ADP-ribose) synthesis upon binding to the AP site containing DNA. PARP1 interacting with AP sites catalyzes DNA strand incision by its AP lyase activity and shows 5′dRP lyase activity. The interaction of PARP1 with AP sites modulates activity of apurinic/apyrimidinic endonuclease 1 (APE1) in the case of DNA substrate containing single or clustered AP sites. The capacity of the other enzyme, tyrosyl-DNA phosphodiesterase (Tdp1), to the AP site cleavage was determined. Tdp1 catalyzes the cleavage of AP site and its synthetic analog, 3-hydroxy-2(hydroxymethyl)-tetrahydrofuran (THF), in DNA by hydrolysis of the phosphodiester bond between the substituent and 5′-adjacent phosphate. The product of the Tdp1 cleavage in the case of AP site is unstable and is hydrolyzed with the formation of 3′- and 5′-margin phosphates. The following repair demands the ordered action of polynucleotide kinase phosphatase, with XRCC1, DNA polymerase β and DNA ligase. In the case of THF, Tdp1 generates break with the 5′-THF and the 3′-phosphate termini [2]. PARP1 stimulates activity of Tdp1 in the AP site cleavage. The protein–protein interactions of PARP1 with APE1 and Tdp1 detected by various techniques were suggested as a key of the modulation of APE1 and Tdp1 activities by PARP1 in the AP site processing.

Project was funded by RFBR (13-04-93107, 13-04-40197-comfi and 14-04-00268), MCB Program of RAS, grant of President of RF for leading scientific schools (420.2014.4)

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Keywords: DNA Repair, apurinic/apyrimidinic sites, PARP1.

WED-311
Perturbation of iron homeostasis promotes the evolution of antibiotic resistance

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Evolution of antibiotic resistance in microbes is frequently achieved by acquisition of spontaneous mutations during antimicrobial therapy. These spontaneous mutations usually emerge from direct, antibiotic specific mutational processes (DNA damage) or might originate from the antibiotic inhibition dependent, oxidative mutagenesis. By employing genome wide screen for evolutionary modulators and long term selection experiments against a DNA gyrase inhibitor (ciprofloxacin) we demonstrated that in addition to the common mutator-phenotypes, inactivation of a central transcriptional regulator of iron homeostasis (Fur) facilitates laboratory evolution of ciprofloxacin resistance in *Escherichia coli* BW25113. To describe the underlying molecular mechanisms, we performed a global transcriptome analysis and demonstrated that the transcriptional pattern regulated by Fur change substantially in response to antibiotic treatment. In accordance with previous literature, we hypothesized that the impact of *fur*-mutation on evolvability under antibiotic pressure is due to the elevated intracellular concentration of free, ferrous iron and the consequent enhancement of oxidative damage-induced mutagenesis. As expected, inhibition of iron transport (1), overexpression of iron storage proteins (2), or anaerobic conditions (4) drastically suppressed the evolution of resistance, while inhibition of the SOS response-mediated mutagenesis had no substantial effect in Fur deficient populations. Importantly, we provide evidence that by binding unincorporated, intracellular iron using a cell permeable iron chelator (phenantroline) (3), the development of resistance was slowed down. Our work clearly revealed the importance of iron metabolism in the *de novo* evolution of antibiotic resistance, a pattern that could influence the development of novel antimicrobial compounds or inhibition of resistance formation.

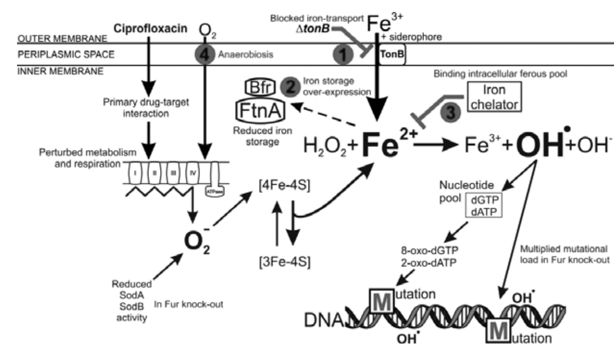

Fig. 1.

Keywords: Antibiotic resistance, Iron homeostasis, Oxidative mutagenesis.

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WED-312
Pre-steady-state kinetics of APE1 in the presence of various divalent metal ions

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Exposure of cellular DNA to reactive oxygen species (ROS), generated either by normal cell metabolism or by chemical and physical exogenous agents, leads to several types of lesions. A DNA lesion may be mutagenic and block replication and transcription. Failure to repair DNA can lead to mutations, genomic instability, premature ageing, mental retardation, or other developmental disorders and cancer. Over several decades, DNA damage and repair mechanisms have been the focus of numerous investigations, including structural, biochemical, and genetic studies on organisms ranging from bacteria to humans.

One of the major pathways to remove DNA lesions is the base excision repair (BER). BER pathway is a multi-step process, and can be reconstituted with a limited number of proteins. It involves a cascade of enzymes, which typically begins with a damage-specific DNA glycosylases that interact with DNA to specifically find target damaged bases and cleave the N-glycosidic bond of the lesion. This generates an abasic (apurinic/apyrimidinic (AP)) site. The toxic AP-intermediates must be recognized and processed by AP endonuclease immediately after the action of a DNA glycosylase. Bifunctional DNA glycosylases can also catalyze a β- or β-δ-elimination reaction (AP-lyase activity) to affect the strand scission after base removal. Short patch BER requires two additional enzymes to replace the damaged nucleotide: DNA polymerase β and DNA ligase III/XRCC1 heterodimer or DNA ligase I.

The endonuclease activity of APE1 has been well characterized for its preference for abasic site-containing DNA and by steady-state kinetic analysis in the presence of divalent cation. In order to understand the kinetic basis for enzyme specificity and efficiency, each step in the reaction pathway needs to be characterized. Here we examined the role of divalent metal ions in APE1 enzymatic pathway. The conformational dynamics of APE1 was investigated by the fluorescence stopped-flow method. The AP endonuclease activity of APE1 was abolished by treatment with EDTA, but was restored on subsequent addition of a molar excess of Mg^{2+} and, to lesser extent, Mn^{2+}, Ni^{2+}, Zn^{2+}, Ca^{2+}, Cu^{2+}. The role of metal ions in the DNA-substrate binding and catalysis was specified.

This work was supported by the Program of the Russian Academy of Sciences "Molecular & Cell Biology" [6.11]; the Grants from Russian Foundation for Basic Research [13-04-00013, 14-04-00531 and 14-04-31174]; the Grant from Russian Scientific Foundation [14-14-00063] and Ministry of Education and Science [SS-1205.2014.4, SP-4012.2013.4].
Keywords: AP endonuclease, base excision repair.

WED-313
Radio-sensitization of normal and tumor cells by DNA repair inhibitors

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Background: The use of specific inhibitors, which prevent double strand breaks (DSB) repair in tumor but not normal cells after ionizing radiation (IR) is a promising therapeutic strategy for cancer treatment. Here we describe the effects of inhibitors of DNA repair on molecular mechanisms triggered by IR. Since these are often p53-dependent, we employed normal human lung fibroblasts (NHLF, p53-wildtype) and compared their DNA damage response to lung carcinoma cell line (H1299, p53-negative). Prior irradiation, the cells were treated by NU7441 (1 μM), a specific inhibitor of DNA-PK; KU55933 (10 μM), a specific inhibitor of ATM, and VE-821 (10 μM), a specific inhibitor of ATR.

Methods: The experimental design consisted of five groups: control+dimethyl sulfoxide (DMSO), IR-treated+DMSO, and combination of IR with each of the inhibitors. We used epifluorescence microscopy for detection of γH2AX, flow-cytometry for cell cycle analysis and Western blotting for assessment DNA repair proteins.

Results: IR in combination with NU7441 caused more DSB than with VE-821 in the later time periods after irradiation in normal cells. Additionally, NU7441 increased G2-arrest in fibroblasts meanwhile KU55933 caused G1-arrest. On the other hand, in H1299 cells the highest amount of γH2AX corresponding to DNA damage was detected in the later time periods after treatment with combination of IR and VE-821, which also led to abrogation of G2-arrest.

Conclusion: Inhibitor VE-821 together with ionizing radiation increases level of DNA damage, shortens G2-arrest, and thus diminishes DNA repair of lung carcinoma cells but not normal lung fibroblasts. These results indicate that targeting ATR in p53-deficient tumors is a promising therapeutic strategy since it does not influence the normal cells.

Keywords: DNA repair, inhibitor, Ionizing radiation.

WED-314
Recombination and the roles of the RAD51-like proteins in *Arabidopsis*

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Repair of DNA double-strand breaks by recombination is key to the maintenance of genome integrity in all living organisms. It can however generate mutations and chromosomal rearrangements, making the regulation and the choice of specific recombination mechanisms of great importance. In addition to end-joining through non-homologous recombination, DNA breaks are repaired by multiple homology-dependent pathways that can be distinguished through their dependence or not on strand invasion catalysed by the RAD51/DMC1 recombinases.

With some exceptions (*Drosophila*, *C. elegans*), it is RAD51/DMC1 -dependent homologous recombination which establishes the chiasmata that physically link pairs of homologous chromosomes in meiosis, ensuring their balanced segregation at the first meiotic division. This is essential for fertility and generate the genetic variation which fuels evolution. The visible manifestation of genetic crossing-overs, chiasmata are the result of an intricate and tightly regulated process involving induction of DNA double-strand breaks and their repair through invasion of a homologous template DNA duplex, catalysed by the strand transfer proteins RAD51 and DMC1 in eukaryotes.

The viability of KO mutants and the strong conservation of recombination proteins/pathways makes the flowering plant *Arabidopsis thaliana* particularly well adapted to studies of the roles of these essential genes in the developing organism. A synthesis of recent results on the roles of RAD51/DMC1 and the RAD51

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mediator proteins in DNA repair and recombination will be presented.

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Keywords: DNA break repair, Rad51, recombination.

WED-315
Stopped-flow FRET analysis of DNA structural dynamics during interaction with mutant forms of human 8-oxoguanine-DNA glycosylase

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DNA bases are constantly modified by numerous environmental and endogenous agents, such as reactive oxygen species (ROS). 8-Oxoguanine is one of the most often generated and highly mutagenic oxidative damage in DNA. This may cause misincorporations of adenine during replication, resulting in C:G to A:T transversion mutations. 8-Oxoguanine-DNA-glycosylase hOGG1, the key DNA-repair enzyme in human cells, recognizes and removes 8-oxoguanine from DNA. This enzyme is bifunctional N-glycosylase/AP-lyase.

Although the structure of hOGG1 bound to DNA is known, the dynamic aspects of 8-oxoguanine recognition and removal are not well comprehended. According to X-ray data the amino acids Cys-253 and Gln-315 of hOgg1 participate in coordination of damaged base extruded from DNA helix to the base-binding pocket. In order to clarify the role of such base coordination and to reveal the elementary steps in overall enzymatic process we have investigated the enzymatic processes catalysed with hOGG1 mutant forms containing substitution of Cys-253 or Gln-315 with bulky amino acids (Cys-253 was changed for either Ile or Leu, Gln-315 for Trp).

The changes in the DNA structure were followed by registration of the FRET (Fluorescence Resonance Energy Transfer) signal using Cy3/Cy5 labels in DNA substrates. Comparison and analysis of shapes and kinetic parameters of the fluorescent curves obtained for non-damaged DNA substrates, or substrates containing 8-oxoguanine, natural AP-site or its synthetic analogue provide information about the dynamics of damage recognition and binding.

Stopped-flow kinetic study for mutant forms C253I, C253L and Q315W revealed that such amino acid substitutions reduce hOGG1 activity but play substantial roles on different stages of enzymatic process. It was shown that although base-binding pocket occlusion distorts the active site of hOgg1 C253I and C253L and greatly decreases the catalytic proficiency of the enzyme, it does not influence significantly the binding step and does not fully prevent 8-oxoguanine and AP-site recognition, sampling and excision. Otherwise, hOGG1 Q315W was not able to place properly the damaged base in the active site and to remove 8-oxoguanine in the course of N-glycosylase reaction. This enzyme form retains the ability to cleave AP-site, although such activity is reduced in comparison with the wild-type.

The work is supported by grants from the Program of the RAS “Molecular & Cell Biology” (6.11), the RFBR (13-04-00013, 14-04-00806), Russian Ministry Education and Science (SS-1205.2014.4, SP-4436.2013.4), RSCF (14-14-00063, 14-14-00628).
Keywords: DNA repair, pre-steady-state kinetics, protein – DNA interactions.

WED-316
Stopped-flow FRET analysis of DNA structural dynamics during interaction with mutant forms of human 8-oxoguanine-DNA glycosylase

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Although the structure of hOgg1 bound to DNA is known, the dynamic aspects of 8-oxoguanine recognition and removal are not well comprehended. According to X-ray data the amino acids Cys-253 and Gln-315 of hOgg1 participate in coordination of damaged base extruded from DNA helix to the base-binding pocket. In order to clarify the role of such base coordination and to reveal the elementary steps in overall enzymatic process we have investigated the enzymatic processes catalysed with hOgg1 mutant forms containing substitution of Cys-253 or Gln-315 with bulky amino acids (Cys-253 was changed for either Ile or Leu, Gln-315 for Trp).

The changes in the DNA structure were followed by registration of the FRET (Fluorescence Resonance Energy Transfer) signal using Cy3/Cy5 labels in DNA substrates. Comparison and analysis of shapes and kinetic parameters of the fluorescent curves obtained for non-damaged DNA substrates, or substrates containing 8-oxoguanine, natural AP-site or its synthetic analogue provide information about the dynamics of damage recognition and binding.

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The work is supported by grants from the Program of the RAS “Molecular & Cell Biology” (6.11), the RFBR (13-04-

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00013, 14-04-00806), Russian Ministry Education and Science (SS-1205.2014.4, SP-4436.2013.4), RSCF (14-14-00063).
Keywords: DNA repair, pre-steady-state kinetics, protein - DNA interactions.

WED-317
Structural and dynamic basis for distinguishing 8-oxoguanine from guanine by three different structural folds in DNA repair glycosylases

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8-oxoguanine (oxoGua) is a potent pre-mutagenic oxidative DNA lesion often encountered in a genome due to leaking aerobic respiration chain and environmental genotoxic stress. Normally, oxoGua is removed from DNA by enzymes belonging to the class of DNA glycosylases, Fpg in bacteria and OGG1 in eukaryotes. While recognizing the common substrate, these proteins share no homology and fall into different structural families: Fpg defines its own family while the structure of OGG1 is similar to endonuclease III. In addition, another enzyme of endonuclease III family, DNA glycosylase MutY (MUTYH in eukaryotes), removes adenine misincorporated by DNA polymerases opposite oxoGua but recognizes the damaged base in a completely different mode through an extra domain similar to NUDIX hydrolases. In order to clarify the source of specificity for the lesion vs non-damaged guanine, we performed molecular dynamic simulations of normal and damaged DNA bound by Fpg from *Lactococcus lactis* and *Geobacillus stearothermophilus*, human OGG1, and MutY from *Geobacillus stearothermophilus*, and supplemented the computation with steady-state and stopped-flow kinetic studies of the corresponding wild-type proteins or mutants in which we modified critical residues at the protein–DNA interface. In both Fpg and OGG1, the ability of the enzyme to evert the damaged nucleotide into a catalytically competent conformation, rather than the events within the active site, seem to be the deciding factor in their substrate specificity; mutant forms of these glycosylases that are unable to evert oxoGua retain their activity on abasic substrates that are intrinsically partially extrahelical. Destabilization of the everted pre-catalytic complex of Fpg or OGG1 by amino acid substitutions or introducing adenine opposite oxoGua may be tolerated due to DNA backbone adjustment. In MutY, we have identified a set of interactions both within the p13 domain responsible for oxoGua specificity and between p13 and other domains that distinguish oxoGua from guanine in the complex. Overall, early events in the path to the catalytically competent complex seem to play a predominant role in oxoGua recognition by DNA glycosylases.

Keywords: 8-oxoguanine, DNA glysosylase, DNA repair.

WED-318
Structural basis for the phosphorylation-dependent recruitment of Tel2 to Hsp90 by Pih1

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The recruitment of client proteins to Hsp90 requires specific co-chaperones that simultaneously bind the client and Hsp90 to mediate their interaction. As part of the RT2P complex, Pih1 is involved in the assembly and regulation of snoRNPs, RNA polymerase II and PI3-kinase-like kinases (PIKKs). Previous reports show that Pih1 is tethered to Hsp90 via its interaction with the TPR-containing protein Tah1, and also that Pih1 interacts in a phosphorylation-dependent manner with Tel2. Here we present the crystal structures of both the Hsp90-Tah1-Pih1 and the Pih1- Tel2 complexes. Structural and biochemical characterization of these interactions show how the C-terminal CS domain of Pih1 interacts with an unstructured region of Tah1, and how a novel N-terminal fold of Pih1 interacts with a phosphorylated Tel2 motif. Furthermore we discuss the implications these observations have on the recruitment of Pih1 to Hsp90 in both yeast and metazoa.

Keywords: Hsp90, Pih1D1, Tel2.

WED-319
The 5′–3′ exonuclease is essential for high constitutive SOS expression in *recA730* mutants of *Escherichia coli*

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The RecA protein is a central component of the recombination machinery which is required for double-strand break (DSB) repair, repair of single stranded gaps (SSG), production of genetic variation during conjugation and induction of the SOS response. In wild-type (wt) *Escherichia coli* strains, DSBs are processed into RecA filaments by the RecBCD pathway, whereas SSGs utilize the RecF recombination pathway [1]. In a *recB1080* mutant, the components of the two recombination machineries act together to produce a RecA filament [2]. There are three enzymatic activities essential for the RecA filament formation: helicase, 5'-3' exonuclease, and RecA loading onto single-stranded DNA [1].

We studied a specific *recA* mutant named *recA73*0 (RecAE38K) which encodes a form of RecA protein that is able to suppresses recombination and DNA repair deficiency in cells where both mechanisms for RecA filament formation are inactivated by mutations in genes for mediator proteins involved in RecA loading [3]. This gain of function mutant also exhibits high constitutive SOS expression (cSOS). The SOS response involves the elevated expression of more than 50 genes with various functions that are induced in response to damage of chromosomal DNA.

By studying the genetic requirements for high cSOS expression in *recA730* mutants, we found that three different genetic backgrounds (wt, *recB1080* and *recB* null), have different genetic requirements. In wt background, the high cSOS expression of a *recA730* mutant is partially dependent on RecBCD function, implying that RecBCD can moderately enhance the already excellent intrinsic abilities of the RecA730 enzyme. In *recB1080* background, the high cSOS expression of the mutant is partially dependent on the helicase activity of the RecB1080CD enzyme and is strongly dependent on the RecJ nuclease. The cSOS expression of a *recA730* mutant in a *recB* null background is dependent on the RecJ nuclease. These results emphasize the importance of the 5′–3′exonuclease for high cSOS expression in *recA730* mutants.

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Keywords: constitutive SOS, *Escherichia coli*, *recA730*.

WED-320
The influence of base-diet on 1,2-dimethylhydrazine induced carcinogenic damage in rat liver

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According to the Hippocrates' theorem …Let food be your medicine and medicine be your food", dietary interventions may induce changes in the metabolic and inflammatory state by improving the antioxidative status and modulating the expression of important genes involved in the chronic disorders. The aim of the present study was to evaluate the preventive properties of long term (14-months) use of biologically active substances-enriched diet (BASE–diet) against tumor alternations induced by injection of 1,2-dimethylhydrazine (DMH) in rats liver. The experiment was conducted on 32 Sprague-Dawley rats divided into two experimental groups (fed with control or BASE-diet, both n = 16). Control diet was a semi-synthetic diet formulated according to the nutritional requirements for laboratory animals. The BASE-diet was enriched with a mixture of polyphenolic compounds, β-carotene, probiotics, and n-3 and n-6 polyunsaturated fatty acids.

BASE diet decreased lipid peroxidation and C-reactive protein activity induced by injection of DMH compared to control group. Also the changes in activity of carcinogen-metabolizing enzymes (CYP1A1 and GST) caused by DMH-treatment in control rats were offset by BASE diet. The microarray technique allowed to identified 308 differentially expressed genes after DMH injection in control rats and 471 differentially expressed genes in rats fed with BASE diet. Analysis carried out using Pathway Studio allowed to identified 46% more differentially expressed genes associated with antioxidant activity in rats fed with BASE diet than in control group. BASE-diet treatment caused also changes in expression of 50 genes involved in direct DNA repair pathway after DMH-treatment.

BASE diet have shown the preventive properties against tumor alternations induced by injection of DMH, evidenced by the clinical results. Transcriptomic results indicate also that long term use of BASE diet caused the activation of defense mechanisms against DMH-treatment.

Keywords: biologically active substances, genes expression, liver.

WED-321
The p400 ATPase regulates homology-directed repair of DNA double-strand breaks independently of H2A.Z histone variant incorporation

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DNA damage signalling and repair take place in a chromatin context. Consequently, chromatin modifying enzymes, including ATP-dependent chromatin remodelling enzymes play an important role in the management of DNA damage and particularly for DNA double strand breaks (DSB). Here, we show that the p400 ATPase (member of the Tip60 complex) play a role in DNA repair by homologous recombination (HR). Indeed, while p400 is not required for DNA damage signalling, DNA double strand breaks repair is defective in the absence of p400. We demonstrate that p400 is important for HR-dependent processes, such as recruitment of Rad51 to DSB, homology-directed repair and survival following DNA damage. In addition, we observed that p400 and Rad51 interact and both favour chromatin remodelling around DSBs. The p400 ATPase activity drives to the incorporation of the histone variant H2A.Z into nucleosome. However, we did not observe any link between H2A.Z incorporation and the phenotypes observed after p400 depletion. Altogether, our data provide a direct molecular link between Rad51 and a chromatin remodelling enzyme involved in chromatin remodelling around DNA double strand breaks. We identified the p400 ATPase as an actor of the regulation of DSB repair by HR independently of its ability to incorporate the histone variant H2A.Z. Thus, p400 could be a brake to the generation of genetic instability.

Keywords: chromatin remodeling, DNA double-strand breaks, DNA repair.

WED-322
UHRF1: a link between epigenetics and DNA damage response?

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UHRF1 is a methyl-CpG-binding protein (MBP) that recognizes both methylated DNA and H3K9me2/3, thus linking DNA methylation and histone modifications. UHRF1 is the only MBP that is essential in mammals. It is a crucial epigenetic regulator that ensures the maintenance of DNA methylation through cell divisions by recruiting and activating the DNA methyltransferase DNMT1 on newly replicated DNA. Besides its interest for basic research, understanding UHRF1 role could have some very significant interest for human health, as UHRF1 is deregulated in many cancers, and thought to be causally involved in the abnormal proliferation of cancer cells.

Given the biological importance of UHRF1, and in order to elucidate its functions, we sought to identify its partners and targets. Proteomics approach revealed important and interesting hits that we validated by microscopy and biochemical experiments. Our results indicate a link between UHRF1 and the DNA damage response – a crucial process for maintaining genomic integrity, which is systematically altered in cancer.

Keywords: Cancer, DNA repair, Epigenetics.

WED-323

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CSV-04 – Host Pathogen Interactions/Bacterial Pathogenesis

WED-324
A bacterial tyrosine phosphatase inhibits plant pattern recognition receptor activation

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The first active layer of plant innate immunity relies on the perception of pathogen-associated molecular patterns (PAMPs) by surface-localised pattern-recognition receptors (PRRs). Many known plant PRRs are receptor kinases, which are annotated as serine/threonine kinases. However, the exact phosphorylation events that lead to receptor activation and initiation of PAMP-triggered immune signaling remain unknown. Here, we report that the Arabidopsis receptor kinase EFR, which perceives bacterial EF-Tu (or the derived peptide elf18), is phosphorylated on tyrosine residues and that this modification is critical for EFR activation upon ligand binding. We identify a single tyrosine residue required for EFR activation, downstream responses and immunity to the phytopathogenic bacterium *Pseudomonas syringae*. Pathogenic bacteria employ type-III secreted effectors to suppress PAMP-triggered immunity and cause disease. The effector HopAO1 is important for the virulence of *Pseudomonas syringae* and possesses tyrosine phosphatase catalytic activity, but its plant targets were still unknown. We found that HopAO1 directly interacts with EFR and FLS2, and blocks elf18-induced EFR activation and immune responses, revealing that HopOA1 targets tyrosine phosphorylation of plant PRR to block their activation. Our results shed light on a novel regulatory mechanism controlling plant immune signaling and highlight a host-pathogen battle to take control of PRR tyrosine phosphorylation that is critical for anti-bacterial immunity.

Keywords: None.

WED-325
A direct interaction between the bacterial nucleomodulin LntA and the chromatin repressor BAHD1 modulates interferon responses to infection

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Listeria monocytogenes is an opportunistic, food-borne bacterial pathogen of human and cattle. Listeriosis can constitute a life-threatening disease in the elderly and in immunocompromised patients; it also has serious outcomes in pregnant women, causing still-birth or frequently lethal neonatal infections. *L. monocytogenes* can cross the intestinal, foeto-placental and blood brain barriers, allowing its dissemination throughout the organism. This facultative intracellular bacterium can enter and multiply in the cytosol of most cell types and spread to neighbouring cells, using an arsenal of virulence factors that target diverse cellular components and subsequently hijack various host cell functions.

We have recently discovered and characterized LntA, a virulence factor from *Listeria monocytogenes* belonging to the emerging family of nucleomodulins. After secretion in the host cell cytosol, LntA enters the infected cell nucleus, interacts directly with a chromatin component, BAHD1, and inhibits its activity [1,2]. BAHD1 participates in the nucleation and spreading of a repressive chromatin complex, which induces gene silencing when recruited at promoters [3]. *Listeria* infection of epithelial cells induced the production of type I and III interferons; however,

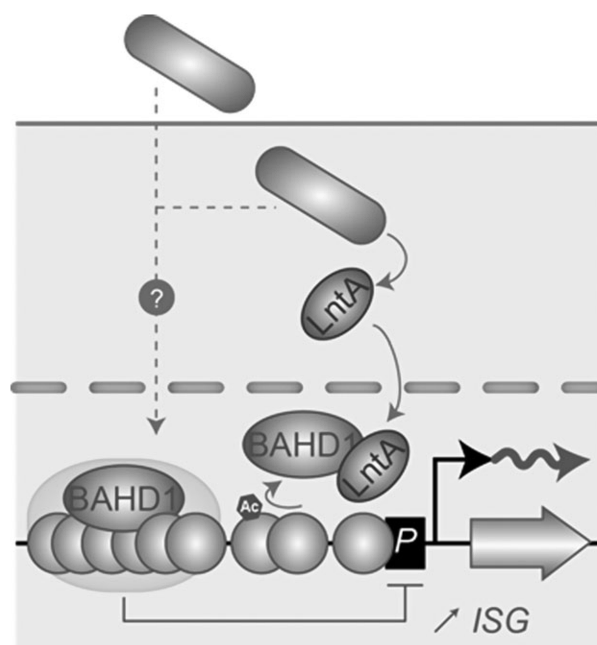

Fig. 1.

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the BAHD1-associated chromatin complex represses the expression of interferon-stimulated genes (ISG), by a so-far unknown mechanism. Upon interaction with LntA, BAHD1 dissociates from the promoters of ISGs, resulting in a reactivation of these genes, and affecting the outcome of *in vivo* infections in mice. Strikingly, the expression of *lntA* is tightly regulated, thereby avoiding uncontrolled activation of host innate immune responses. Altogether, the LntA-BAHD1 interplay fine-tunes the expression of ISGs during the progression of infection.

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Keywords: chromatin, interferon, *Listeria monocytogenes*.

WED-326

A dormancy protein Rv0574c is required for cell wall integrity and virulence of *Mycobacterium tuberculosis* H37Rv

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Virulence of *Mycobacterium tuberculosis* is intimately related to its distinctive cell wall. The proteins involved in synthesis of the polymer, poly-α-L-glutamine (PLG) in the cell wall of virulent mycobacteria have not been explored adequately. The genetic locus Rv0574c codes for a conserved protein, which is a member of 48 genes dormancy regulon of *M. tuberculosis*. The focus of this study is to investigate whether this locus, coding for polyglutamate synthase like protein has any role in synthesis of PLG, in the context of mycobacterial virulence. Rv0574c is expressed maximally in the late log phase of bacterial growth, which coincides with maximum synthesis of this polymer. Evaluation of Rv0574c gene expression in *M. tuberculosis* demonstrated its activation under conditions, e.g. hypoxia, nitric oxide, acid and CO_2, prevalent in the tubercular granuloma. PLG synthesis in the cell wall of the wild type bacterium was also increased marginally under these conditions. To investigate the matter further, we produced a knockout mutant of the gene by allelic exchange. Phenotypic characterization of the strain showed that PLG content was substantially reduced in the cell wall compared to the wild type bacteria. The mutant grew normally in the enriched culture medium, but was attenuated in THP-1 macrophages and in the organs of BALB/c mice. This was in agreement with histopathological evaluation of the lungs, showing slow growth and less severe pathology than the wild type and the complemented counterpart. Additionally, the mutant was more sensitive to detergents like SDS, enzymes like lysozyme, mechanical disruption and drugs, concomitant with drastic reduction in ability to form biofilm. The lipid and protein content in the cell wall of wild type, the mutant and the complemented strain was estimated and found to be unperturbed, supporting the hypothesis that changes in the cell wall integrity of the mutant are due to effect on PLG only and not on any other constituent of the cell wall. In summary, this study demonstrates that the protein encoded by Rv0574c locus, by virtue of depleting PLG in the cell wall, weakens bacterial resistance and compromises pathogenicity of *M. tuberculosis*.

Keywords: *Mycobacterium tuberculosis*, poly-α-L-glutamine, Rv0574c locus.

WED-327

A human proteome microarray identifies that the heterogeneous nuclear ribonucleoprotein K (hnRNP K) recognizes the 5′ terminal sequence of the hepatitis C virus RNA

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Stem-loop I (SL1) located in the 5′ untranslated region of the hepatitis C virus (HCV) genome initiates binding to miR-122, a microRNA required for hepatitis HCV replication. However, proteins that bind SL1 remain elusive. In this study, we employed a human proteome microarray, comprised of $\sim$17,000 individually purified human proteins in full-length, and identified 313 proteins that recognize HCV SL1. Eighty-three of the identified proteins were annotated as liver-expressing proteins, and twelve of which were known to be associated with hepatitis virus. siRNA-induced silencing of eight out of 12 candidate genes led to at least 25% decrease in HCV replication efficiency. In particular, knockdown of heterogeneous nuclear ribonucleoprotein K (hnRNP K) reduced HCV replication in a concentration-dependent manner. Ultra-violet-crosslinking assay also showed that hnRNP K, which functions in pre-mRNA processing and transport, showed the strongest binding to the HCV SL1. We observed that hnRNP K, a nuclear protein, is relocated in the cytoplasm in HCV-expressing cells. Immunoprecipitation of the hnRNP K from Huh7.5 cells stably expressing HCV replicon resulted in the co-immunoprecipitation of SL1. This work identifies a cellular protein that could have an important role in the regulation of HCV RNA gene expression and metabolism.

Keywords: host-pathogen interaction, proteome chip, RNA-protein interaction.

WED-328

A protein extract from *Jatropha curcas* seed cake has in vitro anti-Toxoplasma activity

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Toxoplasma gondii is a parasite of great medical and veterinary importance, which has worldwide distribution and causes toxoplasmosis. There are few treatments available for toxoplasmosis and the search for plant extracts with anti-Toxoplasma activity is of utmost importance for the discovery of new active compounds. The objective of this study was to investigate the action of the protein extract from *J. curcas* seed cake on developing tachyzoites of *T. gondii*-infected Vero cells. The protein extract (*Jc*CE) was obtained after solubilization of the *J. curcas* cake with sodium borate buffer (100 mM, pH 10), centrifugation and dialysis (*cutoff: 12 kDa*) of the supernatant with the extracting

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buffer. Typically, *Jc*CE had around 0.75 mg protein (mgP)/mL and inhibitory activity against papain [28.2 inhibitory units (IU)/mg P] and trypsin (50.7 IU/mgP). *Jc*CE was used for the *in vitro* assays at 0.01; 0.1; 0.5; 1.5; 3.0 and 5.0 mg/mL concentrations for 24 h. At 3.0 mg/mL, *Jc*CE reduced the percentage of infection and parasites to 26.2% and 18%, respectively, but had no effect on the morphology of Vero cells. During treatment with *Jc*CE parasitophorous vacuole-containing tachyzoites had progressive disorganization. These results suggest that *Jc*CE interferes with the intracellular growth of *T. gondii*. Supported by: CNPq and FAPEMA.

Keywords: anti-*T. gondii*, *Jatropha curcas*, protease inhibitor.

WED-329

A putative arginine-ornithine antiporter ArcD in *Streptococcus gordonii* controls *de novo* arginine biosynthesis, low pH tolerance and biofilm formation

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Background: Arginine deiminase system (ADS) is a major ammonia-generating pathway and plays important roles in oral biofilm ecology. ADS catabolizes arginine to ornithine, ammonia, and CO_2 with the concomitant production of ATP. *Streptococcus gordonii,* which is one of the most abundant ADS-positive species in oral cavity, has *de novo* arginine biosynthesis pathway to control total arginine metabolism in conjunction with ADS. *S. gordonii* expresses a putative arginine-ornithine antiporter (ArcD) formimg a component of ADS, whereas the function of ArcD is yet to be investigated. The aim of this study is to elucidate the role of ArcD in *S. gordonii*.

Materials and methods: We generated an ArcD deletion mutant (ΔArcD) by replacing *arcD* ORF in *S. gordonii* DL1 (Challis) with *ermAM* ORF, and examined its growth and biofilm formation under various pH conditions in a chemically-defined medium (CDM). Biofilm microstructure and bacterial surface morphology were microscopically observed. Following the incubation in CDM for various periods, the bacterial internal metabolites were comprehensively analyzed with CE-TOFMS.

Results: Growth of ΔArcD reached more slowly to an identical plateau with WT at mild acidic condition (pH 6.0), whereas it was significantly suppressed below pH 5.5. Exogenous arginine enhanced WT growth at pH of 7.0, while ΔArcD showed suppressed growth regardless of arginine existence. Microscopic observation demonstrated that ΔArcD formed sparse and loose biofilms with less biovolume than WT and faild to express pilus-like surface appendages. Metabolome analysis revealed that decrease of ornithine, citrulline and arginine enhanced the early phase of *de novo* arginine biosynthetic pathway from glutamate to *N*-Acetyl-Ornithine via *N*-Acetyl-Glutamate, catalyzed by ArgDBJC in WT. In contrast, lack of ArcD suppressed the arginine biosynthesis with retention of ornithine, citrulline and arginine.

Conclusion: These results indicate that ArcD is involved in the control of *de novo* arginine biosynthesis to maintain homeostasis in *S. gordonii* and may be also necessary for proper expression of a pilus-like cell surface component and biofilm formation.

Acknowledgement: The authors report no conflicts of interest related to this study.
Keywords: arginine, bacterial metabolism.

WED-330

A role for peroxisomes on the cellular antiviral response to Cytomegalovirus infection

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Viruses are able to develop specific mechanisms of evasion of the host cell antiviral defense. The Cytomegalovirus (CMV) encodes vMia, a protein responsible for circumventing two important cellular responses to viral infection: apoptosis and RIG-I/MAVS signaling. Although still poorly understood, its anti-apoptotic activity involves the recruitment of Bax to mitochondria and blocking of the mitochondrial outer membrane permeabilization. The RIG-I/MAVS signaling pathway is initiated by the recognition of the viral genome by the cytoplasmic protein RIG-I. Upon viral stimulation, this protein interacts with the mitochondrial antiviral signaling adaptor (MAVS), leading to a signaling pathway that culminates in antiviral defense. Although always assumed to localize exclusively to mitochondria, it has been recently demonstrated that MAVS is also present at peroxisomes and that these two organelles perform different but complementing functions within the antiviral response. vMia has been shown to dampen signaling downstream from mitochondrial MAVS and was also shown to trigger mitochondria fragmentation, a phenomenon proven to be essential for the signaling inhibition. However, no studies were yet performed taking into account a possible effect of vMia on the peroxisomal MAVS-dependent antiviral signaling cascade.

Our studies have uncovered a novel peroxisomal localization for CMV's vMia. Interestingly, we have also demonstrated that vMia interacts with Pex19, the cytoplasmic chaperone responsible for the transport of peroxisomal membrane proteins to this organelle. These results suggest that peroxisomes, similarly to mitochondria, may act as an important platform for vMia-dependent evasion of the cellular antiviral defense against CMV.

Keywords: Cytomegalovirus, Peroxisomes, vMia.

WED-331

Adhesive and antimicrobial properties of *Lactobacillus acidophilus* strain Er 317/402 "Narine" for its use in some types of blood cancer

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In the pathogenesis of leukemia the role of microorganisms is not excluded, the leading role being played by pathogenic bacteria, *Staphylococci, Escherichia coli* etc [1].

We used the probiotic strain *L. acidophilus* Er 317/402 "Narine". Antimicrobial properties of bacteria were determined by diffusion method in agar and by serial dillutions method. As test microorganisms were taken *E. coli, Staphilacoccus aureus, Salmonella typhimurium, Shigella flexneri, Vibrio cholera El-Tor, Yersinia pestis, Brucella abortus* etc. To identify the adhesion properties and adhesins in bacterial cells hemagglutination reaction with red blood cells of animals and humans was used. CFA I and CFA II type of fimbrial antigens of adhesion were determined in the reaction of D-mannose-resistant hemmaglutination

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with II A group of human, chickens, sheep and bovine erythrocytes. 3% suspension of erythrocytes was used in the experiment. Identification of isolates strain in children with acute leukemia was carried out.

Probiotic strain of *L. acidophilus* Er 317/402 "Narine" was shown to possess high adhesion and antimicrobial properties. The latter were established for a wide range of test-microorganisms including pathogenic ones. It was revealed that *L. acidophilus* Ep 317/402 "Narine" had a CFA I kind of adhesins. From the children with acute leucosis were isolated and identified *St. aureus, St. epidermidis, E. coli*, which differed from the phage types. It was determined that the pathogenic microorganisms were isolated at a stage of acute leucosis, while saprophyte microorganisms – at a stage of remission. The quantity of *Escherichia* was decreasing in blood when *L. acidophilus* strain Er 317/402 "Narine" was enterally applied, while on the 10–12th days after acidophilotherapy, hemolytic escherichia were not detected.

The results obtained indicate that probiotic strain of *L. acidophilus* Er 317/402 "Narine" exhibits high adhesion and antimicrobial activity and it is recommended in treatment of childhood acute leucosis.

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Keywords: adhesion, lactic acid bacteria, leukemia.

WED-332
Amidate prodrugs of 9-[2-(Phosphonomethoxy)ethyl]adenine (PMEA) as inhibitors of adenylate cyclase toxin from *Bordetella pertussis*

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Adenylate cyclase toxin (ACT) is the key virulence factor of *Bordetella pertussis* that facilitates its invasion into the mammalian body. 9-[2-(Phosphonomethoxy)ethyl]adenine diphosphate (PMEApp), the active metabolite of the antiviral drug bis(POM) PMEA (adefovir dipivoxil), has been shown to inhibit ACT. The objective of this study was to evaluate six novel amidate prodrugs of PMEA, both phenyloxy phosphonamidates and phosphonodiamidates, for their ability to inhibit ACT activity in J774A.1 macrophage cell line. The two phenyloxy phosphonamidate prodrugs exhibited higher inhibitory activity (IC50 = 22 and 46 nM) than the phosphonodiamidates (IC50 = 84–3960 nM). The inhibitory activity of the prodrugs correlated with their lipophilicity and the degree of their hydrolysis into free PMEA in J774A.1 cells. Although they did not inhibit ACT as effectively as bis(POM)PMEA (IC50 = 6 nM), they were significantly less cytotoxic. Moreover, they all reduced apoptotic effects of ACT and prevented an ACT-induced elevation of intracellular [Ca2+]i. The total amount of bis(POM)PMEA (including the hydrolytic products mono(POM)PMEA and PMEA) transported across Caco-2 monolayers was higher than that of the studied amidate prodrugs. On the other hand, the amidate prodrugs were less susceptible to degradation in Caco-2 cells than bis(POM)PMEA. As a consequence, high amount of intact amidate prodrug is expected to be available to target macrophages *in vivo*. This feature makes nontoxic amidate prodrugs attractive candidates for further investigation as novel antimicrobial agents.

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Keywords: None.

WED-333
AMPK/Sirtuin1 activators inhibit herpes simplex virus type 1 replication and protect neurons from neurodegenerative events triggered during infection

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Herpes simplex virus type 1 (HSV-1) is ubiquitous, neurotropic, and the most common pathogenic cause of sporadic acute encephalitis in humans. Data suggest that it is able to establish latency in the CNS, and that this condition would not be harmless. In addition, it has been estimated that in approximately 70% of the population over 50 years old, the virus enters the brain and infects neurons, suggesting the existence of recurrent reactivations. Currently, it is unclear whether a neuron, that undergoes viral reactivation, survives and resumes latency or is killed. Taking in consideration that the stress sensor AMP-dependent kinase (AMPK) and Sirtuin 1 (Sirt1) are involved in neuroprotection, we recently demonstrated that HSV-1 modulates the AMPK/Sirt1 axis differentially during the course of infection interfering with pro-apoptotic signaling and regulating mitochondrial biogenesis. Therefore we evaluated the effects of AMPK/Sirt1 modulators such as resveratrol and AICAR on viral replication efficiency, neuronal viability and neurodegenerative events triggered during neuronal HSV-1 infection. In agreement with our previous results, we found that 1 mM AICAR (a specific AMPK activator) and different concentrations of resveratrol increased viability in infected neurons, and inhibited to some extent the viral replication and the expression of HSV-1 immediate-early genes (ICP27), early (ICP8) and the latency associated transcript (LAT). Moreover, pretreatment of neurons with resveratrol significantly reduced the increase in tau hyperphosphorylation triggered by HSV-1 infection. These results highlight the potential relevance of pharmacological activators of the AMPK/SIRT1 axis in diminishing HSV-1 productive infection and cellular damage associated with reactivation episodes at neuronal level.

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Keywords: AMPK, Neurodegeneration, Virus.

WED-334
Antimicrobial studies on porous hydroxyapatite used in the environment and biological applications

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Due to non-biodegradable behaviour and their incapacity of metabolization and decomposition, PTTE like Pb, Cu, Cd, Zn and Hg are the main contaminants of soils and ground or surface waters. Their progressive accumulation in the human body can cause significant health problems, inducing chronic illness which untreated, can lead to a painful death.

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The aim of this study was to synthetize new porous nanoparticles based on methyltrimethoxysilane coated hydroxyapatite (MTHAp) for environment and biological applications. The functional groups present in the prepared powder were identified by FTIR spectroscopy. All the diffraction peaks of MTHAp powders could be assigned to the standard characteristic peaks of hexagonal hydroxyapatite and no secondary phases were detected, indicating that the phase of the samples was of pure HAp.

Removal performance of Pb^{2+} ions by the MTHAp composite powders was investigated by batch experiments, monitoring the change of Pb^{2+} ion concentration in the aqueous solution. For this experiments, 5 g of MTHAp composite sample was added to 500 ml aqueous solution with various initial Pb^{2+} ion concentrations and pH values. The initial Pb^{2+} ion concentrations of the aqueous solutions were controlled and the values were set in the range 0.1–0.9 $g \cdot L^{-1}$ by dissolving lead nitrate [$Pb(NO_3)_2$] in deionized water.

The powders after the removal of Pb^{2+} were a mixture of $Ca_{2.5}Pb_{7.5}(PO_4)_6(OH)_2$, $Pb_2Ca_4(PO_4)_2(SiO_4)$ and $Ca_{10}(PO_4)_6(OH)_2$.

This study showed that MTHAp with $x_{Ag} = 0.5$ presented an antimicrobial activity against *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 25922). The *in vitro* bacterial adhesion study indicated a significantly decreased number of *E. coli* and *S. aureus* on MTHAp with $x_{Ag} = 0.5$. Furthermore, for $x_{Ag} = 0$ the MTHAp stimulated the growth and multiplication of the tested *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 25922) bacterial strains.

The antimicrobial effects of the MTHAp powders after the removal of Pb^{2+} were investigated against Pseudomonas sp a gram-negative bacteria that live in soil and decomposing organic matter. A significantly number of Pseudomonas sp bacteria were observed in the Pb-contaminated MTHAp for all Pb concentrations. Our studies have shown that the strain- Pseudomonas sp. presented a resistance at Pb.

Keywords: bacterial strains, Pb-contaminated MTHAp, silica matrix.

WED-335

Application of proteomics for identification of virulence factors associated with poultry host preference of *Staphylococcus aureus* strains

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Staphylococcus aureus in an opportunistic pathogen of humans and animals. It secretes a range of proteins into extracellular milieu, many of which act as virulence factors giving the bacterium the ability to effective host colonization and disease development. *S. aureus* exhibits a huge phenotypic and genetic diversity, however genomic studies show a clear distinction between the strains from human and animal origin. Unfortunately, biochemical basis of host preference and proteome components responsible for this phenomenon are largely unidentified.

Our previous studies revealed the correlation between the genotype of poultry isolated *S. aureus* strains and their virulence in chicken embryo but not nematode model [1], what may suggest the existence of host specific virulence factors. This study have been conducted to verify whether the differences in virulence of the strains are reflected at the proteome level. For this purpose extracellular proteomes of well characterized poultry-originated virulent (VS) and non-virulent (NVS) *S. aureus* strains were compared. Extracellular proteins were precipitated, labeled with fluorescent dyes and subjected to two-dimensional difference gel electrophoresis in pairs VS *vs.* NVS.

The proteome images among the strains were highly diverse. In total, 465 differentiating protein spots were cut out from the gels, trypsin digested and subjected to mass spectrometry (MS) analysis. The MS analysis revealed that 80 and 135 proteins exhibited higher abundance in VS and NVS, respectively. However, only a few proteins were identified in all VS and all NVS proteomes. Interestingly, 42 proteins were classified as differentiating in both types of proteomes. Closer inspection of protein localization on the gels and sequence coverage during MS identification indicated different posttranslational processing and/or degradation which may affect their biological activity. The high number of identifications of alpha-hemolysin and lipases as differentiating in VS and NVS proteomes suggest the importance of these known virulence factors in *S. aureus* host preference as well as turns the attention to proteases as possible agents switching between virulent and commensal phenotype.

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Acknowledgements

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Keywords: None.

WED-336

Assesment of oral cavity microbiota – potential risk factors of local/general opportunistic infections in Polish patients with congenital malformations

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Introduction: The oral cavity is an open system with complex and dynamic relations between human host and microbiota. Cleft lip and palate is one of the most common congenital malformations. In Poland, it appears every year in 800 newborns, as a single disorder or syndrome. Environmental and genetic factors, both have influence on development of this pathology. As treatment of patients with cleft malformations is comprehensive and long-lasting, an information on microorganisms colonizing oral cavity is essential to decrease a risk of medical complications. We examined composition and genetic characteristics of species of the oral cavity microbiota in cleft patients, in term of host-microorganism relationships and a risk of local and general invasion.

Material and methods: Sixty patients, 14 to 23-years old, with and without congenital malformations, treated orthodontically were examined; data from clinical, microbiological and genetic examinations were analyzed.

Results: There were differences in the conditions of the patients' oral cavities, expressed by the values of bleeding index, dental plaque index, highest in orthodontic patients with malformations. Different strains of bacteria, yeast-like fungi and protozoan were found. Among others, strains of *E. coli*, *Enterobacter cloacae*, *E. agglomerans*, *Candida* sp., *C. albicans* were often identified. Seven non-Enterobacteriaceae strains, including *P. aeruginosa*,

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were also detected. Relatively often *Enterococcus faecalis* and *E. faecium* occurred; five strains of *Staphylococcus,* also MRS were found.

Conclusion: Although many microorganisms may be found in the oral cavity, there are no standards for its microbiological examination regarding their potential role in local/general infections. Analysis of the data implicates that there are co-infections with different opportunistic/pathogenic strains and, potentially, a serious risk of local and general complications in orthodontic patients with congenital malformations. It should be emphasized that the human oral cavity may act as a major, not fully known, source of strains that can induce clinically important infections. The examination of the oral cavity, in term of presence of opportunistic bacteria and fungi, particularly in persons with congenital malformations, is highly recommended before/during treatment.

Keywords: oral cavity microbiota, opportunistic species, congenital malformations.

WED-337
Atomic structure of a bacterial filament: the type III secretion system needle

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Bacterial filaments such as pili or secretion needles are complex macromolecular assemblies that play an essential role in host pathogen interaction. Cryo-electron microscopy combined with crystal subunit structures has recently provided a powerful hybrid approach to study such nanomachines, resulting in low- and medium-resolution models. However, such approaches can not deliver atomic details, especially the crucial subunit-subunit interfaces, due to the limited cryo-EM resolution. For many bacterial filaments, the mechanisms for molecular assembly are still unknown.

Here, I will show that state-of-the-art Solid-State NMR methodology (1–4) is able to reveal the supramolecular interfaces and ultimately the complete atomic structure of a bacterial filament. Our approach is demonstrated on the *Salmonella typhimurium* Type III Secretion System Needle, a filamentous assembly that mediates the injection of pathogen effector proteins to the cytosol of host cells during bacterial infection. Using an integrated approach combining Solid-State NMR, STEM, and computational modeling, we present an atomic resolution model of the needle (5) in its native filamentous state. Additional data based on Solid-State NMR and immuno-labeling techniques on a homologous needle from *Shigella flexneri* have allowed us to propose a common architecture for the type III secretion system needles (6).

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Keywords: NMR Spectroscopy, secretion system, structural biology.

WED-338
Bacterial effector CagA induces factors involved in EMT phenotype of gastric epithelial cells in experimental *in vitro* infection with *Helicobacter pylori*

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Following adhesion of *H. pylori* to gastric epithelial cells, the bacterial oncoprotein CagA is translocated intracellularly, through a Type IV secretion system (T4SS) and deregulates cellular polarity, inducing the appearance of a scattering phenotype that resembles to the EMT. Hierarchic phosphorylation of CagA by Src and Abl kinases, on repetitive EPIYA motifs, located at the carboxyl-terminus of the protein plays a pivotal role in this transition. In clinical isolates the type of EPIYA motifs has been shown to vary depending on the surrounding sequence namely, EPIYA-*A*:EPIYAKVNK, EPIYA-*B*:EPIYAQVAKK and EPIYA-*C*:EPIYATIDDLG. In this study, we investigated the potential involvement of CagA protein, after *H. pylori*-infected gastric epithelial cells, in the activation of MMP-3, which has been suggested to be required for the change in cell shape, inducing EMT. We utilized isogenic *H. pylori* mutants, based on the reference P12 strain, expressing CagA protein with variable numbers (n = 0–3) of functional terminal EPIYA-C and phosphorylation-deficient EPIFA-C motifs, as well as the corresponding P12 *cagA*-knock out and *cagE*-knock out (T4SS-defective) strains. These strains were used to infect gastric epithelial cells (AGS and MKN45) *in vitro* and MMP-specific transcriptional activation was measured by RT-quantitative Real Time PCR, at several time points. MMP expression in total cell lysates and cell culture supernatants was also determined by western blot analysis at 24 h post-infection. Increased transcriptional activation of MMP-3 gene was observed in AGS cells infected by *H. pylori* strains, appeared to be linked to expression and translocation of CagA and was proportional to the number of functional (EPIYA-C) phosphorylation motifs. Although, MMP-3 expression in total cell lysates of *H. pylori*-infected AGS and MKN45 cells was associated to CagA expression and translocation, it was found to be unrelated to the number of EPIYA motifs and their state of phosphorylation. In contrast, the levels of secreted MMP-3 in cell culture supernatants appeared to be CagA phosphorylation-dependent, suggesting that metalloproteinase release in the extracellular matrix may be related to CagA phosphorylation-dependent intracellular signaling downstream events. We also checked MMP-10 expression, due to its 82% amino acid homology to MMP-3 and observed that its expression in total cell lysates of *H. pylori*-infected AGS cells was infection-independent. An analysis of the molecular pathways of MMP-3, involved in epithelial to mesenchymal transition of gastric epithelial cells, leading the cells to a metastatic phenotype, during chronic *H. pylori* infection, is under way.

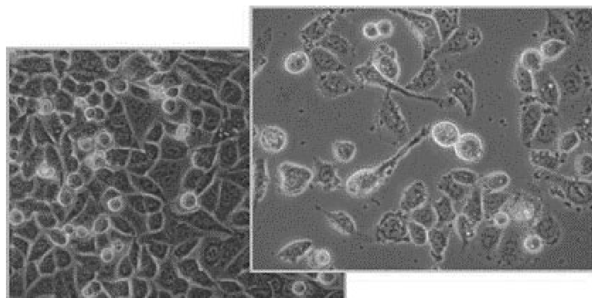

Fig. 1.

Keywords: EMT, *Helicobacter pylori*, MMP-3.

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WED-340
Candida albicans enolase interacts with neutrophil proteins involved in the formation of neutrophil extracellular traps in response to candidial infections.

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Introduction: Neutrophils constitute the first line of host defense at the site of microbial infection, where the formation of extracellular traps (NETs) is one of mechanisms exploited by the host to combat pathogens. NETs are formed by decondensed chromatin, decorated with granular proteins such as myeloperoxidase, elastase, histones, lactoferrin and azurocidin.

Candida albicans yeasts belong to the physiologic microflora of healthy individuals but can convert into a dangerous pathogen in immunocompromised patients. It is equipped with numerous virulence factors, of which the major roles are played by the cell wall adhesive proteins and the secreted hydrolytic enzymes.

Objective: Analysis of possible involvement of a major *C. albicans* cell wall protein, enolase in the entrapment of fungal cells in NETs via their proteinaceous components.

C. albicans enolase is a vital glycolytic enzyme and also a highly conserved immunodominant antigen presented on the fungal surface where it belongs to a large group of moonlighting proteins, loosely bound with the cell wall.

Results: The analysis of chromatographically separated *C. albicans* cell wall proteins allowed to classify enolase as one of the major proteins capable of interacting with granular components of NETs. After purification to homogeneity, enolase was found to bind myeloperoxidase, lactoferrin and azurocidin but not histones. The complexes formed were characterized in terms of thermodynamic parameters with the use of surface plasmon resonance (SPR) measurements. For the catalytic process this enzyme requires bivalent metal ions which were also found to stabilize the enolase interactions with proteinaceous components of NETs. For the strongest binding of enolase to myeloperoxidase, an influence of this interaction on the enzyme activities of both proteins was analyzed. The enolase activity was inhibited, in contrast to the preserved activity of myeloperoxidase, known as critically important for antimicrobial defense of the host.

Conclusions: The production of NETs at the place of infection is one of possible mechanisms for the neutralization of microbial pathogens by the host. This process can involve the direct immobilization of pathogen by the decondensed chromatin, but also some pathogen surface proteins such as enolase can be tightly bound by proteinacous component of NETs, thereby increasing the efficiency of local antimicrobial defense.

Keywords: *Candida albicans*, enolase, Neutrophil extracellular traps.

WED-341
Chaperon activity of heat shock protein 90 (HSP90) is required for the production of paramyxovirus polymerases

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HSP90 is an essential chaperon protein which is known to facilitate the folding, the stabilization and/or the activation of client proteins. HSP90 is a highly conserved and abundant cellular protein which can be induced upon stress. When studying the thermosensitivity phenotype of Measles virus (MeV) vaccine strain in chicken cells, a correlation between the level of expression of HSP90 and the viral growth was found. Further experiments showed that HSP90 appeared to be strictly required for an efficient viral growth of vaccine and wild-type MeV strains both *in vitro* and in an *ex vivo* model of a mouse brain infection. In infected cells, polymerases and nucleocapsids containing the viral genomes are concentrated in structures named “viral factories”. Immunostaining of infected cells showed a colocalization of HSP90 in these viral structures. In the absence of HSP90 activity, the viral transcription was inhibited. Using different approaches, we show that the production of viral polymerase, composed of the large protein L and its cofactor the phosphoprotein P, in a soluble and functional form transiently requires HSP90 activity. In the absence of HSP90 activity, the L protein is mostly degraded by the proteasome or insoluble. Moreover, both HSP90 and P protein were found to be required to produce L protein in a stable and soluble form. Interestingly, mature polymerases do not required HSP90 activity for their function, indicating that HSP90 acts as an authentic compulsory chaperon for MeV polymerase. The HSP90-dependence has also been seen for Nipah virus which belongs to another viral genus (*Henipavirus*) in the *Paramyxoviridae* family indicating that this mechanism may be conserved in the whole family.

Keywords: Hsp90, Polymerase, Virus.

WED-342
Characterization of a novel phase variable Type IIS restriction endonuclease from *H. pylori* strain 26695

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Helicobacter pylori is a Gram-negative bacteria that infects about 50% of the human population, and is responsible for 5.5% of all gastric cancer, per year. *H. pylori* shows extraordinary genetic diversity and this property is key to its success as a human pathogen. Genome sequences of various clinical strains of *H. pylori* reveal an abundance of DNA restriction endonucleases and methyltransferases (R-M systems). Recent studies have shown that components of R-M systems are involved in transcriptional regulation of several genes including virulence, besides their role in regulation of DNA uptake in bacteria. Interestingly, many genes encoding R-M enzymes are strain specific and phase variable in *H. pylori*. The *hp1366-hp1367-hp1368* ORFs in the *H. pylori* strain 26695 codes for an active Type IIS R-M system. The *res* gene is encoded by *hp1366* ORF (HpyAII) which is a novel Type IIS phase variable restriction endonuclease that recognizes the sequence 5′GAAGA3′/5′CTTCT 3′ and cleaves 8 bp downstream on the top strand, and 7 bp downstream on the bottom strand. In *H. pylori* J99 strain the homolog of *hp1366* is inactive due to phase variation. The *hp1366* gene is highly strain specific among Indian strains and other sequenced strains, and hence it would interesting to study its role in the adaptation and possible host-pathogen interaction of *H. pylori*.

The *hp1366* gene of a putative Type IIS R-M system from 26695 strain of *H. pylori* was cloned and purified to near homogeneity. HpyAII prefers for a two-site substrate over one-site substrate for maximal activity. HpyAII is less stringent in metal ion requirement and shows higher cleavage activity with Ni^{2+} over Mg^{2+}. This sheds light on the importance of Ni^{2+} ions, as various studies have shown that *H. pylori* maintains a pool of Ni^{2+} for successful colonization of the host. Why HpyAII prefers Ni^{2+} over Mg^{2+} for maximal activity and what *in vivo* significance it

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can confer on the activity of HpyAII is an interesting question. Mutational analysis of the putative residues of HNH motif of HpyAII confirms that the protein has an active HNH site for the cleavage of DNA. However, mutation of first His residue of the HNH motif to Ala does not abolish the enzymatic activity, instead causes loss of fidelity compared to wild type HpyAII. The novelty of this observation lies in the fact that mutation of first His residue of the HNH motif in all other known HNH motif containing enzymes completely abolishes enzymatic activity. Mutation at a single amino acid residue leading to the loss of fidelity provides probable insight into the evolution of restriction enzymes. This study further exemplifies our understanding about the role of R-M systems in *H. pylori* biology.

Keywords: endonuclease, *Helicobacter pylori*, phase variation.

WED-343
Characterization of bacteriophage Pet-CM3-4 infecting *Cronobacter* spp.

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Cronobacter spp. (formerly known as *Enterobacter sakazakii)* is an opportunistic pathogen associated with severe infections in neonates and infants which can lead to necrotizing enterocolitis, bacteremia and neonatal meningitis, with high fatality rates. An infection in neonates is often associated with the consumption of contaminated dried infant formula. To improve food quality, is important to find an efficient method for pathogen elimination. For such a purpose, bacteriophages could be the appropriate tool in the protection of infant formula from this pathogen.

The aim of this work was to characterize *Cronobacter* specific bacteriophage Pet-CM3-4 as a suitable candidate for application in food control. The bacteriophage was isolated from sewage water on *C. malonaticus* 161007/29 indicator strain. Phage was purified from single plaque by ultracentrifugation in CsCl gradient. Based on whole genome sequencing this phage belonged to the T4-like group of *Myoviridae* family. The whole DNA was 172 kbp long containing 39.8% GC pairs. The sequence had the highest similarity to the CC31 enterophage. The phage had the broad host specificity, it was able to infect 36 from 50 tested *Cronobacter* strains belonging to species *C. sakazakii*, *C. malonaticus*, *C. dublinensis*, *C. turicensis*, *C. muytjensii* and *C. condimenti*. The efficiency of plating reached 0.04–105% comparing with titer on the indicator strain. Inhibition of bacterial growth by Pet-CM3-4 was also observed in liquid medium, the highest effect was detected using high phage concentrations and in the presence of low amounts of bacteria. In these conditions bacterial growth was not observed for the first eight hours, but overgrowth of the phage resistant bacteria occurred in some strains during overnight cultivation. Therefore application of the Pet-CM3-4 phage in the mixture with some other phages will be more practicable in food control applications.

Keywords: bacteriophages, *Cronobacter*.

WED-344
Cloning and expression analyses of an endo-β-(1-6)-D-galactanase gene in two races of *Colletotrichum lindemuthianum* with different life style

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Microorganisms produce cell-wall-degrading enzymes as part of their strategies for plant invasion/nutrition. The endo-β-(1-6)-D-galactanase catalize the hydrolysis of β-(1-6)-galctosyl side chains of arabynogalactans (AGPs). Since the AGPs of the plant cell wall are classified between signal molecules, we think that their degradation is related to defense in plants. We performed the cloning and the expression analysis of the endo-β-(1-6)-D-galactanase gene *Clebg* in two races from *C. lindemuthianum* with different life style, pathogenic and saprophytic. Additionally, we performed a phylogenetic analysis of galactanases from Colletotrichum species.

Clebg gene of both races, pathogenic and saprophytic, contained an open reading frame consisting of 1398 bp (347 amino acids). The deduced amino acid sequence of both proteins showed similarity with other known endo-β-(1→6)-galactanases. The genetic expression of *Clebg* from *C. lindemuthianum* was analyzed by qPCR in mycelium grown in culture medium with glucose for 8 h, and Mathur′s medium containing arabinogalactan, xylan or cell wall from *Phaseolus vulgaris* as carbon source at 0, 2, 4, 6, 12, 24, 48 h and 3, 4, 5, 7 and 9 days. Expression of *Clebg* do not showed catabolic repression in presence of glucose. When mycelium of both races grew with plant cell wall, an increase of relative expression of *Clebg* (~8 fold) was detected in the pathogenic race (1472) but not in saprophytic race (0). This suggest that endo-β-(1-6)-D-galactanase might be a virulence factor. In culture medium with xylan or arabinogalactan, the pathogenic race (1472) showed major relative expression of *Clebg* (~6-12 fold respectively) compared with the saprophytic race (~4–4.5 fold respectively). These results suggest that other components of the cell wall besides AGPs and xylan are inducers of expression of *Clebg* in the pathogenic race but not in the saprophytic race.

Phylogenetic analysis of endo-β-(1→6)-galactanases of *Colletotrichum* species reveled clustering into clades related to host preference: monocots or dicots, where the *C. lindemuthianum* was grouped with the galactanase of *C. orbiculare*, which are both pathogens of dicots. It has been observed that the pathogenic fungi of monocot plants are better adapted to degrade the cell walls of monocot plants, and pathogens of dicot plants are better able to degrade the cell walls of dicot plants, a phenomenon that reflects host preference. This suggest that the galactanases from *C. lindemuthianum* species envolved according to their invasion/nutritional strategy and host preference.

Keywords: arabynogalactans, *Colletotrichum lindemuthianum*, endo-β-(1-6)-D-galactanase.

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WED-345
Combating norovirus-dependent gastroenteritis through RdRp inhibitors

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Noroviruses are members of the *Caliciviridae* family of positive sense RNA viruses, which cause rapid onset diarrhea and vomiting. Currently Norovirus infection is responsible for 21 million gastroenteritis yearly cases in the USA. Nevertheless no effective vaccines/antivirals are yet available to treat Norovirus infection. Since the activity of RNA-dependent RNA polymerase (RdRp) plays a key role in genome replication, the enzyme is considered a promising target for antiviral drug development.

In this context, we identified Suramin and NF023 as Norovirus RdRp inhibitors[1] that, however, are hampered by pharmacokinetics/toxicity problems. To overcome such problem, we analyzed the potential inhibitory role of naphthalene di-sulfonate (NAF2), a fragment derived from these two molecules[2] and of the related molecule pyridoxalphosphate-6-(20 -naphthylazo-60 -nitro-40,80 -disulfonate) tetrasodium salt (PPNDS)[2,3]. The crystal structures of human Norovirus RdRp/NAF2 and RdRp/PPNDS complexes revealed a new binding site that differs from that characterized for NF023/suramin. To further map the new potential inhibitory site, we focused on structurally related molecules that were synthesized following structure-driven information[4]. The synthesis process of Suramin-derivative compounds, led to the isolation of lower molecular weight intermediates hosting one sulphonate head. The crystal structures of both hNV/mNV-RdRps in complex with one of these compounds were analyzed, providing new knowledge on the interactions that a small fragment can establish with NV-RdRps, and establishing a platform for structure-guided optimization of potency, selectivity and drugability.

This work is supported by the European Commission SILVER project, withinthe 7th Framework Program Cooperation Project Grant Agreement No. 2606444.

Reference

Croci R, Pezzullo M, et al. "Structural bases of Norovirus RNA dependent RNA polymerase inhibition by novel Suramin-related compounds" PlosONE, article in press 2014; doi: 10.1371/journal.pone.0091765.

Keywords: Antivirals, enzyme crystal structure, RNA-dep RNA-Polymerase inhibitors.

WED-346
Comparative analysis of virulence of a novel, avian-origin H3N2 canine influenza virus in various host species

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Background: A novel avian-origin H3N2 canine influenza A virus (CIV) that showed high sequence similarities in hemagglutinin and neuraminidase genes with those of non-pathogenic avian influenza viruses was isolated in our routine surveillance program in South Korea. We previously reported that the pathogenicity of this strain could be reproduced in dogs and cats. Here, we aimed to determine the comparable infectivity and pathogenicity of the avian-origin H3N2 CIV in different animal species, including chickens, pigs, mice, guinea pigs, and ferrets.

Materials and Methods: Chickens, pigs, mice, guinea pigs, and ferrets were inoculated with A/Canine/Korea/01/2007 (H3N2), and then the samples collected form the animals were examined for antibody response, virus quantification by viral shedding, and pathologic lesions.

Results: The CIV infection resulted in no overt symptoms of disease in these host species. However, sero-conversion, virus shedding, and gross and histopathologic lung lesions were observed in guinea pig and ferrets but not in chickens, pigs, or mice.

Discussion and Conclusion: Based on the genetic similarity of our H3N2 CIV with currently circulating avian influenza viruses and the presence of α–2,3–linked rather than α–2,6–linked sialic acid receptors in the respiratory tract of dogs, we believed that this strain of CIV would have avian virus-like receptor specificity, but that seems to be contrary to our findings in the present study. Further studies are needed to determine the co-receptors of hemagglutinin or post-attachment factors related to virus internalization or pathogenesis in other animals.

Keywords: canine influenza virus, H3N2, interspecies transmission, sialic acid, model animals.

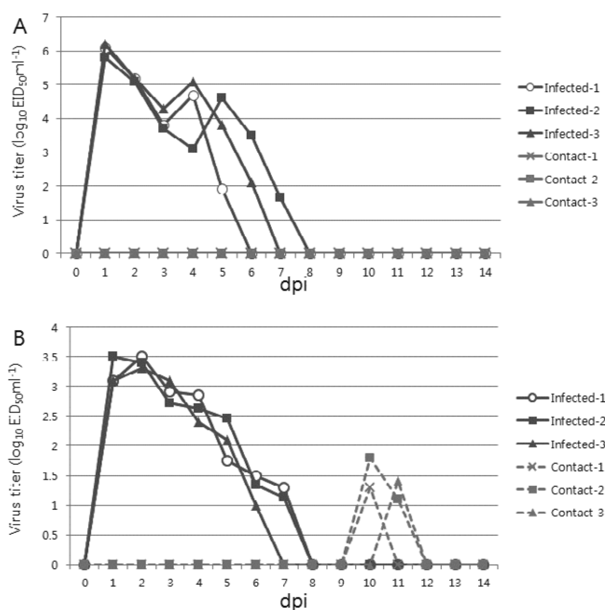

Fig. 1. Nasal shedding of H3N2 CIV from intranasally infected guinea pigs (A) and ferrets (B) and from directly contacted naïve animals. Guinea pigs and ferrets were intranasally inoculated with $10^{7.1}$ EID_{50} of virus, and naïve animals were paired with each infected animal species 6 hours post-inoculation. Nasal swabs were collected daily until 14 dpi, and relative viral titers were determined by a commercial one-step real-time reverse transcriptase-PCR (RT-PCR) kit.

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WED-347
Comparison of the thermotolerance genomic island present in *Cronobacter* spp.

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Cronobacter (formerly *Enterobacter sakazakii*) is a wide-spread opportunistic foodborne pathogen associated with serious infections in preterm neonates and immunocompromised adults. Rehydrated infant milk formula is the most common source of infection. *Cronobacter* is particularly tolerant to osmotic stress and desiccation and some strains of this genus are also tolerant to elevated temperatures. Thermotolerant strains are able to survive heating during infant formula reconstitution, therefore represent increased risk of infection. Up to 10% of *Cronobacter* strains contain a 18 kbp genomic island, which is responsible for increased thermotolerance. However, despite of the presence of the genomic island, differences in thermo-surviving ability between some strains were observed.

The aim of our study was to compare gene content of the thermotolerance islands from five *Cronobacter* and one *E. coli* strains and to establish its influence on thermal resistance. We observed that the island contained a cluster of conserved genes (*orf*B-Q), most of them had significant homologies with bacterial proteins involved in some type of stress response, including heat, oxidation and acid stress. By sequence comparison, two types of the genomic island were present in strains. Four strains contained full length sequenceand two other strains possessed shortened version of the island (genes *orf*B-D, *orf*O-P). The thermotolerance of all island-positive strains was higher than that of the mutant lacking the island. Measurements were also in agreement with the type of the island, strains containing complete region displayed higher thermotolerance than strains containing only partial island. Similar results were obtained in study of *E. coli* cells transformed with plasmids containing complete and shortened versions of island from *Cronobacter*. One exception were *C. sakazakii* NTU 696 and NTU 701 strains, despite of the similar organization of the islands, *C. sakazakii* NTU 696 possessed decreased thermal tolerance. However, this difference was caused by determinants outside from the thermotolerance region as transformation of *E. coli* with plasmids containing whole island from *C. sakazakii* NTU 696 or NTU 701 both increased thermotolerance. In conclusion, we propose that genes located at the beginning of this genomic island (*orf*C-D) contribute noticeably to the ability of the cells to withstand elevated temperatures, the other genes (*orf*H-Q) increase the thermotolerance of strains in lesser extent.

Keywords: *Cronobacter*, thermotolerance island.

WED-348
Compartive genomics of *Entrococcus faecium*

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Enterococci are Gram-positive bacteria that inhabit the gastrointestinal tract of humans and animals as commensal flora. In recent years two species, *E. faecalis* and *E. faecium* have become an increasing medical concern by virtue of their ability to gain and spread antibiotic resistance. We report the sequencing of vancomycin-resistant isolates of *E. faecium* from pig, chicken and cow. The assembled genomes were studied to determine if these strains differed from human isolates and to identify whether they had acquired genes for colonising each animal host. Phylogenomics of *E. faecium* were also performed to investigate the relationship between animal and human strains. The genomes of the chicken, pig and cow isolates differed in size (2.5–3.3 Mb) with the size difference due to acquisition of horizontally acquired elements (mostly phage, transposons and insertion sequences), for example the chicken isolate genome contained 10 prophages. A megaplasmid present in all three isolates was integrated into the genome of the chicken isolate. Comparison of the genomes also identified putative niche adaptation genes with a variety of proposed functions, including carbohydrate utilisation.

Keywords: None.

WED-349
Contribution of secreted aspartic proteases of pathogenic yeast *Candida albicans* to the neutralization of antimicrobial function of human high molecular weight kininogen

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Candida albicans is nowadays the most common fungal pathogen to humans. Its virulence depends on the production of multiple virulence factors, among which the secreted aspartic proteases (SAPs) have been proven to play major roles at various stages of candidial infections. This family comprises ten closely related proteolytic enzymes which display broad substrate specificity at different pH and have been shown to cleave numerous peptides and proteins of the host, with deregulating effect on the biochemical homeostasis of the host organism.

Human high molecular weight kininogen is a multifunctional glycoprotein composed of six domains, with the best recognized functions of a cysteine protease inhibitor (the D2 and D3 domains) and a precursor of vasoactive proinflammatory peptides, the kinins (the D4 domains). Only recently, it has been discovered to possess two distinct internal sequences in the domains D3 (NAT26) and D5 (HKH20) which can be excised by various proteases, such as neutrophil elastase, to release strong antimicrobial peptides (AMPs).

Taking into consideration constantly growing interests in the contribution of AMPs in the host defense against fungal infections, the current study was undertaken to verify a hypothesis that the diverse group of ten SAPs can neutralize the antifungal activity of kininogen-derived AMPs against *C. albicans*. It was shown that synthetic NAT26 and HKH20 significantly differed in susceptibility to SAP action. The helical, positively charged peptide, NAT26, was effectively cleaved by all SAPs but SAP10, whereby loosing its fungicidal properties, while the histidine- and lysine-rich peptide, HKH20, was cleaved and inactivated only by SAP9. The early products of SAP9-dependent cleavage of NAT26 and HKH20 included the NATFYFKIDNVKK plus ARVQVVAGKKYFI and the HKHGHGHGKHKNK plus GKKNGKH mixtures, respectively, and on their expense the NATFYFKID and HKHGHGHGK peptides appeared at the later phase. Similar peptides, devoid of antifungal activity, were also found among the products, obtained after fragmentation of the intact kininogen molecule by SAPs.

Taken together, the current results strongly suggest that SAP9 plays the exceptional, among all SAPs, role in the neutralization of recently discovered fungicidal activity of human kininogen.

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Keywords: antimicrobial peptides, kininogen, secreted aspartic proteases.

WED-350
Cytotoxicity and expression pattern of thermostable direct hemolysin (TDH), a main virulence factor of *Vibrio parahaemolyticus*

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Vibrio parahaemolyticus, a gram-negative marine bacterium, is a worldwide cause of food-borne gastroenteritis. Thermostable direct hemoysin (TDH) is a factor responsible for Kanagawa phenomenon caused by pathogenic *Vibrio parahaemolyticus*. Two tdh genes, *tdhA* and *tdhS*, were identified in the genome sequence of wildtype *V. parahaemolyticus*, RIMD2210633 and TDH-deficient mutant strains were constructed. They showed a complete abolishment of hemolytic activity, an attenuated cytotoxicity against human cell lines, and a significant reduction in mouse lethality. Cytotoxicity of *V. parahaemolyticus* against cell lines occurred independently from caspase, as shown in an experiment using pan-caspase inhibitor. TDH-induced cell death demonstrated characteristics of necrosis, and was diminished by necrostatin-1, an inhibitor of necroptosis. Reporter fusions between the tdh protmotors and the luciferase gene indicated that the *tdhA* gene is expressed higher than *tdhS*, and expression of both tdh genes was induced by presence of crude biles or an iron chelator in the medium. Two global regulators, ToxR (cholera toxin transcriptional activator) and IscR (Fe-S cluster regulator), were found to control expression of the *tdhA* gene. These studies on TDH identified physiological signals and/or transcription factors responsible for modulation of pathogenesis of *V. parahaemolyticus*.

Keywords: thermostable direct hemolysin, *Vibrio parahaemolyticus*.

WED-351
Degradation of proteinaceous components of neutrophil extracellular traps by aspartic proteases secreted by pathogenic yeasts, *Candida albicans*

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Introduction: A dimorphic yeast-like fungus, *Candida albicans* is an opportunistic pathogen in humans. It can be detected in up to 70% of healthy individuals as a normal component of endogenous microflora but in patients with weakened immune system or imbalanced microbial environment causes disorders with variable severity. The manifestation of the pathogenic nature of *C. albicans* depends on its numerous virulence factors, with one of the most important role played by secreted aspartic proteases (SAPs).

Neutrophils serve as the first line of defense and the critical part of the innate immunity. Apart from the phagocytosis and extracellular degranulation, they are able to kill microbes through the release of neutrophil extracellular traps (NETs) that consist of DNA and antimicrobial proteins such as histones, myeloperoxidase, lactoferrin and azurocidin.

Aims: Classification of the major proteinaceous components of NETs in terms of their susceptibility to proteolytic action of SAPs – identification of potential SAP targets as well as proteins that are resistant to this degradation, thereby being potential weapons in the fight against *C. albicans*.

Results: The identification of proteinaceous components of NETs that can be the substrates for SAPs was conducted with the use of supernatants from the cultures of two morphological forms of *C. albicans*. Five potential targets for SAPs were selected. Four of them were histones (H2A, H2B, H3 and H4), known to constitute the main NET components, while the fifth one was an antifungal protein, calprotectin. To verify the obtained results, a number of degradation assays with purified enzymes were carried out. It was demonstrated that SAPs displayed variable ability to degrade components of NETs, with histones being the most susceptible to the proteolysis, mainly by the action of SAP1, SAP2, SAP3 and SAP9. The citrullination of histones, a posttranslational modification likely to occur during the NET formation, significantly slowed down the SAP-dependent degradation of these proteins. The proteolysis of calprotectin occurred at a limited rate, while myeloperoxidase, lactoferrin and azurocidin were resistant to the degradation by any SAP.

Conclusions: The results of the current study suggest that *C. albicans* may use SAPs for the neutralization of the fungicidal forces of NETs. On the other hand, the resistance of myeloperoxidase, lactoferrin and azurocidin to SAPs suggests a role of these proteins in the fight against *C. albicans*, this major fungal pathogen to humans.

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Keywords: None.

WED-353
Differential intestinal colonization of *Escherichia coli* Nissle 1917

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Escherichia coli Nissle 1917 (here after EcN) is a probiotic isolated during World War I and since then utilized to treated intestinal disorders, like chronic constipation and colitis. Since its discovery, EcN has been widely used to shorten the duration of diarrhea in children and to alleviate intestinal inflammation in patients with inflammatory bowel disease and ulcerative colitis. EcN is such a great probiotic because is highly protective against intestinal pathogens and trigger both pro- and anti-inflammatory local cytokines. Also, EcN has also been shown to mitigate experimental colitis in mice while also reducing expression of the pro-inflammatory cytokines. EcN oral administrated can also induce systemic humoral immunity in infants as well as induce specific antibodies in the mucosa. The unknown aspects of EcN are its intestinal colonization preferences, levels of mucosal penetration and the influence of the microbiota on EcN colonization. Here we evaluated EcN intestinal lumen and mucus colonization preferences using the colitis mice model versus mice with normal intestinal microbiota. We determined that EcN colonized the ciego and colon, mainly in the proximal and middle in streptomycin treated mice. EcN did almost not colonize the small intestine in streptomycin treated mice in contrast to normal mice. EcN colonize the entire intestine in not

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treated mice, however its colonization was transient in contrast to microbiota-depleted mice. In all cases, EcN penetrate the mucus layers system. The mucus layer system organization is probably instrumental for a balanced and symbiotic relation between host and bacteria. The Muc2 mucin is the major protein component of the mucus layer and this mucus system occurs in two distinct layers: an inner layer that is devoid of bacteria and an outer layer that is the habitat of the microbiota. Using purified mouse Muc2 mucin from different regions along the intestinal tract we determined that that EcN has a chemotactic responses to Muc2 mucin from the duodenum, proximal and middle colon. These results suggest that EcN has a preference for colon, and Muc2 and microbiota influence this differential colonization.

Keywords: *E. coli* Nissle, intestine, colonization.

WED-354
DNA binding activities of DicA, H-NS and Cnu on the promoter region of the *dicA* gene in *Escherichia coli*

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Cnu, a small protein (71 amino acids), complex with H-NS and the complex binds DNA. Over production of a variant of Cnu, CnuK9E and H-NS complex has been reported to cause filamentous growth of WT *E. coli* cells at 37 °C but not at 25 °C (PLoS ONE 7(9): e45236, 2012). The charge reversal from positive to negative at 9th residue of Cnu might have caused unstable binding activity of DicA to its own promoter only at 37 °C, resulting in filamentous growth of the host cells. In this study, we have investigated the DNA binding activities of the three proteins, Cnu, H-NS and DicA to the promoter of *dicA*, *PdicAC*. We employed 'Crude EMSA': A crude protein preparation was subjected to EMSA (Electrophoretic Mobility Shift Assay). The specific DNA binding activity of DicA to *PdicAC* was detected from the crude protein preparation of the WT *E. coli* cells. To examine the role of Cnu and H-NS in DNA binding activity of DicA to *PdicAC*, we analyzed the mobility shift of the DicA bound 89 nucleotides-long *PdicAC* DNA fragment in crude protein preparations from mutant strains where the gene for Cnu or H-NS was deleted. We also performed Crude EMSA by adding the missing protein (purified) to the mutant crude protein. The binding reactions were performed at 2 different temperatures, 25 °C and 37 °C. The followings are the results we obtained from these experiments.

1. DicA binding to *PdicAC* occurs at a specific site (20 nucleotides) called *Oc*, and this binding can occur without H-NS or Cnu both *in vivo* and *in vitro*.
2. In order for a stable DNA-Protein complex to be formed on *PdicAC*, the DicA protein should be bound to *PdicAC* for H-NS and Cnu to bind.
3. The nucleoprotein complex of the DicA-HNS-Cnu bound on *PdicAC* is stable at 25 °C but becomes unstable at 37 °C.

Keywords: DicA, H-NS, Cnu, filamentous growth, temperature-dependent DNA binding.

WED-355
Dogs' susceptibility to human influenza viruses (A/H1N1, A/H3N2, and B): a mixing vessel to threaten human health?

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Consecutive outbreaks of epidemics of canine influenza virus from avian (H3N2) and equine origin (H3N8) among dogs in various countries have raised concern regarding the role of canines in the zoonotic transmission and novel recombination of influenza viruses. We investigated the pathogenicity and transmissibility of the human seasonal H3N2, pandemic (pdm) H1N1 (2009) and B influenza viruses in dogs so as to elucidate the probability of reverse zoonosis of human influenza viruses in canines. Further, seropositive samples from dogs, collected between 2007 and 2011, were screened for these viruses. Dogs inoculated or exposed to humn H3N2 virus developed symptoms of viral infection and we confirmed transmission of the virus from inoculated to naïve dogs. In contrast, the transmissibility of pdm H1N1 from dogs inoculated with this virus was limited. Further, evidence of pdm H1N1 infection as well as dual infection with pdm H1N1 and canine H3N2 virus were found in sera collected from field dogs during 2010 and 2011. We found no evidence of pathogenicity and viral reproduction after inoculation with influenza B virus. Our results indicate that dogs could potentially be potential hosts for human origin flu viruses (pdm H1N1 and seasonal H3N2), which would imply the possibility of dual infections with those human influenza viruses and canine-specific influenza virus.

Keywords: dogs, influenza virus, Mixing vessel.

WED-356
Dynamic modularity of host protein interaction networks outcome for *Salmonella Typhi* infection

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The availability of large scale protein-protein interaction networks represents the backbone of molecular activity within cells, thus providing an opportunity to comprehensively understand the mechanism of diseases. Integration of expression data with protein-protein interaction network may provide clues to the dynamic features of these networks. *Salmonella Typhi* infection that leads to typhoid fever is a leading concern for human health in the developing world. Although previous studies had identified putative therapeutic targets of typhoid fever, the underlying molecular mechanism of pathogenesis remains unclear.

We developed a network-based comparative analysis approach that integrates protein-protein interactions with gene expression profiles to reveal conditional specific subnetworks. Applying this method to a group of typhoid fever-infected Nigerian children, we identified Salmonella infection specific subnetwork. We found that a number of hub proteins of this subnetworks tend to be differentially expressed between different biological conditions. The difference in dynamics of that network in typhoid fever in compared to other disease conditions further suggest a potential molecular model of typhoid fever.

Here, we propose a method to analyse the interaction networks in different biological states specifically in infection dis-

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eases. It successfully reveals condition specific subnetwork of human protein-protein network; more importantly, these network dynamics provide new insights into the pathogenesis of typhoid fever. The revealed components (both hub and their selected interactors) of subnetwork might be used as potential drug targets and provide new directions for typhoid fever prevention.

Keywords: *Salmonella typhi*, Protein-protein interactions, Subnetwork.

WED-357

Dynamic regulation of Pathogenicity Islands and multiple antibiotic resistance loci in *Salmonella typhimurium*

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Salmonella enterica serovar typhimurium (*S. typhimurium*) is a common food borne pathogen capable of causing diseases ranging from the self-limiting gastroenteritis to the life-threatening systemic infections. For successful infection, the bacterium employs flagella, fimbriae, and Type 3 secretion systems encoded on loci, called Salmonella *Pathogenicity* Island (SPI)-1 and SPI-2. SPI1 is critical for the bacterium to gain entry into the host cell, while SPI2 is essential for survival once inside the host. In response to the immune response from the host, *Salmonella* activates the genes expressed in the *mar/sox/rob* regulon which are responsible for enabling the bacterium to survive in the harsh and toxic intra-cellular environment.

In this work, we present evidence that the expression of SPI1 and SPI2 are controlled by the *mar/sox/rob* regulon in *Salmonella*. We demonstrate that this enables the cell to control the timing of activation and deactivation of the SPIs, both at the population and single-cell level. We also demonstrate that feedback from the *mar* regulon is an active part of this control strategy, and is responsible for switching off SPI1 and switching on SPI2, once the bacterium gains entry into the host.

Keywords: gene regulation, *Salmonella typhimurium*, SPIs.

WED-358

Dynamics of linker residues modulate the nucleic acid binding properties of the HIV-1 nucleocapsid protein zinc fingers

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The HIV-1 nucleocapsid protein (NC) is a small basic protein containing two zinc fingers (ZF) separated by a short linker. It is involved in several steps of the replication cycle and acts as a nucleic acid chaperone protein in facilitating nucleic acid strand transfers occurring during reverse transcription. Recent analysis of three-dimensional structures of NC-nucleic acids complexes established a new property: the unpaired guanines targeted by NC are more often inserted in the C-terminal zinc finger (ZF2) than in the N-terminal zinc finger (ZF1). Although previous NMR dynamic studies were performed with NC, the dynamic behavior of the linker residues connecting the two ZF domains remains unclear. This prompted us to investigate the dynamic behavior of the linker residues. Here, we collected ^{15}N NMR relaxation data and used for the first time data at several fields to probe the protein dynamics. The analysis at two fields allows to detect a slow motion occurring between the two domains around a hinge located in the linker at the G35 position. However, the amplitude of motion appears limited in our conditions. In addition, we showed that the neighboring linker residues R29, A30, P31, R32, K33 displayed restricted motion and numerous contacts with residues of ZF1. Our results are fully consistent with a model in which the ZF1-linker contacts prevent the ZF1 domain to interact with unpaired guanines, whereas the ZF2 domain is more accessible and competent to interact with unpaired guanines. In contrast, ZF1 with its large hydrophobic plateau is able to destabilize the double-stranded regions adjacent to the guanines bound by ZF2. The linker residues and the internal dynamics of NC regulate therefore the different functions of the two zinc fingers that are required for an optimal chaperone activity.

Keywords: HIV-1 Nucleocapsid, NMR Spectroscopy, Protein dynamics.

WED-359

Efficacy of *Parthenium hysterophorus* for subduing the cause of *Fusarium* wilt in potato

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Fusarium wilt, caused by *Fusarium solani,* is an economically important disease of potato in Pakistan. Presently, pathogenic potential of *F. solani* was studied by inoculating potato plants with three *F. solani,* strains to screen the most virulent isolate among *F. solani* FCBP-016, *F. solani* FCBP-434 and *F. solani* FCBP-470. Pathogenicity test depicted that *F. solani* FCBP-434 was the most pathogenic isolate with variation in genetic level that was determined by RAPD-PCR. *F. solani* FCBP-434 was 55.66% different with both isolates. This disparity in genetic constitution might be cause of high pathogenicity. Afterwards, antifungal bioassays were conducted to confirm mycotoxic potential of root, shoot and leaf of *Parthenium hysterophorus* against *F. solani* FCBP-434 using 1–4% concentrations of aqueous, methanol and n-hexane extracts. Bioassays revealed that growth of *F. solani* FCBP-434 was greatly inhibited at 1 and 2% concentrations of aqueous and methanol leaf and stem extracts while 3 and 4% concentrations of n-hexane extract proved more effective in suppressing the growth. Among root extracts, higher concentrations of aqueous and n-hexane exhibited more promising results by causing reduction of 85 and 74%, of the growth, respectively, whereas in methanol extract again lower concentrations were more inhibitory.

Keywords: aqueous and organic solvents, Biological control, Fusarium wilt.

WED-360

Elucidating the genetic and molecular basis of gut immunocompetence variation in a *Drosophila melanogaster* population

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Drosophila melanogaster is emerging as a valuable model system to study the intestinal response to infection. Many aspects of this response have been uncovered using classical genetic approaches including pathogen recognition, the induction of antimicrobial peptides, the production of reactive oxygen species, and the restoration of gut homeostasis. However, little is known about the rel-

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ative contribution and relevance of each mechanism in a natural population. We have performed enteric infection with the entomopathogenic bacterium *Pseudomonas entomophila* of 140 lines from the Drosophila Genetic Reference Panel (DGRP), and observed extensive variation in survival following infection. To investigate the genetic basis of this variation, we performed a genome-wide association analysis, which allowed the identification of multiple loci with small effects. In parallel, we explored global differences in intestinal gene expression before and after infection in resistant and susceptible lines and found distinct transcriptome signatures between the two phenotypic groups. The main findings of this study will be presented and discussed in details.

Keywords: DGRP population, GWAS- Transcriptome variation, Immuno-competence variation.

WED-361
Elucidation of immune recognition of zwitterionic polysaccharides by synthetic oligosaccharides

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Capsular polysaccharides (CPSs) are crucial for the survival of bacteria in a mammalian host. CPSs are constantly exposed to the environment, interact with and actively modulate the host's immune system. Zwitterionic polysaccharides (ZPSs) are found on the surface of commensal and pathogenic bacteria and contain both positive and negative charges in each repeating unit. ZPSs are key players in the immunomodulatory function of commensal bacteria and induce an immune response in a T-cell dependent manner [1]. However, the precise mechanisms of immunomodulation by ZPSs are still ill-defined.

To unravel the biological implications of ZPSs, structurally defined oligosaccharide probes are needed that can be chemoselectively conjugated to suitable reporter molecules. However, the presence of a free amino group in the unusual monosaccharide 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (D-AAT) is incompatible with most common conjugation methods[2].

We describe here the synthesis and immunological evaluation of zwitterionic oligosaccharides that can be chemoselectively coupled to reporter moieties, such as microarray surfaces. By applying these conjugation-ready glycans in immunological settings, we elaborate on the role of the rare sugar D-AAT on the recognition of ZPSs by the immune system.

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Keywords: commensal, polysaccharide, synthetic oligosaccharides.

WED-362
Evaluation of detergent efficiency in controlling *Enterococcus faecalis* propagation

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Enterococcus faecalis, a gram-positive bacterium ubiquitous in the environment and normal inhabitant of the gastrointestinal tracts of humans and other mammals, has now emerged as a major health problem. The threat is exacerbated by *E. faecalis* ability at surviving adverse conditions, including prolonged stay on environmental surfaces. Its proneness to acquire resistance to various antibiotics by horizontal gene transfer mechanisms and the potential retention of antibiotics resistance in *E. faecalis* biofilms after treatment, have furthered the difficulty in controlling this pathogen's propagation. Here we report *E. faecalis* tolerance to sodium N-lauroylsarcosinate (sarkosyl) and sodium dodecyl sulfate (SDS), two anionic detergents that are routine household chemicals.

A clinical isolate of *E. faecalis* and *Escherichia coli* strains HB101, HB101(pBR322), HB101(pBR325) were grown in Luria-Bertani (LB) broth or on LB-agar with various concentrations of either detergents and various antibiotics concentrations. Results showed that the *E. faecalis* strain exhibited plasmid-mediated resistance to kanamycin (kan) (minimum inhibitory concentration (MIC) 2 mg/ml) and tetracycline (tet) (MIC 50 μg/ml). Rapid plasmid isolation was facilitated by the use of sarkosyl and produced two small plasmids (~ 7 and 5.7 kb) as identified by gel electrophoresis in 1% agarose. The plasmid DNA thus prepared would transform *E. coli* and render it resistant to kan (MIC 2 mg/ml) and tet (MIC 50 μg/ml). Compared to *E. coli*, *E. faecalis* was very sensitive to sarkosyl and SDS, with a sarkosyl MIC of 0.1% (10% for *E. coli*) and a SDS MIC of 0.05% (6% for *E. coli*). Attempts to cure *E. faecalis* with SDS or acridine orange remained unsuccessful, but 3% curing was observed after growth in the presence of sarkosyl, reducing kan MIC to 50 μg/ml and tet MIC to 25 μg/ml. In contrast, 35% curing of plasmid-bearing *E. coli* was observed after growth in the presence of either SDS or acridine orange, but none was observed after growth in the presence of sarkosyl. A mutant *E. faecalis* strain with accrued sarkosyl resistance (MIC = 0.6%) was isolated after growth of the bacterium in the presence of 0.06% sarkosyl. The mutant did not exhibit cross-resistance to SDS, but it grew faster than the original strain and still carried kan and tet resistance plasmids. While the findings reported here showed the usefulness of sarkosyl in handling *E. faecalis*, they also emphasized the remarkable capacity of *E. faecalis* to resist harsh treatment and the potential emergence of strains with increasing tolerance to detergents commonly used for cleaning purposes in households. This, in turn, further increases the challenge of finding ways to control the spread of *E. faecalis* -propagated infections.

Keywords: *Enterococcus faecalis*, plasmid, sarkosyl.

WED-363
Exclusive potential of tagetes erectus for the management of alternaria diseases of tomato

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Tomato (*Lycopersicum esculentum* Mill.) is one of the most economically important vegetable crops in the world. It is estimated that diseases reduce tomato production to a greater extent worldwide. Natural plants derived compounds contribute a lot in fight against pathogens. In the current study the tomato plants were inoculated with *A. alternata* FCBP-573, *A. alternata* FCBP-479

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and *A. alternata* FCBP-349 for the scrutiny of the most pathogenic isolate among these. *A. alternata* FCBP-573 was screened out as the most pathogenic isolate. RAPD analysis confirmed that *A. alternata* FCBP-573 had variability in its genetic constitution with other two isolates; thus this disparity in genetic constitution might be a cause to stir up more pathogenicity in this isolate. Therefore, *A. alternata* FCBP-573 was selected as the most pathogenic isolate and subjected to biological control through *Tagetes erectus* L. In antifungal bioassays different plant parts of *T. erectus* with 1–4% concentrations of aqueous, methanol and n-hexane extracts of each part were evaluated against *A. alternata* FCBP-573. Results revealed that the growth of *A. alternata* FCBP-573 was greatly inhibited at 4% concentration of methanol extract followed by aqueous and n-hexane extract. Among different plant parts tested, root extract exhibited more promising results by causing 81–92% reduction in biomass. The study concludes that aqueous and organic extracts of ornamentals have potential to obstruct dreadful effect of pathogenic fungi by suppressing their growth. *T. erectus* conferred vital and surprisingly stable compounds having inhibitory potential against *A. alternata* FCBP-573.

Keywords: *Alternaria alternata*, *Tagetes erectus*, tomato.

WED-364
Exploiting the *Burkholderia pseudomallei* acute phase antigen BPSL2765 for structure-based epitope discovery/design in structural vaccinology

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Structure-based antigen engineering is frequently used in the vaccine development process to specifically modify protein antigens of a pathogen, to enhance their immunogenic properties, with the aim of improving their protective efficacy. Such approaches may entail engineering epitope-containing regions of the protein, or simply the epitope sequences themselves in the form of synthetic peptides.

In this context, we are involved in a multi-disciplinary ‘Genome-to-Antigen’ (GtA) project that aims to identify *Burkholderia pseudomallei* antigen candidates to enter into the vaccine discovery pipeline. 3D antigen structures form the basis for the application of *in silico*-based B-cell epitope predictions, combined with experimental validation and immunological testing. Overall, we aim to connect the understanding of structural properties at atomic resolution, to the reactivity properties of the protein (or specific epitopes) in an immunological context.

Among other antigens, we solved the crystal structure of *B. pseudomallei* acute phase antigen BPSL2765 in the context of a structural vaccinology study, in the area of melioidosis vaccine development. Based on the 3D-structure, we applied a recently developed method for epitope design that combines computational epitope predictions with *in vitro* mapping experiments. We successfully identified a consensus sequence within the antigen that, when engineered as a synthetic peptide, was selectively immune-recognized to the same extent as the recombinant protein in sera from melioidosis-affected subjects. Antibodies raised against the consensus peptide were successfully tested in opsonization bacterial killing experiments and antibody-dependent agglutination tests of *B. pseudomallei*. Our strategy represents a step in the development of immunodiagnostics in the production of specific antibodies and in the optimization of antigens for vaccine development, starting from 3D structural principles.

Keywords: *Burkholderia pseudomallei* antigen, structural biology, structure-based antigen engineering.

WED-365
Expression of mycobacterial glycosyltransferase GlfT1 in fusion with MBP

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Mycobacterium tuberculosis is a gram positive bacterium causing chronic illnesses. Tuberculosis is characterized by a disease persistence and difficulties with treatment. Fight against multi-drug resistant strains requires new antituberculosis drugs.

The mycobacterial cell wall is a complex of arabinogalactan, mycolic acids and peptidoglycan. Arabinogalactan consists of 30 5- and 6-linked β-d-galactofuranose (Gal*f*) units. It anchores in peptidoglycan by a α-l-rhamnopyranosyl-(1→3)-*N*-acetyl-α-d-glucosaminyl-phosphate linker. Two to three arabinan chains with 31 d-arabinofuranose (Ara*f*) units each, composed of 5-linked α-d-Ara*f* with branching by 3,5-α-d-Ara*f*, are bound to the galactan. (1) Mycobacterial GlfT1 is a membrane associated galactofuranosyl transferase cosynthesizing arabinogalactan (1). This enyme transfers the first and the second Gal*f* units from donor UDP-Gal*f* to the C50-P-P-GlcNAc-Rha acceptor, creating b-(1–>4) and b-(1–>5) linkages. GlfT1 is a member of the inverting GT-2 family and belongs to the GT-A superfamily (2). Glycosyltransferases are in difficult to express and purify.

A new MBP-GlfT1 construct has been created. Fusion to maltose binding protein (MBP) is useful for difficult targets. Various purification strategies have been combined delivering the protein in a quality for structural studies. The protocol prevents proteolysis, aggregation and chaperone inpurities. Glycosyltransferase assay confirmed activity after purification.

The research leading to the results obtained financial contribution from the EU under the 7th Framework Programme by CEITEC (CZ.1.05/1.1.00/02.0068) project from European Regional Development Fund

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Keywords: Cell wall, Glycosyltransferases, *Mycobacterium tuberculosis*.

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WED-366

Fecundity as one of possible factors contributing to the dominance of the wMel genotype of Wolbachia in natural populations of *Drosophila melanogaster*

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Alphaproteobacteria of the genus *Wolbachia* are common intracellular endosymbionts of a variety of insects (O'Neill et al. 1997). *Drosophila melanogaster* is infected with a single *Wolbachia* strain (*w*Mel), the proportion of infected individuals may range from as high as 40–60% to as low as 8% in populations from different regions (Solignac et al. 1994; Verspoor and Haddrill 2011). Among the known diversity of *Wolbachia* infecting *D. melanogaster*, a single genotype, *w*Mel, within the *w*Mel strain has been found to dominate over other genotypes world-wide (Riegler et al. 2005). The reasons for *w*Mel domination over the other genotypes remain unrevealed.

We have analyzed the infection frequency and genotypes of *Wolbachia* in 13 natural populations of *D. melanogaster* Ukraine (Chornobyl, Poliske, Chernobyl Nuclear Power Plant (CNPP), Kyiv, Motovylivka, Varva, Pyriatyn, Uman', Odesa, Yalta, Kharkiv, Inkerman, Drogobych). Sampling was carried out in late summer (August-September) of 2011-2013. Infection by *Wolbachia* was justified by PCR-analysis of the DNA from F1 individuals derived from wild-caught flies as described at O'Neil et al 1992 and Zhou et al 1998 and by genotype analysis as according to Riegler et al. (2005).

The results showed that the prevalence of infection ranges from 0.27 to 0.79. In some populations it remains stable over three sampling years (Kyiv), in others it significantly varies in different years (Uman', Odesa). For populations of Odessa and Kyiv we analyzed prevalence of infection during the period of imago fly-out (May-November), which showed a stable frequency in a given period in 2012 and considerable fluctuations in 2013. The analysis of genotypes showed the dominance of *Wolbachia* genotype *w*Mel in all populations in Ukraine. However, there is a stable population in Uman' showing rare genotype *w*MelCS, and in some populations (Varva, Odesa, Kyiv, Uman'2) a few individuals with the genotype *w*MelCS were found as well. This result indicates the genotype *w*MelCS is being maintained in population, but at a very low frequency which can indicate its much greater prevalence in nature.

Flies infected with *w*Mel demonstrated a significantly higher number of produced eggs (17.59 ± 1.96) per female compared with those infected with the genotype *w*MelCS (8.65 ± 1.24; $t = 8.94$, $p < 0.05$), which suggests that *w*Mel can confer some fitness advantage to its infected host by elevating the host's fecundity.

Our results suggest that different *Wolbachia* genotypes may confer differential fecundity in the infected host females, which we hypothesize, could be one of factors contributing to differential dispersal of the bacteria in *D. melanogaster* populations worldwide.

Keywords: *Drosophila melanogaster*, genotyping, Wolbachia.

WED-367

Fibronectin, a multifunctional human glycoprotein, tightly binds to proteins exposed on the cell wall of pathogenic yeasts, *Candida parapsilosis* and *Candida tropicalis*

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Candida parapsilosis and *Candida tropicalis* are currently considered as some of the most important fungal pathogens in humans amongst non-albicans *Candida* species. Both cause severe, often life threatening infections in immunocompromised individuals, but *C. parapsilosis* is more common infectious agent within neonates and patients with prosthetic devices, stents, or undergoing the parenteral nutrition while *C. tropicalis* is responsible for severe candidiasis in intensive care units patients, individuals with cancer and those treated with broad-spectrum antibiotic therapy.

One of the initial steps essential for the development of candidial infections is the adherence of fungal cells to the extracellular matrix (ECM) proteins, particularly to multifunctional, high-molecular weight glycoprotein, fibronectin, which is also involved in clotting, wound repair and host cell adhesion. Fibronectin adsorbed by yeasts provides a bridge to deeper tissues, facilitating the colonization of human organism by pathogens. The aim of the current work was to characterize fibronectin binding at the surface of *C. parapsilosis* and *C. tropicalis*, and to indicate the major fungal cell wall components involved in these interactions.

Our study showed the strong adsorption of fibronectin by *C. tropicalis*, as compared to significantly weaker binding of this ECM protein to *C. parapsilosis* cell surface. In both species, the fibronectin binding to filamentous, pseudohyphal forms was much stronger than that to unicellular yeast forms. Analysis of fibronectin binding by pseudohyphae with enzymatically decomposed major constituents of the fungal cell wall, i.e., glucan, mannan and cell wall proteins (CWPs), strongly suggested a predominant role of CWPs in this adsorption phenomenon. This hypothesis was also confirmed by a direct binding assay, using microplate-immobilized fibronectin and biotinynaled CWPs extracted with beta-1,3-glucanase. Moreover, with the use of affinity chromatography and chemical cross-linking coupled with mass spectrometric analysis, the major individual fibronectin-binding CWPs were identified, including amidase, hexokinase and elongation factor 2 in *C. parapsilosis* and fructose-1,6-bisphosphatase and transaldolase in *C. tropicalis*.

The detailed mapping of the interactions between ECM components and fungal surface can contribute to deeper understanding the pathogenesis of infections caused by these two emerging fungal pathogens.

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Keywords: extracellular matrix, candidiasis, adhesion.

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WED-368
Fragilysin as a sheddase: identification of released proteins

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Fragilysin (BFT) is a metalloprotease that is secreted by enterotoxigenic *Bacteroides fragilis*. There are three closely related fragilysin isoforms of identical length (BFT-1, -2, -3). In our study we have obtained recombinant BFT-1, -2, -3 in heterological system of *E. coli*. All the recombinant protein samples had biological activity when tested on HT-29 cells. We observed cell rounding and revealed E-cadherin cleavage after HT-29 cells treatment with fragilysin isoforms. We hypothesized that E-cadherin is a substrate for fragilysin. Nevertheless, all the recombinant fragilysins did not cleave recombinant E-cadherin obtained in *E. coli* and in Expi293 cells.

For identification of potential fragilysin substrate on cell surface we used LC-MS analysis of cultural medium after HT-29 cells treatment with BFT-2. We have identified 791 proteins by ProteinPilot software v 4.5 (833 proteins by Mascot search engine v2.2.07) in the cultural medium of HT-29 cells after BFT-2 treatment, compared to 552 proteins (636 by Mascot) in the control medium. Analysis of proteins released after BFT-2 teatment showed presence of several membrane proteins. Among them there are cell adhesion proteins (members of cadherin superfamily), membrane receptors, including ones involved in regulation of cell contact and adhesion, growth control, tumor invasion and metastasis. Beyond that, there were proteins with functions that are still unknown.

Keywords: *Bacteroides fragilis*, fragilysin, metalloproteinase.

WED-369
From monomer to tetramer: crystal structures of related restriction endonucleases AgeI and BsaWI

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Type II restriction endonucleases recognize short 4–8 bp nucleotide sequences and cleave phosphodiester bonds within or close to their target site. Due to their high specificity restriction endonucleases became invaluable molecular tools in genetic engineering and attractive models for studying protein-DNA interactions. Structural and bioinformatics studies revealed that group of restriction endonucleases, which share CCGG motif within their target sites, is evolutionary related. Seven crystal structures of restriction endonucleases, recognizing five variants of the CCGG containing were solved and structure comparison revealed that all these enzymes have a similar fold and CCGG recognition determinants. On the other hand, the DNA cleavage mechanisms of this small family represent the variety of the restriction endonucleases: they are active as dimers or tetramers, require the binding of one, two or three DNA targets for their optimal activity.

Here, we present crystal structures of two new members of this family AgeI (recognition sequence ACCGGT) and BsaWI (WCCGGW, W stands for A or T). These enzymes are composed of two domains: the N-terminal helical domain and the C-terminal catalytic domain. Despite the low sequence similarity (~15% identical aa), the domain structures of AgeI and BsawI are very similar. However, the relative position of the domains within the AgeI and BsaWI differs and results in differences of DNA binding and cleavage mechanisms. The N-terminal domain of AgeI is folded over the C-terminal domain and AgeI is a monomer, which dimerises only upon binding to the symmetric DNA target. Differently, the N-terminal domain of BsaWI is folded onto the C-terminal domain of the other BsaWI subunit, thus making a tight dimer. Moreover, the BsaWI dimers form tetramers through the contacts by the C-terminal domains. The BsaWI tetramer binds and cleaves two DNA targets. Interestingly, at high protein concentrations the BsaWI dimers can form inactive networks of proteins by the C-terminal domains' contacts with the different dimers.

This work was supported by the Research Council of Lithuania (grant MIP-41/2013 to Giedre Tamulaitiene).
Keywords: DNA cleavage, restriction endonuclease, X-ray structure.

WED-370
Functional characterization of a thrombospondin structural repeat containing rhoptry protein from *Plasmodium falciparum* merozoites

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Plasmodium falciparum invades a variety of host cells as it completes its complex life cycle in humans and mosquitoes. Host cell invasion requires multiple molecular interactions between host receptors and parasite ligands. A family of parasite proteins that contains the conserved thrombospondin structural repeat motif (TSR) has been implicated in receptor binding during the invasion of both hepatocytes and erythrocytes. In this study we have characterized the functional role of a TSR containing blood stage protein referred to as *P. falciparum* thrombospondin related apical merozoite protein (PfTRAMP). Both native and recombinant PfTRAMP bind normal human erythrocytes as well as erythrocytes treated with neuraminidase, trypsin or chymotrypsin suggesting that it binds a novel host receptor to mediate erythrocyte invasion. PfTRAMP is localized in the rhoptry bulb and is secreted during invasion. Adhesion of released microneme protein EBA-175 with glycophorin A (gly A), its receptor on erythrocytes, provides the signal that triggers release of PfTRAMP from the rhoptries. Purified rabbit antibodies against PfTRAMP blocked erythrocyte invasion by *P. falciparum* in growth inhibition assays suggesting that PfTRAMP plays an important functional role during erythrocyte invasion. Combination of antibodies against PfTRAMP with antibodies against microneme protein EBA175 provides an additive inhibitory effect against invasion. These observations suggest that targeting multiple conserved parasite ligands involved in different steps of the invasion process may provide an effective strategy for the development of vaccines against blood stage malaria parasites.

Keywords: None.

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WED-371

Functions of regulatory proteins encoded by the German cockroach densovirus: studying by transgenic *Drosophila melanogaster* model system and yeast two-hybrid technique

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Densovirus of German cockroach, BgDV1, belongs to a group of densoviruses that are insect infecting parvoviruses (Parvoviridae family, Densovirinae subfamily).

BgDV1 possess three regulatory proteins: NS1, NS2, and NS3, which are expressed during the virus productive infection. There exists significant basis to speculate that densovirus regulatory proteins play pivotal role in many processes of viral pathogenesis. Functional role of NS1 may be predicted based on the comparison with proteins of vertebrate parvoviruses and includes replication initiation, genome transcription and encapsidation regulation; the functional role of the NS2 and NS3 remains completely unknown and we predict it only by the comparison with protein databases.

In order to shed light onto the functions of BgDV1 NS proteins and to study their potential cytopathological effects, we aimed at developing a new model system simulating the virus host organism and based on a set of transgenic *Drosophila melanogaster* strains. Three of them contain in their genome NS1, NS2, and NS3 genes and are thus expressing one of the NS proteins under the control of GAL4-UAS system. Two other strains contain regions of two BgDV1 promoters allowing for the investigation of the NS impact on their activity. The model system can be further supplemented by additional strains expressing other virus proteins or containing the whole BgDV1 genome for the purposes of testing virus NS functions.

Utilizing the *Drosophila* model system developed we demonstrated that the expression of NS3 alone can cause the pathological effects. Namely, the flies expressing NS3 in all tissues of an adult organism showed slower development, extremely low eclosion rate, and decreased fertility if compared with the corresponding F1 progeny expressing NS1 or NS2 proteins. The highest mortality was observed during the pupae stage. We speculate that NS3 protein ability to induce the pathological effects can be mediated by its ability to transactivate the host cell promoters.

To further test this hypothesis we utilized the GAL4-based yeast two-hybrid system and when testing only one plasmid encoding GAL4 DNA-binding domain fused with one of the NS1, NS2, or NS3 proteins to restore potential transcription activator domain, we showed that NS3 protein only was able to induce the transcription of reporter gene.

Using the two-hybrid system we also studied the ability of BgDV1 proteins to interact with each other. We showed that NS2 protein is able to interact with itself forming homodimers. This ability may be rendered by the coiled-coil domain we bioinformatically predicted in the protein.

Keywords: densovirus pathology, protein interactions, transcription regulation.

WED-372

G4-DNA inhibitors of HIV entry

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We report here new anti-HIV DNA aptamers that inhibit an attachment step of the viral entry pathway. Their supposed mechanism of action involves interaction with the HIV envelop glycoproteins gp120 on the surface of virions, which prevents gp120 from binding CD4 receptors on the cellular membrane. The new DNA aptamers are monomolecular G-quadruplexes (GQs). Multiple studies of the last decade confirm anti-HIV activity of various GQs targeted against gp120, HIV-integrase or both. However, all known active structures are intermolecular GQs, and their potential folding variability (ambiguity) is an obvious disadvantage. Monomolecular structures are easier to handle and refold correctly after denaturation. The advantages of monomolecular GQs are generally acknowledged, therefore, certain attempts have been made, for instance, to link covalently the strands of tetramolecular aptamers to gp120. We report and compare for the first time several active monomolecular GQs, both natural and chemically-modified (thiophosphoryl), and discuss the impact of a particular folding type (antiparallel and parallel) on GQ inhibitory activity. Four-tetrad parallel native and various thio-modified GQs appear to be efficient HIV entry inhibitors (IC_{50} in the nanomolar range).

Our results suggest that a number of monomolecular GQs could be used in the development of new anti-HIV therapeutics or microbicides.

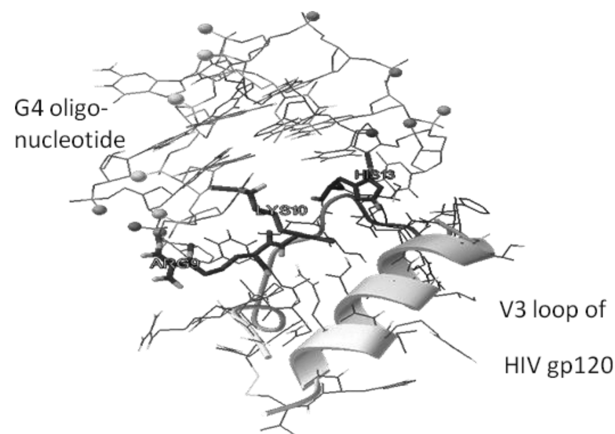

Fig. 1. Model, representing possible interaction of the gp120 V3 loop and the thiophosphoryl-DNA GQ (thio-GGTTGGTGTGGTTGG, IC_{50} about 70 nM).

This work was supported by Russian Science Foundation [14-25-00013].

Keywords: aptamers, G-quadruplexes, HIV.

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WED-373
Genome-wide analysis identifies gain and loss/change of function within the small multigenic insecticidal Albumin 1 family of *Medicago truncatula*

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Background: Albumin 1b peptides are small disulfide-knotted insecticidal peptides. To date, their diversity among the Fabaceae has been essentially investigated through biochemical and PCR-based approaches. The availability of high-quality genomic resources for the model species *Medicago truncatula* (*Mtr*) allowed for a genomic analysis of this protein family aimed at (i) deciphering the evolutionary history of this legume-specific protein family and (ii) exploring the genomic biodiversity within this species for novel bioactive molecules.
Results: Investigating the *Mtr* genome revealed a remarkable and hitherto unique expansion, mainly through tandem duplications, of A1 genes retaining nearly all of the canonical structure at both gene and protein levels. Phylogenetic analysis resolved six clades among the 53 homologous unigenes detected, and revealed that the ancestral molecule was most probably the insecticidal one giving rise to, among others, a clade of short disulfide-bonded peptides, non-insecticidal and involved in root nodule signaling (A1b-nodulins). Expression meta-analysis revealed many silent genes and a wide tissue distribution of the A1 transcripts/peptides within plant organs. *Mtr* did not use the original peptide as a form of seed insecticidal defense, but it has given rise to two other clades expressing derived insecticidal function in roots/nodules. Evolutionary rate analyses highlighted branches and sites with positive selection signatures, including two sites shown to be critical for insecticidal activity (Da Silva *et al. JBC* 2010). Seven peptides were chemically synthesized and folded *in vitro*, then assayed for their biological activity. Among these, AG41 (*aka MtrA1013* isoform, encoded by the orphan TA24778 contig.), showed an unexpectedly high insecticidal activity.
Conclusion: Our study highlights the unique burst of diversity of this peptide family within the Trifoliae clade compared to the other taxa for which full-genomes are available: no A1 member in *Lotus* and only a few in soybean and in *Cajanus*. Such expansion is reminiscent of the situation described for other disulfide-rich peptide families (NCR) discovered within the same species.
Keywords: biopesticides, disulfide-knotted insecticidal peptides, model species *Medicago truncatula*.

WED-374
Getting an insight into the antibacterial activity of the plant derived alkaloid (-)-roemerine against *E. coli* cells

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Seeking new antibacterials that weaken bacterial resistance mechanism(s) is one of the remedies to counteract increasing antibiotic resistance. Plant-derived alkaloids are in the limelight for screening potential drug candidates due to their substantial biological activities that includes antibacterial, antifungal, antimalarial and anti-HIV activities. Unfortunately, to a large extent different multidrug resistance pumps reduce their effects. Hence it is important to identify their targets in designing new molecules. (-)-Roemerine is an alkaloid synthesized by several plant families possessing antimicrobial activity. Yet its mechanism of action of this alkaloid remains unclear. To this end, the effect of this alkaloid has been investigated using a proteomics approach. Upon treatment with 100 microgram/ml (−)−roemerine, we have identified 15 differentially expressed proteins in *E. coli* TB1 cells. 9 of these proteins were down-regulated whereas 6 were up-regulated. Among the up-regulated proteins were proteins involved in cell adhesion, outer membrane component, phosphoenolpyruvate-dependent sugar phosphotransferase system, bacteriosin transport, amino acid transport and cell redox homeostatis. The down-regulated proteins were involved in carbohydrate transport, asparagine metabolic process, protein biosynthesis and amino acid biosynthesis. These results give clues on how the bacterial defense mechanism becomes ineffective. This work has been supported by TUBITAK-MAG Project with the number 113M052.
Keywords: (-)-roemerine, *E. coli*, proteomics.

WED-375
Gingipains, cystein proteases produced by *Porphyromonas gingivalis*, disregulate the system controlling activity of kallikreins

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Porphyromonas gingivalis is the main etiological agent of periodontitis, also implicated in the development of rheumatoid artritis and aspiration pneumonia. The latter life-threatening condition could be caused by inhalation of bacteria into respiratory system by accidental aspiration of saliva or during medical procedures. Although the importance of *P. gingivalis* in development of disease is well documented, the exact mechanism still remains elusive. Animal studies showed massive destruction of lung tissue, what may suggests upregulated activity of proteases responsible for degradation of extracellular matrix proteins. One of the largest group of such enzymes is kallikrein-related protease family (KLKs). Due to the fact that their action is tightly controlled by production as inactive zymogens and expression of specific protein inhibitors from SPINK (serine protease inhibitor Kazal-type) family, dysregulation of this control systems may lead to excessive proteolytic activity within tissue. The aim of presented study was to verify if gingipains, main extracellular proteases produced by the pathogen, could activate proforms of KLKs on one hand, and degrade their inhibitors on the other. For that purpose we created and developed a novel system based on soluble proteolytically-resistant carrier protein with exposed amino acid sequence representing cleavage site responsible for activation of KLKs. Moreover, we found out that gingipains are able to degrade SPINK6 and domens of SPINK5 leading to their inactivation.

We conclude that ability of gingipains to activate proKLKs together with hydrolysis of SPINK inhibitors may in part explain massive degradation of tissue leading to severe impairment of host homeostasis and defense systems enabling spreading and progression of disease.
Keywords: aspiration pneumonia, gingipains, kallikreins.

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WED-376

Hepcidin is a war tool between uropathogenic *E. coli* and renal host cell during urinary tract infection

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Introduction: Urinary tract infection (UTI) is mainly due to uropathogenic *Escherichia coli* (UPEC), which have the ability to adapt to iron limitation, acidic pH of urine and strong host defenses of the urinary tract. Hepcidin is a 25 amino acid peptide that regulates iron homeostasis but it has also been shown to exhibit an antimicrobial activity. The liver is the major source of bloodstream hepcidin although this peptide was recently found to be expressed in several epithelial barriers that are frequently confronted to pathogen infection, including renal tubules. We have recently shown that hepcidin controls iron excretion by regulating its reabsorption in distal nephron (B. Moulouel et al., kidney Int, 2013). However, whether hepcidin plays a role in the protection against UTI, has never been investigated.

Methods: Two different bacterial strains (CFT073 and K12) were tested and the impact of their infection was analyzed in WT and hepcidin Knockout (Hepc−/−) mice. We measured renal cytokines levels by multiplex ELISA and Bacterial growth with a Tecan Infinite reader. Structural architecture of CFT073 was analyzed by electronic microscopy. The expression of hepcidin in liver and kidney were estimated by RT-qPCR. BMP/SMAD and IL6/STAT3 signaling pathways that control hepcidin expression were investigated in mice, and in renal mIMCD-3-and hepatic HepG2-infected cells.

Results: (i) Using microdissected renal tubules, we showed that hepcidin is preferentially expressed in distal nephron tubules. (ii) Significant UPEC infection was observed in the Hepc−/− kidneys compared to control. However, pre-treatment of WT mice or of CFT073 with hepcidin synthetic peptide, significantly blocked the UTI. (iii) Incubation of CFT073 with Hepcidin synthetic peptide inhibited considerably their growth and disturbed their cell envelope with obvious cell lysis. (iv) In Hepc−/− kidneys, iron was accumulated in the medulla, both renal atp4a and atp12a pumps were repressed and urine alkalinized. (v) In infected Hepc−/−, excess iron was widely consumed by CFT073, and the inflammatory response was significantly attenuated. (vi) CFT073 significantly repressed renal hepcidin despite large increase of TLR4- and IL6-signaling. Infection of mIMCD-3 and HepG2 cells with fixed CFT073, also inhibit hepcidin expression. This effect was mediated via reduction of SMAD-signaling both in mice and cell lines.

Conclusions: These data strongly suggest that renal hepcidin may be one of the targets that UPEC trigger to become highly pathogenic and evade renal host defenses during UTI.

Keywords: Hepcidin, iron metabolism, Urinary Tract infection Uropathogenic *E. coli*.

WED-377

Higher parasite burden in asymptomatic individuals in endemic areas of Visceral Leishmaniasis

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Background: In an area endemic for Visceral Leishmaniasis (VL), subclinical or asymptomatic infections play a crucial role in progression of disease. We determined the parasite load by quantitative PCR (qPCR) in healthy infected population living in an area endemic for Visceral Leishmaniasis. We enrolled 13366 persons from 11 villages of highly endemic region of Bihar, India.

Materials and Methods: We conducted two sero-surveys by rK-39 ELISA and DAT with 1 year time interval for identification of incident infected healthy (Asymptomatic) individuals. Parasite load by using TaqMan based qPCR were done on peripheral blood using kDNA specific primers and probe on these sero-converted individuals and its matched control populations. Individuals having parasite load greater than 1 genome/ml of blood was considered as positive by qPCR. Follow-up visit to the homes of each individual were made to monitor the disease conversion in this cohort.

Results: Between seroconversion and qPCR were accessed by kappa value. Total 235 persons were converted their serology within 12 month intervals. Of these 235 sero-converters 105 (44.6%) individuals were also positive by qPCR. However, similar number of controls groups (87/ 237, 37%) also showed positivity by qPCR. The agreement between sero-converter and qPCR was poor (k = 0.12). And among all individuals only one was progress to disease.

Conclusion: So there is no major difference in qPCR result between sero-negatives and sero-converters and among all individuals only one were converted into disease that has parasite load 146 parasite genome/ml of blood. These findings suggest the usefulness of parasite load in healthy individuals living in an endemic area of Bihar and contribute as a good tool for VL elimination programme.

Keywords: Asymptomatic, Seroconversion, Visceral Leishmaniasis.

WED-378

Host-pathogen interrelations in human corneal infections difficult to diagnose and treat

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Several species of the genus *Acanthamoeba* that complete their life cycles in natural environments of various parts of the world including Poland can infect man and exist as pathogens. Humans are exposed to both, the non-pathogenic and pathogenic strains of *Acanthamoeba*. Some strains of the amphizoic organisms present a serious risk to human health as causative agents of a vision-threatening corneal infection, *Acanthamoeba* keratitis that develops particularly in contact lens wearers. In the pathogenesis, extracellular proteases, among others, are host tissue degradation determinants in *Acanthamoeba* infections. The amoebae may act as carriers for more than 20 bacterial species pathogenic for humans from genera *Legionella, Pseudomonas, Mycobacterium, Escherichia, Listeria* that are able not only to survive but even proliferate within the amoebae, thus play an important role in

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the dispersion of potentially pathogenic microorganisms. Recently, it is considered that vision-threatening *Acanthamoeba* keratitis emerging disease in more than 85% is linked with isolates of the T4 genotype. The disease is difficult to treat due to misdiagnosis as a viral infection with *Herpes simplex*, fungal with *Fusarium spp*. or keratitis caused by *Pseudomonas aeruginosa*. Here, we present complicated, difficult to diagnose and treat incidences of keratitis suspected of fungal, bacterial and/or amoebic aetiology in three Polish patients.

We analyzed selected data in terms of usefulness of *in vivo* and *in vitro* investigations for the diagnosis and therapeutic management. The severe pain, epithelial defects and reduced visual acuity appeared in affected eyes of all patients. Active epithelial inflammations and hyper reflective tissue in corneal ulcer were revealed by slit-lamp. A diagnosis of corneal infection in terms of mixed bacterial, fungal and A*canthamoeba* or amoebic only aetiology has been made by microbiological investigations. Use of corneal scrabs cultured *in vitro* and PCR techniques allowed to detect mixed infections of eye with *Pseudomonas aeruginosa* and *Acanthamoeba sp*. in one patient and identify mixed corneal infection with yest-like *Candida sp*. and *Acanthamoeba* sp. in the other one. Hyper reflective objects visualized by *in vivo* confocal microscope in the affected eye of third patient have determined by *in vitro* cultures as severe amoebic infection. Complex aetiology, late detection of corneal infections, factors influencing diagnostic and therapeutic difficulties in the cases analyzed are discussed.

Keywords: bacterial, fungal, amoebic keratitis, in vitro cultures, genotype determination.

WED-379
Host stress hormone noradrenaline interferes with *Pseudomonas aeruginosa* social behaviors in an iron dependent manner

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Bacteria are able to recognize and respond to host-related signaling compounds, as hormones, by mechanisms that are widely unknown. Even if there is a great interest on deciphering pathogens-host communication during infection, very few data regarding the role of catecholamine stress hormones on the opportunistic pathogen *Pseudomonas aeruginosa* can be found. In this study we investigated the impact of the neurohormone Noradrenaline (NA) on the physiology and social behavior of this pathogen, using both phenotypic and molecular approaches. In order to best mimic the host internal medium, all experiments were performed in RPMI 1640 cell culture medium, supplemented or not with 10% serum. The viable cell count results proved that NA is able to significantly promote growth only in serum-containing minimal medium, displaying a toxic effect in serum-free minimal medium because of its iron binding affinities in different circumstances, as revealed by ICP-MS (Inductively Coupled Plasma Mass Spectrometry) analysis. The iron-uptake mechanism was also incriminated for the NA effects on Quorum Sensing (QS) controlled social phenotypes as swarming motility, pyoverdine and pyocyanine production and also biofilm formation. NA has proved to stimulate *P. aeruginosa* growth by an endogenous siderophore-independent mechanism, supporting and promoting the growth of a siderophore deletion mutant (*P. aeruginosa PAOI ΔpvdD/pchEF*), by acting itself as an exogenous pseudosiderophore. Nevertheless, our molecular data demonstrate that NA interferes with bacterial ferrienterobactin iron uptake Two Component Systems Pir and Pfe, modulating *pirA* and *pfeA* gene expression in *P. aeruginosa*. None of the observed phenotypes could be totally reversed by using alpha- and beta-adrenergic antagonists: phentolamine and propranolol. Our results demonstrate that iron uptake modulation represents a key element in NA signaling in *P. aeruginosa*, and supports the idea that a special adrenergic receptor is not absolutely required for NA induced response in bacteria.

Keywords: bacterial behavior, host-pathogens communication, stress hormones.

WED-380
Hydroxychavicol isolated from *Piper betle* targets cutaneous fungal infections

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Superficial cutaneous fungal infections are caused by yeasts (e.g. *Candida* species and *Malassezia* species), dermatophytes, and non–dermatophyte species of filamentous fungi /dermatomycoses. They are among the most common infections in the world and dermatophytes are the major causative agents. However, despite enormous advances in the treatment of these infections in recent years, nail infections (onychomycosis) are difficult to eradicate, with recurrence reported in up to 10 to 53% of cases. Similarly, in some cases, chronic mucocutaneous candidiasis, which is consists of persistent and recurrent infections of the mucous membranes, skin, and nails, along with a variety of other manifestations. A current challenge for health care providers is to prevent the recurrence of these infections after a successful treatment. Therefore, the search for new, more effective therapies continues to be pursued. Topical therapy has been recently proposed to treat dermatophytosis and onychomycosis as well as other nail disturbances without subsequent occurrence of relapses and re–infections. Interestingly, the ointments of the *Piper betle* L., leaves extract have been used for the treatment of various skin diseases. Our *in vitro* and *in vivo* as well as toxicological data indicates that hydroxychavicol would be a promising antifungal agent of topical use for the treatment of cutaneous candidiasis and dermatophytosis (tinea corporis) because it is highly effective in achieving mycological eradication without subsequent occurrence of relapses along with its significant cutaneous retention capacity (prophylactic efficacy) and low toxicity. The mechanisms of action of hydroxychavicol appear to originate from the inhibition of ergosterol biosynthesis and the disruption of membrane integrity.

Keywords: fungicidal, Candida biofilm, prophylaxis, dermatophytosis, relapse, cutaneous candidiasis.

WED-381
Identification of an exported Heat Shock Protein 70 in *Plasmodium falciparum*

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With the emerging evidence that physiological state of the parasite is different in culture than in patient, we initiated clinical proteomics studies in malaria few years back with an idea to identify proteins which are significantly/specifically expressed in the real disease scenario. Being a chaperone biology group we were excited to identify an Hsp70 family protein, PfHsp70-x, to be uniquely present in the clinical stages. Adding to our surprise

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and its clinical relevance, we found that this gene was a pseudogene in 3D7 strain of *Plasmodium falciparum* while a functional counterpart was present in all other strains. Therefore, we believed PfHsp70-x to be involved in some biochemical pathway which is operational exclusively in the actual disease condition.

Ongoing initiatives involving genome sequencing brought a twist in the field when PlasmoDB released its updated version with re-annotated sequence of many genes. Original annotation of PfHsp70-x described it as a gene with one intron which upon getting spliced resulted in the formation of a pre-mature stop codon. The SNP present in PfHsp70-x, which was responsible for it to be a pseudogene, was now found to be a genome sequencing error. Thus, 3D7 also had A in place of T like other strains of *P. falciparum*. This generated an ATG codon and resulted in the formation of an alternate start site. The re-annotated coding sequence of PfHsp70-x now began within the predicted intron sequence and encoded the entire ORF. The new PfHsp70-x sequence lacked intron and encoded for a full length Hsp70 having sequence conservation with other PfHsp70s. Interestingly, the additional sequence added from the intron at the N-terminus represented an ER signal peptide and thus it could enter the secretory pathway.

Sub-cellular fractionation of infected erythrocytes revealed that PfHsp70-x is present in the parasitophorous vacuole (PV) as well as the erythrocyte cytosol. IFA further supported this finding as PfHsp70-x showed punctate distribution in the erythrocyte cytosol and around the PV apart from signal within the parasite. However, only a small fraction of PfHsp70-x was exported beyond the parasitophorous vacuolar membrane (PVM) into the erythrocyte cytosol, suggesting its potential involvement in processes on either side of PVM. A major population of the exported PfHsp70-x showed overlap with MAHRP1, a Maurer's cleft marker, in the erythrocyte periphery. This suggested that PfHsp70-x associates with Maurer's clefts and might be involved in protein sorting and trafficking activities.

Hsp70s generally work in association with Hsp40s. However, until recently only PfHsp40s were known to get exported. Our study provides answer to the long standing question of lack of an exported Hsp70 and thereby fills an important gap in malaria chaperone biology.

Keywords: heat shock proteins, Malaria, protein trafficking.

WED-382
Identification of potential periodontal pathogens based on genetic specificity

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Background: Periodontal disease is a destructive, inflammatory disease of the supportive structures of the teeth and oral bacteria and their products are the causes of tissue destruction. So far about 600 species of oral microorganisms have been identified. Among them, only about 10 species were identified as causative agents of periodontal diseases and these are mainly anaerobic, gram-negative bacteria. Periodontal pathogen should possess virulence factors relevant to the inflammation on periodontal tissue. In this study, it compares the degree of genetic similarity with all of the species of oral microorganisms using the destructive virulence factors as genetic marker.

Observations: The genetic sequences of 11 of destructive virulence factors and 653 species of oral microorganisms were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and data for constructing database were parsed with the JAVA programming language. Using 'Pair-wise Alignment', between the each of 5 of major periodontal pathogens the degree of genetic similarity was compared and the minimum value of the results was 69.48% as a thresholds. The degree of genetic similarity between the each of destructive virulence factors and each oral microbial was also compared and analyzed. With the result data, we created a phylogenetic tree using the ML method. Among the results it should be noted that 14 species of oral microorganisms have been found as potential pathogens of periodontal disease. These are consisted of estimated periodontal pathogen in other studies and normal oral microflora of health population. Their own pathogenecity is very low but they are opportunistic pathogens. When the balance of the normal flora is broken in the mouth and abnormal host immune system occurs, their pathogenecity will become very strong.

Conclusion: The results of this study revealed some potential periodontal pathogens among species, whose relevance with periodontal disease was previously remained uncertain. And this will provide basic information for accurate diagnosis and identification of causal relationships about periodontal disease. It is hoped that this research will be further developed for the practical use.

Keywords: periodontal pathogens.

WED-384
In vitro characteristics of tobravirus nonstructural 16K protein

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Using computer prediction and circular dichroism, we demonstrated that the nonstructural 16K protein of tobacco rattle virus (TRV, tobravirus), a suppressor of post-transcriptional gene silencing, consists of two structural parts: an ordered N-terminal region containing two putative α-structure "zinc fingers", and a fully disordered C-terminal region with two independent bipartite nuclear localization signals (NLSs) (Ghazala et al., 2008). The protein possesses RNA-binding and oligomerization activities. We have shown that the protein has at least three RNA-binding sites, interacts with small RNAs and binds to single-stranded sRNAs more efficiently than to double-stranded (ds) sRNAs. We identified plant coilin as a major cellular partner of the viral 16K protein. Coilin acts as the structural scaffolding protein of Cajal bodies (CBs), which are dynamic subnuclear compartments. According to our results (Shaw et al., 2014) coilin/CB deficient *Nicotiana benthamiana* RNAi knockdown plants have intensified TRV infection, resulting in persistent severe systemic symptoms. We have demonstrated that the TRV 16K protein and plant coilin directly interact *in vitro* and have mapped the sites of the interaction using Far-Western assays and a set of deletion and point mutant proteins. The 16K protein is a multifunctional protein that participates in several biological activities *in vivo*. The *in vitro* properties could reflect many roles of the 16K protein in the infection cycle. This work was supported by RFBR, the grant 13-04-01467a.

Keywords: None.

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WED-385
In vivo toxicity studies of superparamagnetic iron oxide nanoparticles in a silica matrix

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The goal of this study was to evaluate the *in vivo* toxicity of superparamagnetic iron oxide nanoparticles in silica matrix (IOSi-NPs) after intratracheal instillation in rats.

Superparamagnetic iron oxide nanoparticles in silica matrix were obtained by dropping into silica xerogel composite the mixture of ferrous chloride tetrahydrate and ferric chloride hexahydrate. The EDAX analysis indicated that the embedded particles were iron oxide. The particle size of iron oxide nanoparticles in silica matrix calculated from X-ray diffraction (XRD) analysis was estimated at around 10 nm. The average size deduced from transmission electron microscopy (TEM) was 12.2 ± 0.7 nm in good agreement with XRD analysis.

Histological evaluation of the nanoparticles effects on rat tissues after a single intratracheal instillation of IOSi-NPs in male Brown Norway rats at various concentrations were realized to clarify the controversial toxicity of nanoparticles.

The lung examination at 24 h after the intratracheal instillation with 0.5 mg/kg of IOSi-NPs the lung parenchyma of the rats showed a preserved alveolar architecture with rare macrophages in the alveolar septa. The pathological micrographs of lung in rats after the intratracheal instillation with 0.5 mg/kg dose of IOSi-NPs show that the lung has preserved the architecture of the control specimen. After the intratracheal instillation of the rats with a 2.5 mg/kg dose of IOSi-NPs, the lung parenchyma of the rats showed preserved alveolar architecture with rare macrophages in the alveolar septa, discreet anisokaryosis and anisochromia of type II pneumocytes with rare nucleoli. Lung parenchyma of the specimen after intratracheal instillation of IOSi-NPs in rats at concentration of 5 mg/kg showed preserved alveolar architecture with macrophages in the alveolar septa, discreet anisokaryosis and anisochromia of type II pneumocytes, with chromocenters and nucleoli. In the lung parenchyma it is also observed focal ectatic capillaries in the alveolar septa.

Our data suggest that IOSi-NPs are not capable of translocating from the lung to the liver via the circulation at all the tested concentrations. The histopathological appearance of the liver after intratracheal instillation of IOSi-NPs in rats show that the different pathological alterations such as enlargement of hepatocytes, hydropic degeneration of hepatocytes, nuclear enlargement and dilatation of the sinusoids were not presented at concentrations of 0.5, 2.5 and 5 mg/kg.

Keywords: *in vivo* assays, intratracheal instillation, iron oxides-silica matrix.

WED-386
Increase in white blood cells correlated with plasma water soluble antioxidants in exposed but seronegative HIV individuals found in a segment of Nigerian population

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Previous work has shown that micronutrient deficiency can cause immune function suppression with a resultant effect on both innate T-cell-mediated immune and adaptive antibody responses, thus altering the balanced host response. Antioxidants could enhance the cytolytic activity of Natural killer cells and increase the amount of Interferon-gamma produced by T cells. Our study thus seeks to investigate the correlation between immune cell responses and the activity of water soluble antioxidants in HIV serodiscordant partners of black origin. Thirty-four (34) serodiscordant heterosexual partners (serodiscordant- seronegative {SSN group} and serodiscordant-seropositive {SSP group}) and 15 seronegative healthy individuals {(SNH group} were recruited for the study. HIV statuses were confirmed using enzyme immuno assay method (immune comb 11) while the total and differential WBC count was assessed by Coulter sysmex haematology analyzer. FACScan flow cytometer was used to measure CD4, CD3 and CD8 cells. Isolation of HIV mRNA was by Nuclisens Magnetic extraction method, and quantification by Nucleic acid sequence- based amplification assay (NASBA). Reduced glutathione and ascorbic acid assays were determined spectrophotometrically. The results showed a significant increase ($P < 0.05$) of total WBC count by 39% in SSN when compared with SSP, similarly, ascorbic acid and glutathione concentration were significantly increased($P < 0.05$) in the SSN by 58.2% and 43.8% respectively. In group B eosinophils and basophil were absent, conversely monocytes were significantly increased ($P < 0.05$) in SSN. FACScan flow cytometric measurement of CD4, CD3 and CD8 cells were also increased in SSN group. The concentration of water soluble antioxidants and total WBC count showed a positive correlation ($P < 0.01$; $r = 0.89$) while mRNA was not detected in SSN. Our study showed for the first time the relationship between water soluble vitamins and immune cells in resistant but HIV exposed individuals of black origin. Since water soluble antioxidants may play a significant role in actively strengthening immune cells to fight against infections especially in HIV disease, it therefore indicates that serodiscordant HIV infection could be used as a model in understanding immunological differences and strengths for effective therapeutic targets.

Keywords: HIV/AIDS, CD4, serodiscordant.

WED-387
Induction of systemic resistance in tomatoes against soil borne pathogen using indigenous *Bacillus* strains

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This study was planned to investigate an ecological safe method to manage soil borne diseases in crops. We used two indigenous *Bacillus* strains including *B. fortis* 162 and *B. subtilis* 174. The bacterial strains were primed with tomato plants and the soil was made sick using active cultures of *Fusarium oxysporum* f. sp. Lycopersici, the causal agent of Fusarium wilt. Both the strains significantly supported plant growth and helped in decreasing soil

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borne disease. However, a little variation was observed in this and *B. subtilis* showed more effective results in comparison to the *B. fortis*. The studies were extended to the evaluation of the total phenolics and defence related enzymes in treated plants to have an idea for the physiological changes after defence induction. Overall findings depicts that the tested bacterial strains forced the plant to activate their defence system. In the next step fractions from *B. subtilis* 174 metabolites were investigated for potential ISR (Induced systemic resistance) determinant/s. Intracellular metabolites and cell free culture filtrates of selected bacterium were analyzed for their capability to induce systemic resistance in tomato under greenhouse conditions. In contrast to intra-cellular metabolites, cell free cultural filtrates (CFCF) elicited ISR in tomato plants as they showed significant reduction in disease index. These CFCF were then fractionated by a series of organic solvents and purified. These fractions were then tested for inducing systemic resistance in tomato using test tube bioassay. ISR determinates were found to retain in ethyl acetate fraction that was then subjected to column chromatography and portioned into ten sub-fractions by step wise elution method. GCMS (Gas Chromatography Mass Spectroscopy) analysis of ISR active sub-fraction confirmed the presence of four compounds including Eugenol, 3-methoxy Butylacetate; Pentachloroanilin and Phthalic acid Dimethyl ester. On analysis of ISR activity of these pure compounds in different concentrations, Phthalic acid Dimethyl ester proved as ISR determinant of B. subtilis IAGS174.

Keywords: Fusarium wilt, systemic resistance, Tomato.

WED-388
Influenza A virus affects the expression of nmda receptor subunits in mice

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Influenza is an acute respiratory infection caused by (-)-RNA influenza viruses. Seasonal epidemics of influenza result in about 3–5 million cases of severe illness and up to 0.5 million deaths annually. Among various complications of influenza are neurological. Particularly according to the clinical observations it was suggested that individuals exposed to maternal influenza A virus infection are at increased risk of developing schizophrenia later in life. Since schizophrenia is in part associated with the dysfunction of N-methyl-D-aspartate receptors (NMDARs) and influenza virus infection is among the affecters of NMDARs we evaluated the impact of influenza A virus infection on the expression of mRNAs encoding the NR1, NR2(A-D) and NR3(A-B)) of NMDARs in mice. We analyzed the mRNA levels of NMDAR subunits, in brain of mice, infected with influenza A/WSN/1933 (H1N1) and A/Aichi/2/1968 (H3N2) viruses at 0,2 LD_{50}, on the 1, 3, 5 and 10 days post infection using real-time PCR method. All seven NR mRNAs were detected in brain of mock animals. The infection with influenza A virus led to the changes in the expression levels of NR2A, NR2B, NR2D and NR3B mRNAs. We also evaluated the influence of A/H7N9 virus on the expression of NMDARs. Further we are going to make the more detailed analysis of NMDARs expression on the mRNA and protein levels during influenza A infection in mice, and also to study other genes, that are up- or down-regulated during schizophrenia.

Keywords: Influenza A virus, NMDA receptor, gene expression.

WED-389
Interaction of *C. perfringens* epsilon toxin with biological and model membranes

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Epsilon toxin (ETX) is a powerful toxin produced by strains of *C. perfringens* classified as types B and D, and is responsible for enterotoxemia in animals. ETX belongs to the heptameric β-pore-forming toxin family that includes aerolysin and *C. septicum* alpha toxin. These toxins are characterized by the formation of a pore through the plasma membrane of eukaryotic cells, consisting in a β-barrel of 14 amphipatic β-strands. ETX shows a high specificity for a few cell lines, like Madin-Darby canine kidney (MDCK) the first sensible cell line identified and the most studied one, G-402 (Human Renal Leiomyoblastoma cell line) and mpkCCDc14 (Mouse cortical collecting duct cell line). The molecular mechanism of ETX has been well characterized *in vitro* using MDCK cells. It is known that ETX induces permeabilization to different ions, organelle alterations and vacuolization, it is internalized in cells and colocalizes with endosomes and lysosomes. Moreover pore formation requires binding and heptamerization of the toxin in cholesterol-rich microdomains. Depletion of cholesterol by inhibitors of cholesterol synthesis damage ETX heptamerization, reducing its cytotoxicity. The aim of our project was to establish the role of lipids in the toxicity caused by ETX and the correlation of its activity in model and biological membranes.

In MDCK cells, using cell counting and confocal microscopy, we have seen that the toxin causes cell death mediated by toxin binding to plasma membrane. However little effect is observed in large or giant unilamellar vesicles.

Keywords: cell death, model membranes, toxin.

WED-390
Isolation of immunoglobulin Y from hen egg and determination of its efficiency against *Salmonella* species

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The World Health Organization recommends not to use the new age antibiotics on animals, plants and aquaculture because of their antimicrobial resistance mechanisms. It further recommends to restrict the use of existing antibiotics. The misuse of antibiotics causes a problem of antibiotic residual in the food chain. It also causes an increase in health problems of individuals, a decrease of effectiveness and reduces low public budgetary. On the other hand, passive immunotherapy that counteracts pathogens helps destroying antimicrobial residual and resistance mechanisms. It can be used even in the presence of infection so it is a good alternative for antibiotic therapy, especially in hazardous periods. In our study we have used a Salmonella-free Ross 308 parent stock. Immunization was achieved using *Salmonella gallinarum, Salmonella pullorum, Salmonella gallinarum* + *Salmonella pullorum* and *Freund's Adjuvant*. Two doses of immunization was given for 21 days. Eggs were collected daily and saved. The amount of total protein was measured by Bradford method. Ammonium sulfate precipitation and dialysis methods were used for IgY purification and proteins were identified by SDS-PAGE. Subsequently in vitro activity of all fractions in inhibiting microbial growth was determined. Especially *Salmonella gallinarum* + *Salmonella pullorum* antibodies were observed to have high activity. In conclusion, we have devel-

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oped a method and process whereby egg yolk antibodies can be obtained in a short time and can be produced as specific antibodies against other antigens than *Salmonella*.

Keywords: Antibody Generation, Bacterial Growth Inhibition, Immunoglobulin Y.

WED-391
Keratins 8 and 18 are required for Listeria invasion of mammalian cells

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To promote infection pathogens interfere with crucial host intracellular pathways. In particular, host cytoskeleton components are preferential targets of infecting bacteria. The study of the cell biology of infection provided insights in the way bacteria manipulate the host cytoskeleton and revealed unsuspected functions of cellular proteins, leading to a deeper understanding of eukaryotic basic cellular processes. *Listeria monocytogenes* is a facultative intracellular gram-positive pathogen adapted to thrive in diverse environments. In humans, *Listeria* is capable to cause listeriosis, a pernicious foodborne disease, largely usurping cytoskeleton functions during host cell infection. Keratins are major components of the cytoskeleton of epithelial cells with structural and regulatory functions. Although keratins were reported to be targeted by pathogens, the molecular and functional details behind keratin involvement in bacterial pathogenesis remain largely elusive.

In this work we studied the importance of Keratin 8 (K8) and its preferential binding partner Keratin 18 (K18) in *Listeria monocytogenes* pathogenesis. Our results demonstrate that both K8 and K18, together with actin and cMet, a major *Listeria* receptor, are found enriched in the vicinity of *Listeria* invading HeLa cells. Depletion of K8 and K18 by an RNAi approach revealed that both keratins are required for *Listeria* entry in Hela cells. Likewise, InlB/c-Met-dependent internalization of *Listeria* is found to be impaired in cells depleted for K8 and/or K18. Strikingly, we found that K8/K18 depletion results in the down-regulation of cMet levels and impaired cMet signaling. Additionally, using beads coated with the InlB invasin, we found that actin recruitment precedes K18 enrichment during InlB-mediated internalization in HeLa cells, suggesting that keratins may be relevant in later stages of *Listeria* uptake.

Together our results point out keratins as important players in *Listeria* pathogenesis. The molecular details of their involvement in *Listeria* infection and cMet signaling are under investigation.

Keywords: Infection, Keratins, *Listeria monocytogenes*.

WED-392
Malaria parasite responds to unfolded protein stress using a novel mechanism

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To establish a successful infection, *Plasmodium falciparum* has to competently remodel erythrocytes, its host compartment. The infected erythrocytes harbor several parasite induced membranous structures. Most importantly, pathogenesis related structures termed knobs which impart cytoadherence appear on the cell surface of the infected erythrocytes. In order to do so, the parasite exports 8% of its genome (around 400 proteins) to various destinations in the host cell. In other words, the parasite heavily relies on secretory functions for its pathogenesis. Additionally, this parasite also experiences significant amount of redox stress during its growth in human erythrocytes. As a result, it is predisposed to unfolded protein or endoplasmic reticulum stress during its life cycle.

Eukaryotes possess a fairly conserved mechanism known as unfolded protein response (UPR) to deal with ER-stress which restores the cellular homeostasis. However, when we looked for the UPR components in *P. falciparum*, we found that this parasite completely lacks the canonical UPR machinery. In accordance with that, our biochemical analysis established that malaria parasite indeed fails to mount a response as it was unable to up-regulate ER chaperones or ER associated degradation (ERAD) components when subjected to DTT mediated ER stress. However, an exported chaperone, PfHsp70-x, otherwise exported to erythrocyte cytoplasm, was found to be retained in the ER, increasing the local availability of this BiP homologue upon DTT-stress and thereby could provide a temporary relief. High throughput transcriptomic and proteomic analysis in response to ER stress, revealed a network of AP2 transcription factors and their targets being activated. Interestingly, a set of sexual stage specific genes became up-regulated when the parasites were subjected to DTT mediated ER-stress. Intrigued by this observation, we looked at the ability of ER stressors to induce stage transition by undergoing gametocytogenesis in response to ER-stress. We indeed found that ER-stress results in a greater than 2 fold induction in the numbers of gametocytes formed suggesting that this parasite makes use of a novel strategy of stage transition to cope with ER-stress.

In conclusion, our study not only highlights lack of a canonical UPR pathway in malaria parasite but also suggests that this parasite utilizes stage transition as an alternate means to escape ER stress.

Keywords: Gametocyte, *Plasmodium falciparum*, Unfolded Protein Response.

WED-393
Mapping Rig-I Like Receptors protein partners upon Chinkungunya virus infection

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The host innate immune response to chikungunya virus is type-I interferon dependant. Today, our knowledge on the molecular players recognizing this virus during infection is still a matter of research. It is known that there are two different mechanisms of recognition, one is IPS-1 (Interferon-b Promoter Stimulator 1) dependent and the other is IPS-1 independent (Schilte et al, 2012). We study the IPS-1 dependant pathway of chikungunya virus recognition via the cytosolic Rig-I Like Receptors (RLR): Rig-I, Mda5 and Lgp2. The role of these cytosolic receptors in chikungunya virus detection is currently controversial.

To clarify our knowledge on the mechanisms of action of RIG-I, MDA5 and LGP2 during infection, we have established HEK293 cell lines that express tagged RLRs. These cell lines have been characterized for:

(i) their capacity to overexpress the respective tagged-RLRs, (ii) the capacity to purify tagged RLRs by affinity chromatography of cell lysates, (iii) their efficacy to produce type-I interferon after in vitro transfection of synthetic RNA (iv) the efficiency of chikungunya virus replication in these tagged RLR cells.

Further, our aim will be to map the protein partners of RLRs upon viral infection. For this we will perform affinity purification of tagged-RLRs and mass spectrometry analysis.

The role of RLRs during chikungunya infection has been shown to be essential for controlling virus dissemination and initiating adaptive immunity against other RNA viruses. Thus it is essential to elucidate the protein complexes that are being formed and that are at the origin of this response.

Keywords: chikungunya virus, innate immunity, Rig-I Like Receptors.

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WED-394
MEG-14: an intrinsically disordered protein

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MEG-14 is a protein encoded by a micro-exon gene (MEG) from the parasite S. mansoni, a causative agent of schistosomiasis. The MEGs are capable to produce a pool of variant secreted proteins by alternative splicing of micro-exons. Bioinformatics analysis of MEG-14 protein sequence suggested the presence a signal peptide in the N-terminus, a transmembrane helix at the C-terminus and a central soluble portion which is mainly disordered. This soluble portion (sMEG-14) was expressed in a heterologous system and used for analysis of the effects of a range of physical-chemical factors of the structure this protein The synchrotron radiation circular dichroism (SRCD) spectroscopy spectrum of sMEG-14 in aqueous solution exhibits a strong minimum at 198 nm and a small positive band at 182 nm, corresponding a high content of disordered structure (>80%). However, disorder-to-order transitions that lead to the induction of a alfa-helix structure in sMEG-14 were noted by the addition of trifluoroethanol (≥50%) with negative peak at 208 and 222 nm and a stronger positive peak at 192 with a shoulder at 175 nm. This ordering effect was also produced by increasing temperature (in a reversible process) and in the presence of negatively charged lipids (like POPG and SDS). Furthermore, dynamic light scattering measurements demonstrate that sMEG-14 protein promoted fusion of vesicles formed by negatively charged lipids, the same effect was not observed for vesicles with zwitterionic or positive charged lipids. Taken together, these results support the classification of MEG-14 as good model of an intrinsically disordered protein, and suggest its interaction with different partners.

Keywords: intrinsically disordered proteins, micro-exon gene proteins, *Schistosoma mansoni.*

WED-395
Membrane destabilization and amyloid oligomers formation by influenza A virus protein PB1-F2 are dependent on bilayer composition

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Influenza is an acute respiratory infection which most severe manifestations are caused by Influenza A viruses of the *Orthomyxoviridae* family. The precise mechanism of Influenza A mediated pathogenicity is still being investigated and it appears that the virulence of the virus can be influenced by each of virus proteins. PB1-F2 is small accessory protein of 75-90 amino acids displays a strong polymorphism and is expressed in most human and avian influenza A strains. PB1-F2 was shown to increase morbidity and mortality though cell-type and viral-strain dependent manner. The protein C-terminal containing a non-canonical mitochondrial targeting signal is believed to be essential for the protein reactivity.

PB1-F2 is an intrinsically disordered protein in aqueous solutions but can adopt α-helical or β-sheet secondary structure depending on the environment hydrophobicity. In a membrane mimic environment or within infected cells PB1-F2 was shown to adopt β-sheet conformation and oligomerize to amyloid fibres.

To further understand the interaction of PB1-F2 with cellular membranes we sought to investigate PB1-F2 domains involved in the protein binding to membrane bilayers and to characterize PB1-F2 conformational changes and self-association occurring upon bindings. For this, we tested interactions of the full length and C- and N-terminal domains of PB1-F2 with liposomes of various lipid compositions and different net charges. We show that recombinant PB1-F2 aggregates and forms amyloid like structures upon binding membranes of negative net charge and that the conversion process depends on the lipid composition. Our results confirm the high reactivity of C-terminal part of PB1-F2 to impair membrane integrity but reveal also the enhancing role of the protein N-terminal domain in membrane structure destabilization. Finally we tested the cytotoxicity of monomeric and oligomerized PB1-F2.

Keywords: None.

WED-396
Microarray-based molecular assay for rapid identification of tuberculosis causative agent, analysis of its pathogenicity and drug susceptibility testing

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Fast spread of Multidrug-resistant and Extensively drug-resistant strains of *Mycobacterium tuberculosis* (TB) is one of the greatest threats to the global control of TB. Multidrug resistance (MDR) is resistance to at least isoniazid and rifampin. Extensively drug-resistant (XDR) TB is MDR TB plus resistance to any fluoroquinolone and at least one of the three injectable second-line drugs (amikacin, kanamycin or capreomycin). Annual growth of XDR TB cases estimated as 28000 worldwide (~9% of MDR TB) and almost all of them cause death. The actual incidence of XDR TB could be underestimated, because second-line drug susceptibility testing is not availible in many countries. To avoid a progressive development of drug-resistant TB worldwide, rapid identification of resistant *M. tuberculosis* complex strains is necessary.

A molecular assay based on original Russian technology of hydrogel microarrays (biochips, www.biochip.ru) has been developed for fast identification of MDR and XDR tuberculosis.

The microarray comprises immobilized oligonucleotides based on the corresponding sequences of IS6110 region that is typical for *Mycobacterium tuberculosis* complex and the sequences of *rpoB, katG, inhA, ahpC, gyrA, gyrB, rrs, eis, embB* genes with the mutations that act as markers for identification MDR and XDR TB as well as the sequences of single nucleotide polymorphisms that identify different TB genotypes such as Beijing, Beijing B0/W148, Haarlem, LAM and Ural which differ from each other by their pathogenicity.

The procedure includes multiplex amplification of analyzed fragments of mycobacterial genome with simultaneous fluorescent labeling of PCR-products followed by hybridization on the developed microarray. The assay allows to identify 104 genetic determinants of TB resistance to rifampin, isoniazid, fluoroquinolones, aminoglycosides/capreomycin and ethambutol. Compared to conventional drug susceptibility testing (Bactec MGIT 960, Becton Dickinson, USA), the sensitivity and specificity of the assay were 97.4% and 96.2% for rifampin; 98.0 and 91.5% for isoniazid, 94.1% and 85.2% for fluoroquinolones; 95.1% and 86.4% for aminogycosides/capreomycin; and 67.4% and 90.5% for ethambutol correspondingly. All analyzed samples defined as Beijing B0/W148 were extensively drug-resistant that confirms

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clinical significance of identification of this strain. Application of the developed method in clinical practice will lead to the coincident medication of tuberculosis with the timely therapeutic correction and will promote prevention of the expansion of extensively drug resistant *M. tuberculosis* strains.
Keywords: Extensively drug-resistant tuberculosis, Hybridisation, Microarrays.

WED-397
Mixed proteome analysis using a long monolithic column for elucidation of mechanisms of *Candida albicans* infection

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Candida albicans is an opportunistic pathogen that causes disease if the host immunity is compromised. The mortality rate of systemic candidiasis is very high because of the lack of effective diagnoses and therapy. Host resistance against pathogenic fungus relies on the ingestion and elimination by macrophage. However, *C. albicans* can escape from attack by macrophage. Therefore, further analysis of the mechanisms of *C. albicans* infection is required for development of treatment protocol.

On the other hand, proteome analysis is an important approach for comprehensive characterization of biological process. Separation technique such as liquid chromatography takes very important role in development of high-efficiency analysis system. Therefore, a long monolithic column which showed excellent performance has been applied to nano-LC/MS system for proteome analysis [1].

In this study, we performed mixed proteome analysis using a long monolithic column for direct analysis of the response of *C. albicans* to phagocytosis without isolation of cells. Proteins directly extracted from cells in co-cultivated mixture with *C. albicans* and macrophages were subjected to reduction, alkylation, and tryptic digestion. Tryptic fragments were injected to high-efficiency proteome analysis system (nano-LC/MS) using a long monolithic column (500 cm long, 0.1 mm ID) [2]. Collected data were used for protein identification and pathway analyses for elucidation the mechanisms of *C. albicans* infection. From these results, phenomena during the interaction of *C. albicans* with macrophage were comprehensively suggested. In *C. albicans*, proteins related to the glyoxylate cycle and hypha formation, etc were up-regulated. In macrophage, proteins related to the immune response were down-regulated [3].

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Keywords: Candidiasis, Mixed proteome analysis, Monolithic column.

WED-398
More Jaz in plant defense response

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Plants are exposed to many forms of stress, including biotic and abiotic factors. Due to their sessile life style, plants defend themselves via physiological adaptations. Jasmonates (JAs) are important hormones involved in plant defense responses. JAs-dependent defense reactions form two major branches. In one branch against insect herbivores, the JAs receptor F-box protein COI1 triggers degradation of JAZ proteins, which in the absence of JAs bind to the transcription factors AtMYC2/3/4 and repress its activity. In Arabidopsis the JAZ family counts 12 members, 10 of which interact with AtMYC2 in yeast. In the other branch of JAs-dependent defense against necrotrophic microbial pathogens, JAs interact with the defense hormone ethylene (ET). We previously identified the AP2-domain transcription factor ORA59 from Arabidopsis, which regulates the JAs- and ET-responsive expression of a set of defense genes, including the plant defensin gene PDF1.2. Interestingly, this branch also depends on the F-box protein COI1, since a coi1 mutant does not express this branch. It is speculated in the plant defense field that JAZ repressors may also control the activity of ORA59. However, ORA59 does not appear to interact directly with members of the JAZ protein family. Recently, we identified a protein interacting with ORA59 via yeast two-hybrid screening of an Arabidopsis cDNA library (ORA59-binding protein 1, OBP1). In the ORA59 activity assay, OBP1 has a moderate repressing activity. Interestingly, even more recently we discovered that OBP1 interacts in the yeast two-hybrid assay with a member of the JAZ family. Biochemical and molecular results showed that OBP1 forms the bridge between ORA59 and the JAZ protein and that this complex represses ORA59 activity in the absence of JAs.
Keywords: None.

WED-399
Multivalent Adhesion Molecule 7 clusters act as signaling platform for host cellular GTPase activation and facilitate epithelial barrier dysfunction

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Vibrio parahaemolyticus is an emerging bacterial pathogen which colonizes the gastrointestinal tract and can cause severe enteritis and bacteraemia. During infection, *V. parahaemolyticus* primarily attaches to the small intestine, where it causes extensive tissue damage and compromises epithelial barrier integrity. We have previously described that Multivalent Adhesion Molecule (MAM) 7 contributes to initial attachment of *V. parahaemolyticus* to epithelial cells. Here we show that the bacterial adhesin, through multivalent, high-affinity interactions between surface-induced adhesin clusters and phosphatidic acids in the host cell membrane, induces activation of the small GTPase RhoA and actin rearrangements in host cells. In infection studies with *V. parahaemolyticus* we further demonstrate that adhesin-triggered RhoA activation is sufficient to redistribute tight junction proteins, leading to a loss of epithelial barrier function. Taken together, these findings show an unprecedented mechanism by which an adhesin acts as assembly platform for a host cellular signaling pathway, which ultimately facilitates breaching of the epithelial barrier by a bacterial pathogen.
Keywords: actin reorganisation, host-pathogen interaction, lipid components.

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WED-400
NMR study of the structure and interactions of two cofactors of the polymerase of human respiratory syncytial virus

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Respiratory syncytial virus (RSV) is a member of the Paramyxoviridae family of non segmented, negative sense single-stranded RNA viruses. Bovine and human respiratory syncytial virus (bRSV and hRSV) are major causes of respiratory diseases in calves and children, respectively, and are prioritized vaccine targets. Our aim is to get answers about the structure and dynamics of different components of the RSV RNA dependent RNA polymerase complex (RdRp) and about their interactions either with other components of the RdRp or with cellular partners, by using Nuclear Magnetic Resonance, as a prerequisite to rational drug design. We are focusing on two RSV proteins: the phosphoprotein, which is the main polymerase co-factor and necessary for both viral transcription and replication, and M2-1. We are aiming at characterizing the structure of P. Indeed, it has been predicted that P contained an oligomerization domain and large disordered N-and C-terminal extensions.

NMR is a unique tool to investigate intrinsically disordered proteins and regions (IDRs).

We have shown that these regions display transient helices which form potential sites for binding partners of P, among which the M2-1 and N proteins. The M2-1 protein of hRSV functions as an essential transcriptional cofactor of the viral (RdRp) complex by increasing polymerase processivity. M2-1 is a modular RNA binding protein that also interacts with the viral phosphoprotein P. These binding properties are related to the core region of M2-1. After solving the structure of the corresponding domain, we showed that partial overlap of the RNA and P interaction surfaces, determined by NMR on the monomeric M2-1(58-177) fragment, accounts for the previously observed competitive behavior of RNA versus P in M2-1 binding.

Keywords: respiratory syncytial virus, polymerase, transcription.

WED-401
Novel mode of action in plant defense peptides

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The multilayered plant immune system relies on rapid recognition of pathogen-associated molecular patterns followed by activation of defense-related genes that results in the reinforcement of plant cell walls and production of antimicrobial compounds. To suppress plant defense, fungi secrete effectors including a recently discovered Zn-metalloproteinase from *Fusarium verticillioides*, named fungalysin Fv-cmp (Naumann, T.A. (2011) Modification of recombinant maize ChitA chitinase by fungal chitinase-modifying proteins. *Mol Plant Pathol*, 12, 365-372). This proteinase cleaves class IV chitinases, plant defense proteins that bind and degrade chitin of fungal cell walls. In this work, we studied plant response to such pathogen invasion and discovered novel inhibitors of fungalysin. We produced several recombinant hevein-like antimicrobial peptides named WAMPs containing different amino acids (A, K, E, and N) at the non-conservative position 34. An additional serine residue in the site of fungalysin proteolysis makes the peptides resistant to the protease. Moreover, approximate equal WAMP-1b, -2 concentration to chitinase concentration was sufficient to block the fungalysin activity and keeping the chitinase active against fungi. Thus, WAMPs represent a novel type of protease inhibitors being active against fungal metalloproteases. According to *in vitro* antifungal assays WAMPs demonstrated direct inhibition of hypha elongation suggesting that fungalysin is playing an important role in fungi development. A novel molecular mechanism of dynamic interplay between host defense molecules and fungal virulence factors is suggested.

AAS and AAV are recipients of the stipend of the President of Russian Federation.
Keywords: hevein-like antimicrobial peptide, fungalysin, Zn-metalloproteinase, chitinase, Triticum kiharae, *Fusarium verticillioides*

WED-402
Oligandrin and β-aminobutyric acid-induced resistance to *Oidium neolycopersici* in tomato plants

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Oligandrin, a 10 kDa elicitin molecule secreted by *Pythium oligandrum*, was demonstrated to share biochemical similarities with other elicitins, however fail to provoke a visible HR-associated necrotic response on leaves of treated tomato plants [1]. Treatment of tomato plants with oligandrin confers enhanced resistence against *Phytophthora parasitica, Fusarium oxysporum* f. sp. *radicis-lycopersici and Botrytis cinerea*. Oligandrin is known to trigger the expression of PR-proteins and to stimulate the activity of the defense related enzymes. Oligandrin might induce signal pathways of salicylic and jasmonic acid and ethylene in parallel. A non-protein amino acid β-aminobutyric acid (BABA), has previously been shown to effectively induce plant resistance against many different oomycetes and against various downy mildews. It has been shown that callose deposition as well as defense mechanisms depending on the phenylpropanoid and the jasmonic acid pathways contributed to BABA-induced resistance [2]. Expression of PR-proteins after treatment with BABA is dependent on used plant species, applied BABA concentrations and the on the application mode. In our study, as a working model we used two *Solanum* genotypes differing in their susceptibility to *O. neolycopersici* pathogen: *S. lycopersicum* cv. Amateur as a susceptible and *S. habrochaites* as a highly resistant genotype [3]. We analysed the expression of 45 defense genes after treatment of oligandrin and BABA with tomato leaves or after inoculation with *O. neolycopersici*. We evaluated germination and growth of pathogen and detected the effect of oligandrin or BABA on pathogen development. Our results showed that the expression of defense genes was increased after inoculation by *O. neolycopersici* in *S. habrochaites* (resistant genotype) comparison with *S. lycopersicum* cv. Amateur. By contrast, BABA slightly induced expression of genes in susceptible genotype but not *in S. habrochaites*. Expression of defense genes was similar after treatment with oligandrin in both genotypes. Data obtained from gene expression

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study correspond with evaluated resistance against *O. neolycopersici*. Our work provides a step to understand the molecular basis of the induced resistance to *O. neolycopersici*.

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Keywords: *Oidium neolycopersici*, oligandrin, β-aminobutyric acid.

WED-403
Outer membrane proteins, lipopolysaccharide and morphological alterations of starved *Shigella* isolated from wastewater

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Bacteria living in habitats of frequently changing conditions like nutrient starvation have evolved very sophisticated responses to adapt to environmental changes. To survive prolonged periods of starvation, many bacteria have developed starvation-survival strategies enabling them to persist in the environment until conditions become favorable for growth. One of the most frequently observed behaviors in the nutrient starvation response of Gram negative bacteria is the size reduction and cell morphology conversion from rod to coccoid shape.

The aim of this work was to evaluate the starvation effect (30 days incubation in laboratory microcosms) on the survival, morphology, Lipopolysaccharide (LPS) and outer membrane proteins (OMPs) profiles of *Shigella* sp. (isolated from wastewater).

Our results showed that *Shigella* sp. is able to adapt and survive under starvation conditions, owing to gradual changes in cellular physiology and morphology. Untreated cells are rod shaped presenting a very clear cell wall and membrane surrounding an expended cytoplasm. Whereas, the starved cells present a coccoid form with condensed cytoplasm. In addition, our data indicate that starvation induced changes in the OMPs and LPS profiles of *Shigella* sp.

These results point out that *Shigella* may sustain their adaptation to changes of external environment by changing their morphology, LPS and OMPs profiles.

Keywords: LPS, OMP, Morphology, *Shigella*, Starvation.

WED-404
Palmitoylation of HIV-1 Tat regulates its capacity to inhibit neurosecretion

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HIV-1 infected cells release the transactivating Tat protein. This small (86 residues) and basic proteins owns seven cysteine residues and is recruited to the inner leaflet of the plasma membrane by a strong and specific interaction with phosphatidylinositol(4,5) bisphosphate (PIP_2). Tat is then directly exported through the plasma membrane using an unconventional secretion process that remains poorly characterized, although Tat secretion is very efficient since about 2/3 of this protein is exported. Tat concentration reaches the nanomolar range in the serum of infected patients. Tat can be endocytosed by target cells and escapes from endosomes by translocation through their membrane to reach the cytosol. It can then trigger various cell responses, depending on cell types.

Here we shoed that Tat, once endocytosed by PC12 cells and translocated to the cytosol, is able to bind PIP_2 at the plasma membrane with which it remains stably associated. Tat thereby prevents the PIP_2-mediated recruitment of proteins involved in neurosecretion and strongly inhibits this process. Why does Tat stays on PIP_2 in neurosecretory cells and is apperently not exported? It seems that it is because Tat is palmitoylated in these cells. Indeed we found, using two independant approches that transfected Tat can be palmitoylated in PC12 cells but not in T-cells that release Tat. We identified the modified cysteine as well as the enzyme that enables Tat acylation among the 23 DHHC mammalian protein acyl transferases. Non-acylable Tat poorly inhibits neurosecretion and is more cytosolic than the WT. Similar data were obtained using 2-bromopalmitate, a palmitoylation inhibitor. Hence, palmitoylation stabilizes the association of Tat with PIP_2 and enables Tat to strongly impair neurosecretion.

Keywords: HIV-1, Palmitoylation, Tat protein.

WED-407
Phylogenetic and three-dimensional analysis of endoxylanases of two races of *Colletotrichum lindemuthianum*, pathogenic and saprophytic, with endoxylanases of *Colletotrichum* sp.

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The genus *Colletotrichum* includes a number of plant pathogens of major importance, causing diseases of a wide variety of mono and dicots plants. *C. lindemuthianum* is an intracellular hemibiotrophic pathogen of bean. After penetration of a host epidermal cell, an infection vesicle is formed and the fungus extends into adjacent cells by large primary hyphae, which invaginate without penetrating the host cell membrane, thus persisting as a biotrophic interaction. Once a large area of the plant tissue has been colonized, necrotrophic hyphae develop and this event closely correlates with the production of a battery of host cell wall-degrading. Among these, xylanases are thought to play a major role in pathogenesis.

Deduced amino acid sequences of xilanases reported for twenty two *Colletotrichum* species, xilanases Clxil1 from a pathogenic (race 1472) and a saprophitic (race 0) race of *C. lindemuthianum* isolated in this study, and a xylanase sequence from *Lentinula edodes* as an external group, were used to generate distance trees. Phylogenetic analysis showed that the xylanases are grouped into four clades, where Clxil1 xylanase from *C. lindemuthianum* Race 1472 and R0 were grouped with xilanase from *C. orbiculare* (goup I) and in another clade with *C. gloeosporioides Nara_gc5_ xil1 and C. gloeosporioides Cg_14_xil1*.

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Results showed an interesting separation of xylanases from *C. graminicola M1_xil2* and *C. higginsianum_xil1* (Group III), both pathogens of monocotyledoneus.

Apparently there is a different source for these enzymes, those from pathogens of monocotyledonous on one side and on the other side enzymes from dicotyledonous pathogens hosts (group I and II) followed by a diversification of these enzymes in more recent evolutionary process (group II).

The results show an basal separation mostly integrated whith xilanases from *C. gloeosporioides Nara_gc5* and *C. gloeosporioides Cg_14,* these enzymes show patterns with larger sequences and carbohydrate-binding modules.

Interestingly, for fungi that have more of a xylanase in their repertoire, these enzymes are not in the same group, as in the case of *Colletotrichum gloeosporioides, C. graminicola* and *C. higginsianum*.

We believe that these xylanases of *Colletotrichum* sp. evolved independently according to its host (mono or dicotiledons), life style and that might have influence on small changes in the tridimensional structure and these perhaps generate more efficient enzymes.

Keywords: *Colletotrichum lindemuthianum*, Endoxylanases, Phylogenetic relationships.

WED-408

Plasmodium falciparum products affect human erythrocyte membrane-bound glycohydrolases activity

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Background: *Plasmodium falciparum* (*Pf*) malaria causes about 600,000 deaths each year, mostly African children, because of complications including cerebral malaria and severe anemia. Pathogenesis of anemia is due to different factors including parasite induced hemolysis, diserythropoiesis and reduced deformability, accelerated senescence and removal of uninfected red blood cells (RBC). Modifications of RBC membrane's properties can be ascribed to increased oxidative stress caused by parasite heme products such as Fe(III)-protoporphyrin IX (hematin) or hemozoin, present at high concentration in the plasma of malaria patients.

Several plasma membrane-bound glycohydrolases of human RBC were recognized to have a role in signaling early membrane alterations both in pathologies related to oxidative stress and in physiological conditions as ageing, suggesting their use as new markers of cellular oxidative stress. However, an association between RBC glycohydrolases alteration and malaria infection has not been previously described.

The aim of the present work was to investigate the ability of malaria parasite products to alter RBC membrane by affecting glycohydrolases activity.

Methods: RBC from human donors were incubated for 24 hours in the presence of supernatants, derived from *Pf* cultures, containing heme products released during parasite growth. RBC were then treated with different concentrations of hematin (20-10-5 μg/ml) or hemozoin (10-5-2.5 μg/ml) purified from *Pf* cultures. Hexosaminidase, β-D-Glucuronidase, α-D-Glucosidase and acidic Sialidase activities were evaluated by fluorimetric assays; membrane fluidity by fluorescence anisotropy method.

Results: A decrease in the activity of the tested enzymes was observed after incubation of RBC in the presence of culture supernatants from different *Pf* strains. Moreover, a significant dose dependent inhibition was observed after treatment with either hematin or hemozoin. At the highest dose used, hematin induced a decrease in the activity of the examined enzymes between 65% and 55%. Likewise a decrease of RBC membrane fluidity was observed following treatment with hematin or hemozoin.

Conclusions: The decrease of glycohydrolases activity and the increase in membrane rigidity are in agreement with accelerated RBC senescence observed in malaria infection and the parasite heme products seems to be the main contributors to these membranes damages. Glycohydrolases alterations could be indeed promising candidates as early markers of RBC damage in malaria infections.

Keywords: Human erythrocyte membrane-bound glycohydrolases, Malaria, Parasite heme products.

WED-409

Pleiotropic role played by the PDZ domains in neuronal signaling pathways

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The human tyrosine phosphatase PTPN4 and the Ser/Thr kinase MAST2 are two enzymes expressed in neurons. While PTPN4 is an anti-apoptotic protein, MAST2 inhibits neurogenesis and neuroprotection. The PDZ domain of these two enzymes is specifically targeted by the cytoplasmic domain of the envelope glycoprotein (G protein) of the rabies virus (RABV) during neuron infection (Préhaud et al., 2010). We have solved the NMR structures of the complexes formed by MAST2-PDZ and PTPN4-PDZ with their respectives endogenous and viral ligands. As a result, the complexes formed by the PDZ of the two enzymes and their respective ligands are disrupted, triggering drastic effect on cell signaling and cell commitment either towards death or survival. By targeting MAST2-PDZ, the G protein of virulent RABV alters the intracellular trafficking of PTEN (Terrien et al., 2012) and promotes survival, whereas the G protein of attenuated RABV induces neuronal cell death by targeting PTPN4-PDZ (Babault et al., 2011). We recently demonstrated that the catalytic activity of PTPN4 is regulated by its PDZ domain and that the viral sequence interfered with this allosteric regulation.

We provided structural and biological evidences that the RABV proteins act as competitors endowed with specificity and sufficient affinity in a vital cellular process. The disruption of critical endogenous protein-protein interactions by viral protein altered drastically intracellular protein trafficking and catalytic activity controlling the cellular homeostasis.

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Keywords: Neurons, PDZ domains, Virus.

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WED-411

Rapid preparation of frequently mutated-proteins of influenza viruses and construction of screening system to discover novel inhibitors

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Recently, many of new mutants of influenza virus, such as H5N1 subtype, have occurred. Hemagglutinin (HA) and neuraminidase (NA), which are membrane proteins of influenza virus, are the main factors that determine the pathogenicity. It has been reported that some NA mutants have tolerance to oseltamivir and zanamivir which are NA inhibitors for effective treatments. It is necessary to develop a novel treatment for the disease caused by new mutants of influenza virus. In order to solve such problems, we focus on yeast cell surface engineering (1). Using this system, it is possible to construct mutant protein library on yeast cell surface easily and comprehensively, because various inconvenient and troublesome steps for protein preparations could be avoided. In addition, it can be easily evaluated whether oseltamivir and zanamivir could be effective against mutant proteins. We succeeded in displaying HA derived from H1N1 subtype and NAs derived from H9N2 and H1N1 subtypes on the yeast cell surface (2). The activity of displayed HA was detected by hemagglutination assay. It was confirmed that displayed mutant NAs which are known to be tolerant to oseltamivir and zanamivir maintained the tolerance. Moreover, the NA- and HA-displaying yeasts constructed in this research can be considered to be useful tools in high-throughput screening of inhibitors toward frequently mutated-influenza viruses. Using the displaying yeasts, a new screening system could be constructed for developing new NA inhibitors (3). Our advantageous system will contribute to prevention against pandemic by influenza viruses.

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Keywords: cell surface engineering, frequent mutation, influenza virus.

WED-412

Review on malaria in Saudi Arabia, current status and future prospects

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Malaria is atimeworn illness as the mankind itself, in many countries, it discompose a dangerous hurdle to economic advancement, furthermore Malaria is arduous to exterminate and its monitoring is conceivable only with consistent efforts of the governmental agencies, general publicandhealthcare personnel. Malariosis is caused by*Plasmodium* spp., a protozoan parasite which make it is responsible for an over of one million people death annually, mostly are children. The demonstrationsof malaria disease are conductedthrough the erythrocytes infection by the parasite asexual stages, so it is believedto be apossibility multisystem disease, as every body's organ is reached by the blood. The essential mode of malaria infection transmission is via the female *Anopheles* mosquito bites, while the other modes of infection include the transplacentally route, infected blood transfusion and very rarely cases by needle-stick injuries. Saudi Arabia is the only country that hosts annually and throughout the year large numbers of pilgrims of different nationalities, that may pose a significant burden in tackling the spread of epidemic diseases. In Saudi Arabia, *Plasmodiumfalciparum* is the predominant species, as well as the *P. vivax* is also reported. The malaria incidence performedalong the Southern Region of the Red Sea coast, down to the border with Yemen. In 2004 Saudi Arabia took the decision to remove this disease especially in the border villages by mapping the malaria foci and the investigation of all infected cases using the clinical diagnosis which based on the patient's sign and symptoms, then by making the free laboratory diagnosis and giving the suitable medication.

Keywords: Anopheles mosquito, Malaria, Saudi Arabia.

WED-413

Role for Tsg101 recruitment by the viral nucleocapsid in modulating packageable DNA or RNA into HIV-1 particles

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HIV-1 agent of the AIDS pandemic is a RNA virus that requires an intermediate DNA phase via reverse transcription (RT) step in order to establish productive infection in the host cell. Within cells retroviral assembly requires thousands of structural Gag proteins and two copies of genomic RNA (gRNA) as well as cellular factors to converge to the assembly sites at the plasma membrane in a finely regulated timeline. In this process, the nucleocapsid domain of Gag (GagNC) ensures gRNA selection and packaging into virions. Then, budding and virus release require the recruitment of cellular ESCRT machinery. Interestingly, mutating GagNC results into the packaging of DNA instead of gRNA by promoting a late occurring step of reverse transcription of the gRNA into DNA. Generally, RT takes place early after entry of wild-type viruses in cells. Late RT occurs in producer cell prior to virus release through an unknown mechanism and is reminiscent of the detection of DNA-containing particles in HIV-1-infected people. In order to decipher the molecular mechanisms involved in controlling late RT, we explored the biogenesis of DNA-containing particles in cells, combining live-cell total internal reflection fluorescence microscopy, electron microscopy and *trans*-complementation assays. Our study reveals that DNA virus production is the consequence of HIV-1 budding defects with large patches of Gag aggregation at the plasma membrane. Targeting Tsg101, a key component of the ESCRT-I machinery, to virus assembly sites restores virus budding and decreases DNA levels in cells and particles in favor to RNA packaging. Altogether, our results highlight the role of

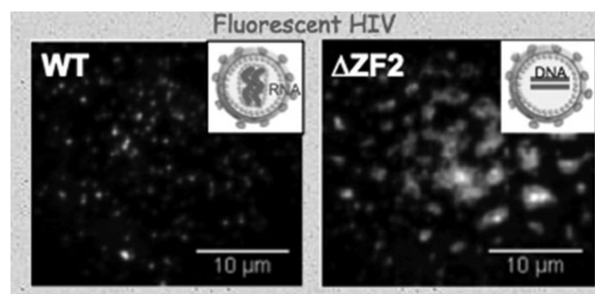

Fig. 1.

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GagNC in controlling RT activity in a spatiotemporal manner, via an ESCRT-I-dependent mechanism.

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Imaging HIV biogenesis at the plasma membrane by *total internal reflection fluorescence* microscopy (TIRFM).

Keywords: ESCRT machinery, HIV biogenesis, TIRF microscopy.

WED-414
Role of a two-component system, AcsS/R, in expression of acetyl-coA synthetase of *Vibrio vulnificus*

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VarS/VarA is a master regulator involved in diverse aspects of bacterial metabolism. An experiment to identify VarS/VarA target gene(s) in *Vibrio vulnificus* revelaed a putative LuxR-type transcriptional regulator as a down-regulated protein in *ΔvarA* mutant. Comparative transcriptome analysis of this *ΔluxR* mutant versus wild-type, indicated that the *acsA* gene encoding acetyl-CoA synthetase was down-regulated in the renamed *ΔacsR* mutant (response regulator for acetyl-CoA synthetase). A putative histidine kinase gene, *acsS* (sensor kinase for acetyl-CoA synthetase) was found upstream of the *acsR* gene. Luciferase activities of the *acsA::luxAB* transcriptional fusion indicated that expression of *acsA* was decreased in *ΔacsR* or *ΔacsS* mutant than wild-type. In addition, expression of the *acsA::luxAB* was repressed by the presence of glucose, which occurs in a CRP-cAMP-independent manner. *ΔacsA* mutant *V. vulnificus* was retarded in bacterial growth in minimal acetate medium. In the same manner, *ΔacsR* and *ΔacsS* mutant strains grew slower than wild-type in the same medium. Growth retardation of *ΔacsR* strain in minimal acetate medium was restored when wild-type *ascR* gene was added to the mutant as a complementation plasmid. Binding of AcsR recombinant protein to the *acsA* promoter was shown by *in vitro*-gel shift assays. These results indicated that AscS/AscR plays a role in controlling acetate metabolism by positively regulating the expression of acetyl CoA synthase.

Keywords: acetyl-coA synthetase, two-component system, *Vibrio vulnificus.*

WED-415
Role of hydrogen peroxide and peroxiredoxins in the resistance of cowpea (*V. unguiculata* [L]. Walp.) to *Colletotrichum gloeosporioides*

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C. gloeosporioides is a major hemibiotrophic pathogen with a broad host range that causes substantial crop losses. Recent data have shown that the hypersensitive response (HR) is an event involved in the resistance of cowpea against *C. gloeosporioides*. Several studies have been undertaken on the defense mechanisms of plants to pathogens, but overall they remain poorly understood. Previously, we have compared changes in protein expression induced in resistant cowpea leaves after infection with *C. gloeosporioides* using a proteomic approach. Based on these results, in this present study we evaluated whether *PRXIIBCD* and *PRXIIE*, genes which encode for peroxiredoxins and are involved in HR, are responsive in cowpea leaves challenged with *C. gloeosporioides* using qRT-PCR analysis. Increase of hydrogen peroxide (H_2O_2) generation was detected in cowpea leaves 2 h after infection (HAI) using DAB-staining. However, spectrophotometric measurements showed that the amount of H_2O_2 increased 12 HAI. Enhanced lipid peroxidation was also observed, but at 2 HAI. qRT-PCR analysis showed that the quantitative level of *PRXIIBCD* transcript expression was up-regulated at 2 HAI and remaining elevated up to 72 HAI. Contrarily, *PRXIIE* transcript did not alter at the early hours after inoculation, but showed increased expression levels at 48 and 72 HAI. The present study indicates that H_2O_2 has an important function in the early defense strategies of cowpea *preventing* the *spread* of *C. gloeosporioides Infection* and possibly as a signaling molecule. Furthermore, *PRXIIBCD* and *PRXIIE* seem to be involved, in combination with other molecules, in the regulation of H_2O_2 levels toward preventing cell death and regulating the cell redox homeostasis, important events in the resistance mechanisms of plants to pathogens.

Keywords: *Colletotrichum gloeosporioides*, Hydrogen Peroxide, Peroxiredoxins.

WED-416
Role of oligomannose-rich apoptotic membranous vesicles in *Escherichia coli* infections

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Escherichia coli (*E. coli*) are known infectious agents in *cystitis* and Crohn's disease by utilizing the same mechanism of binding with receptor molecules on the epithelia of oropharyngeal, gastrointestinal and urinary tracts – the oligomannose-specific lectin (adhesin) FimH on the tip of type 1 fimbriae. Thus, bacteria utilize the sugar decoration of cells, the glycocalyx, to colonize the cell surface, wherever cells are in contact with the outside environment, as for example in the case of epithelial cells. *E. coli* are more prone to infect patients having a disease with an increased apoptotic rate. Recently we demonstrated [R.Bilyy, T. Shkandina, et al, JBC, 2012] that apoptotic cells release two distinct types of apoptotic cells-derived microvesicles (ACMV): either derived from endoplasmic reticulum (ER), or from the plasma membrane (PM). Both exhibited an altered glycoprofile: desialylated glycotopes resulting from surface-borne sialidase activity on PM-derived ACMV, while surface exposure of oligomannose glycoproteins results from surface exposure of internal ER membranes.

Here we studied the possibility of *E. coli* to utilize mannose-rich ACMV for host cell colonization and studied the application of a new class of monovalent mannosides for preventing binding of AIEC towards host cells. (i) We have developed an ELISA-based method for testing affinity of synthetic inhibitors towards FimH using oligomannose glycans of RNAse B proteins as substrate. This test enables quick evaluation of the intensity of lectin binding to the substrate, as well as a fast test of compounds that inhibit this process [Brument S; at all J Med Chem, 2013]. In this way specific FimH inhibitors acting in the nanomolar range have

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been selected. (ii) Co-incubation, of bacteria that expose FimH lectin, with host cells led to formation of oligomannose-positive ACMV and these ER-derived ACMV were bound by FimH lectin. Also by using a combination of fluorescent and DIC microscopy we demonstrated the direct binding of *E. coli* with mannose-rich ACMV of human *HeLa* cell after co-incubation period of 2 h. Changes of surface oligomannose-rich glycans of epithelial cells after influence of FimH adhesins were confirmed by MALDI TOF MS/MS screening of ACMV glycans in the untreated conditions and after the action of bacterial FimH lectin. (iii) We used *Jurkat* human T-cells and human intestinal epithelia *Caco-2* cells in co-culture models with *E. coli* and demonstrated that thiazolylmannoside-based inhibitors can effectively abrogate FimH-induced apoptosis. Moreover, addition of thiazolylmannoside-based inhibitors prevented binding of *E. coli* towards host cells in bacteria-cells co-cultures at 500 nM concentration.

Keywords: apoptotic cells-derived microvesicles, ELISA, *Escherichia coli*.

WED-417

Salmonella co-opts host cell chaperone-mediated autophagy for intracellular growth

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Salmonellosis is one of the most common food borne diseases in humans and presents a major public health and economic burden worldwide. *Salmonella* serovars are facultative intracellular pathogens which are invasive for host cells where they establish an intracellular, membrane-bound replicative niche known as the *Salmonella*-containing vacuole (SCV).

The SCV has been regarded as a nutrient deprived compartment. However, despite apparent nutrient limitation within the SCV, *Salmonella* is still able to replicate in the SCV. Here, we provide evidence for a unique mechanism whereby intracellular *Salmonella* gains access to the host cell cytosol from within its membrane-bound compartment to acquire nutrients. Our study shows that *Salmonella* Typhimurium acquires small peptides by co-opting the host cell chaperone mediated autophagy (CMA)-dependent cytosolic protein turnover pathway. CMA is a selective host cell protein turnover pathway active in all cell types and w is involved in the transport of cytosolic proteins into lysosomes for degradation. An estimated 30% of all cytosolic proteins are turned over through this mechanism. Here we show for both intracellular *Salmonella* and in purified SCVs that the SCV is associated with the key components of the CMA pathway, and inhibitors of CMA affect the intracellular growth of peptide-dependent mutants of *Salmonella*. Furthermore, we show that acquisition of the key CMA components is selective, with recruitment of only one isoform of one host protein component, and exclusion of other lysosomal proteins.

These results reveal a novel means whereby an intracellular pathogen can access the host cell cytosol to acquire nutrients from within its membrane-bound compartment. We suggest these results may provide an explanation for relapse infections resulting from persistent *Salmonella* infections, and suggest a possible means of targeting antibacterials against intracellular pathogens.

Keywords: Chaperone mediated Autophagy, Salmanellosis, Salmonella Containing Vacuole.

WED-418

Salmonella enterica cells accumulate RND-family efflux pump inhibitor Phenylalanyl-arginyl-beta-naphthylamide

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Increased activity of efflux pumps is one of the major reasons of bacterial resistance to antibiotics. Prevention of the efflux of administered antibiotics using the pump inhibitors could increase effectiveness of the treatment. Interaction of RND-family efflux pump inhibitor phenylalanyl-arginyl-b-naphthylamide (PAβN) with *Salmonella enterica* ser. Typhimurium cells was assayed using a novel PAβN-selective electrode. We showed that PAβN has a high affinity to lipopolysaccharide (LPS), the major constituent of bacterial outer membrane. EDTA stimulates binding of PAβN to *S. enterica* cells and *E. coli* LPS. Because of the membrane voltage, like other lipophilic cations, PAβN accumulates also in the cytosol of EDTA-treated bacteria. However, energization of the cells by addition of glucose does not induce efflux of PAβN. Polycationic antibiotic Polymyxin B releases both the accumulated and the LPS-bound inhibitor.

Keywords: efflux pumps, Phenylalanylarginyl-beta-naphtylamide, *Salmonella enterica*.

WED-419

Screening of Kazakhstani wheat varieties for the presence of Lr-genes and their effectiveness

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Leaf rust caused by *Puccinia recondita* Desm. is very harmful for wheat yield and widely expanded in many cereal-producing countries. A search for effective Lr-genes and their introduction into culture are necessary to create new varieties with high level of resistance to pathogen.

The goal of the research was to analyze the collection of commercial bread wheat varieties and isogenic lines of Thatcher variety for the presence of *Lr* genes and their effectiveness against local pathotypes of *P. recondita*. The analysis was carried out on 45 commercial varieties and 39 lines of Thatcher variety, using four *Lr* markers: *Lr*1, *Lr*9, *Lr*10 and *Lr*19. The experiment was conducted on DNA markers associated with Lr-genes and phytopathological tests in greenhouse and in the field.

For each marker the frequency of occurrence in the research group was defined. The least occurring and least effective of the four markers was *Lr*19 gene. Varieties with the given gene were susceptible to three pathotypes of *P. recondita*, which are widely distributed on the territory of Kazakhstan, and revealed low level of resistance in field experiments. The same result was shown for the varieties with *Lr*1 marker in their genome. The only exclusion was the resistance of this gene towards low virulent pathotype KHP/F (South-Eastern Kazakhstan). Varieties with *Lr*10 gene were also slightly resistant. In monogenic condition this gene was effective only for pathotype KHP/F. The most effective of all analyzed genes was *Lr*9 gene. Varieties with this gene were characterized by high level of resistance to all three pathotypes; they also showed high resistance during field

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experiments. It is important to mention varieties that combine *Lr*9 and *Lr*10 genes in their genome. When evaluating plant lesions in points according to Mains and Jackson scale, the varieties that contained both *Lr*9 and *Lr*10 genes, were characterized by higher level of resistance in comparison with the plants with only *Lr*9 gene. Kazakhstan varieties with these characteristics are Yegemen and E-792.

Keywords: None.

WED-421

Secreted aspartic proteases of *Candida parapsilosis*, SAPP1 and SAPP2, deregulate the major systems of host homeostasis, including the complement cascade, the fibrynolysis pathway and the kallikrein-kinin system

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Candida yeasts are causative agents of human disorders (candidiases) of variable severity, ranging from relative mild superficial infections to life threatening systemic diseases. Although one species, *Candia albicans*, is responsible for most cases of candidiasis, the role of "non-albicans" *Candida* species has progressively increased in last decades. The major pathogen from this latter group is *Candida parapsilosis* which is frequently isolated from blood cultures, especially in neonates, transplant recipients and patients receiving parenteral nutrition. The major virulence factors of *C. parapsilosis* are secreted aspartic proteases (SAPPs) which can help the pathogen to acquire nutrients, disseminate and also to evade the host defense mechanisms, e.g., by deregulating the major homeostatic systems of the host which are based on triggering the proteolytic cascades.

The current study aimed at characterizing the influence of two major secreted aspartic proteases of *C. parapsilosis*, SAPP1 and SAPP2, on three proteolytic systems of the human host, including the complement cascade, the fibrinolysis pathway and the kinin-generating system. It was shown that SAPP1, but not SAPP2, degraded the complement C3 protein in a non-specific manner to form small inactive peptides. On the other hand, both SAPP1 and SAPP2 seemed to specifically digest the component C5. The products of C5 degradation appeared to be similar to the C5a and C5b fragments, although their activity had still to be confirmed. The analysis of plasminogen digestion excluded the contribution of SAPP1 and SAPP2 in the activation of fibrinolytic pathway. We showed that SAPPs degraded not only plasminogen but also its active form, plasmin, thereby definitively inhibiting the fibrinolysis. In the current study it was also found that SAPP1 non-specifically digested the components of the kallikrein-kinin system, i.e., the plasma prekallikrein and its active form, kallikrein, suggesting that at sites of infections caused by *C. parapsilosis* the production of kinins, the universal mediators of inflammation, can be effectively ceased.

The results of the current study confirm the hypothesis that the deregulation of major homeostatic systems of the host by fungal proteases is an important mechanism of *C. parapsilosis* virulence.

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Keywords: None.

WED-422

Sequence-structure-function relationships in the GmrSD family of Type IV restriction enzymes

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GmrSD belongs to the Type IV restriction systems. It constitutes the first-discovered modification-dependent restriction endonuclease which specifically targets and digests glucosylated hydroxymethylcytosine (glc-HMC) DNA. Nuclease activity was first reported to be dependent upon the presence of two proteins: GmrS and GmrD which can form a double (in *Escherichia coli* CT596 strain) or single-polypeptide (in *E. coli* UT189 strain) structure. GmrS was initially predicted to act as an endonuclease and NTPase while GmrD was proposed to bind DNA. Enzyme activity requires also nucleoside triphosphates (NTPs) hydrolysis (favors UTP) and is stimulated by calcium [1,2]. However, the two GenBank entries for *E. coli* CT596 proteins have been recently replaced with a single record, suggesting that GmrSD system has the single-polypeptide structure in both *E. coli* strains.

We present the results of an extensive bioinformatics analysis of sequence - function - structure relationships in the GmrSD protein family. We show that the GmrS and GmrD proteins are composed of DUF262 and DUF1524, respectfully and thus we propose the assignment of function to this uncharacterized domains. In contrast to previous reports, our protein fold-recognition analysis revealed the GmrD to possess the HNH endonuclease fold, while GmrS to be an NTPase. For both proteins, we have constructed three-dimensional models of the catalytic cores which revealed residues potentially important for structure and/or function. We have also found that the GmrSD system exists in its single-polypeptide form rather than as a heterodimer but still in some organisms the GmrS and GmrD domains are separated. Our genomic context analysis of GmrSD homologs proved them to be encoded in regions enriched in defense and gene mobility-related elements. Finally, phylogenetic reconstructions of GmrS and GmrD proteins suggests strong co-evolution of these two domains. Altogether, our results provide a stepping stone towards understanding of the GmrSD mechanism of action.

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Keywords: protein function prediction, Restriction-Modification systems, sequence analysis.

WED-423

Single cell measurements of intracellular signaling, and motility, in activated macrophages

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Macrophages are cells of the vertebrate innate immune system. They are the only cells able to eat colloidal scale particles and bacteria. Macrophages move around the body to explore the environment and with their receptors they are able to detect the presence of pathogens. When this happens a complex net-

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work of signal pathways is triggered on. In this particular state the macrophage is "activated". The aim of our research is the characterization of the activation process in macrophages, via the investigation of both the NF-kB intracellular signaling pathway and the observation of motility and morphological cell phenotypes.

Regarding the intracellular signaling we propose a single cell approach to a better understanding of the TLR4 receptor and its role in the activation of the NF-kB pathway inside macrophage cells. We developed custom-build image segmentation software that enables the detection of NF-kB translocation within the cell. This method allows us to have quantitative direct measurements and to discriminate common trends and differences between different individual cells. On a bigger scale, from the point of view of cell motility, we investigate if the migratory behavior of macrophages changes de- pending on the different activation agents in order to fulfill specific biological needs. With this aim we conducted experiments to observe cells behaviors after stimulation from LPS (a molecule present on the outer membrane of gram-negative bacteria) and IFN-γ (used by macrophages for intercellular communication).

Keywords: Cell motility, NF-kB pathway.

WED-424
Singlet molecular oxygen generation by light-activated DHN-melanin of the fungal pathogen *Mycosphaerella fijiensis* in Black Sigatoka disease of bananas

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In pathogenic fungi, melanin contributes to virulence, allowing tissue invasion and inactivation of the plant defence system, but has never been implicated as a factor for host cell death, or as a light-activated phytotoxin. Our research shows that melanin synthesized by the fungal banana pathogen Mycosphaerella fijiensis acts as a virulence factor through the photogeneration of singlet molecular oxygen O2 (1**D**g). Using analytical tools, including elemental analysis, ultraviolet/infrared absorption spectrophometry and MALDI-TOF mass spectrometry analysis, we characterized both pigment content in mycelia and secreted to the culture media as 1,8-dihydroxynaphthalene (DHN)-melanin type compound. This is sole melanin-type in *M. fijiensis*. Isolated melanins irradiated with a Nd:YAG laser at 532 nm produced monomol light emission at 1270 nm, confirming generation of O2 (1**D**g), a highly reactive oxygen specie (ROS) that causes cellular death by reacting with all cellular macromolecules. Intermediary polyketides accumulated in culture media by using tricyclazole and pyroquilon (two inhibitors of DHN-melanin synthesis) were identified by ESI-HPLC-MS/MS. Additionally, irradiation at 532 nm of that mixture of compounds and whole melanized mycelium also generated O2 (1**D**g). A pigmented-strain generated more O2 (1**D**g) than a strain with low melanin content. Banana leaves of cultivar Cavendish, naturally infected with different stages of black Sigatoka disease, were collected from field. Direct staining of the naturally infected leaf tissues showed the presence of melanin that was positively correlated to the disease stage. We also found hydrogen peroxide (H2O2) but we cannot distinguish the source. Our results suggest that O2 (1**D**g) photogenerated by DHN-melanin may be involved in the destructive effects of *Mycosphaerella fijiensis* on banana leaf tissues. Further studies are needed to fully evaluate contributions of melanin-mediated ROS to microbial pathogenesis.

Keywords: Melanin, *Mycosphaerella fijiensis*, singlet oxygen.

WED-425
Structome analysis of rapid freeze-substituted *Mycobacterium tuberculosis* cells on serial ultra-thin sections by transmission electron microscope

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Introduction: Structome is a coined word by combining 'structure' and '-ome', and defined it as the quantitative and three-dimensional structural information of a whole cell at the electron microscopic level. We've performed transmission electron microscopy (TEM) of *Mycobacterium tuberculosis* (MTB) cells processed through rapid freeze-substitution (RFS), in which can preserve exquisite ultrastructure comparable to live cell. In this report, we quantitatively examined structural properties of MTB cells by RFS and serial ultra-thin section (SUS) and performed Structome analysis.

Materials and methods: MTB, H37Rv strain was cultured and freeze-substituted as described (2, 3). The SUSes were cut to a thickness of 55 nm, mounted on a grid with formvar support film, stained and examined with JEOL JEM-1230 and the images were analyzed with Fiji (ImageJ) software.

Results: Five MTB cells were cut into 24, 35, 69, 55 and 63 SUSes. Cell profiles were measured and the average data were as follows, length: 2.71 ± 1.05 μm, diameter of cell and cytoplasm were 0.345 ± 0.033 μm and 0.293 ± 0.025 μm, respectively. Cross section area of cell and cytoplasm were 0.115 ± 0.030 μm^2 and 0.092 ± 0.024 μm^2, respectively. Surface area of outer membrane (OM) and plasma membrane (PM) were 3.04 ± 1.26 μm^2 and 2.64 ± 1.18 μm^2, respectively. Volume of cell, OM, periplasm, PM, and cytoplasm were 0.286 ± 0.113 fl (=μm^3), 0.006 ± 0.002 fl, 0.057 ± 0.021 fl, 0.018 ± 0.008 fl and 0.204 ± 0.085 fl, respectively. Total ribosome number was $1,313 \pm 841$ (ranging from 49 to 1,997) and density was 597.9 ± 361.7 / 0.1 fl.

Conclusion: This is the first report of structome analysis in MTB cells prepared with RFS and examined SUS by TEM. These data must contribute to understanding of structural properties relating to the antigenicity, acid-fastness, and the mechanism of drug-resistance relation to the ratio of the targets to the corresponding drugs.

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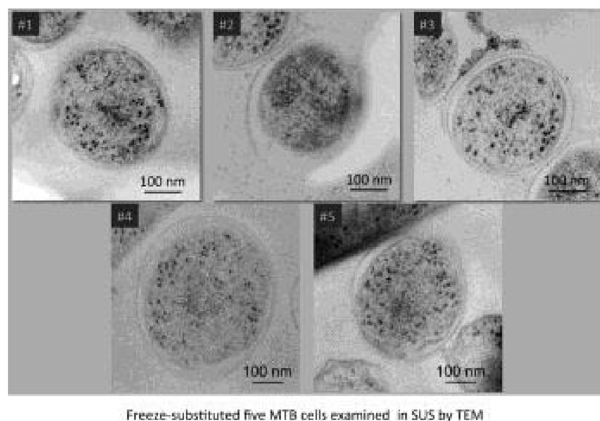

Fig. 1. Freeze-substituted five MTB cells examined in SUS by TEM.

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Keywords
Mycobacterium tuberculosis, serial ultra-thin sectioning of freeze-substituted samples, transmission electron microscopy.

WED-426
Structural and functional insights into ObcA

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Bacterial quorum sensing (QS), a cell-to-cell communication in many *Proteobacteria*, coordinates the gene expression for population-wide characteristics. Recently, a novel concept of QS was recognized, in which QS provides further benefits at the population level by regulating the production of public goods. The function of public goods could be beneficial to all members of the group including QS-deficient, exploitative individuals. In the genus *Burkholderia*, oxalate was identified as an excreted public good for the group. Particularly, its acidity regulates the pH of the environment, avoiding a possible sudden collapse of the bacterial population caused by an alkaline pH in the stationary phase of their growth. Therefore, QS-mediated oxalogenesis is a cellular event indispensible for the survival of bacteria in the stationary phase. ObcA is the first of two enzymes in oxalate biosynthetic component (obc) in *B. glumae*. ObcA-dependent catalysis presents several interesting and unexpected features. It uses oxaloacetate and acetyl-CoA as substrates, metabolites also utilized by citrate synthase in the TCA cycle, and produces an anionic tetrahedral oxaloacetate-CoA adduct, which differs from the formation of the citroyl-CoA intermediate catalyzed by citrate synthase. The proposed CoA adduct from ObcA is degraded into oxalate, acetoacetate and CoA in ObcB-dependent catalysis. These structural features are absent in other structurally irrelevant but functionally related acetyltransferases, in which an acetylated substrate and CoA are commonly produced as product via a tetrahedral intermediate. Our structural and functional analyses revealed an unprecedented mechanism of oxalogenesis.

Keywords: bacterial quorum sensing, oxalogenesis, Structure Determination.

WED-427
Structural basics for complex formation between HIV-1 integrase and its cellular co-factor Ku70

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Today, the treatment of HIV infection mostly relies on a combination of inhibitors of viral enzymes (HAART) and as a result, drug resistant viral strains are eventually generated. However, a new approach for HIV drug design is being currently developed that is focused on the disruption of functional interactions between viral enzymes and their cellular co-factors. This approach has been proven to be applicable when compounds that specifically disrupt the interaction between HIV-1 integrase (IN) and its cellular co-factor LEDGF inhibited viral replication in cell culture.

Recently, a few reports showed that Ku70, which is a part of the DNA-PK complex, prevents HIV-1 IN from degradation potentially by a direct interaction. It is known, that in cells depleted of Ku70 levels of viral replication are significantly lowered. Thus, the inhibition of Ku70-IN interaction might affect viral replication. A detailed structure of Ku70/IN complex would greatly facilitate drug design. Unfortunately, only single domains of IN can be effectively crystallized. In this regard, it is important to estimate the minimal subdomains within IN and Ku70 that are required for complex formation.

A full-size N-His_6-tagged HIV-1 IN was purified form *E. coli* as well as its C-terminal domain (IN_220-270 a.a.). At first, two versions of Ku70, Ku70_wt and a protein carrying the first 430 amino acids of Ku70 (Ku70_430), were purified also from *E. coli* both carrying a GST-tag on their N-termini. Ku70_wt and Ku70_430 both formed stable complexes with IN with Kd approximated at 80 nM as determined by GST and His6 pull downs. Ku70_wt and Ku70_430 undergo proteolysis during expression with formation of one major N-terminal product (1-319 a.a., identified by mass-spectrometry). Interestingly, this proteolytic product when individually purified interacts with IN_wt as strongly as do larger proteins with Kd approximated at 70 nM. This deletion mutant of Ku70 also equally binds the C-terminal domain of IN. The X-ray structure of Ku70 shows that Ku70_430 contains the N-terminal domain and the DNA-binding ring stabilized by a β-barrel formed by N- and C- terminal a.a., while Ku70_319 contains just the N-terminal domain and a part of the DNA-binding loop. To investigate the function of DNA-binding loop in complex formation we constructed and purified additional Ku70 deletion mutants: Ku70_309, Ku70_294, Ku70_273 and Ku70_250, where the later comprised the sole N-terminal domain of Ku70. All of them co-precipitated with IN_wt. Therefore, the DNA-binding ring in Ku70 is not an essential structure for IN/Ku70 complex formation. Also, we successfully minimized the fractions of Ku70 and IN that are required for complex formation.

Keywords: HIV-1 integrase, Ku70, Protein – protein interactions.

WED-428
Structural dynamics of Perfringolysin O after incorporation into the lipid membrane

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Perfringolysin O (PFO) is a toxic protein produced by *Clostridium perfringens* that binds to cholesterol-containing membranes.

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The interaction of perfringolysin O with cholesterol-rich membranes initiates a series of structural changes which result in the formation of a ring-shaped-barrel pore, leading to cell lysis. The pore formation mechanism is a three-step process consisting of binding of water-soluble monomers to the membrane, assembly into oligomeric prepore complex on the membrane surface and finally insertion of monomer amphipathic β-hairpins into the membrane, resulting in conversion of a prepore complex into a β-barrel pore.Previous studies have uncovered the scenario of events in this multistage structural transition to a considerable detail, but the underlying molecular mechanisms are not fully understood yet.

Using hydrogen-deuterium exchange coupled with mass spectrometry (HDX-MS) method we have obtained a detailed list of all regions which undergo structural changes caused by the interaction with lipid membranes and oligomerization, thus providing a new experimental constraints for molecular modeling of the pore formation process.

Obtained results indicate that HDX-MS can be useful method applied for study of structural changes of PFO derivatives during interaction with lipid membranes. Structural insight into the properties of PFO mutants can bring a new information helpful to elucidate the role of particular domains in conformational changes during formation of lytic pores.

Keywords: HDX-MS, pore-formation, toxin.

WED-429

Structure-function relationship of the first strand transfer in human immunodeficiency virus type 1 (HIV-I)

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HIV-1 can develop resistance to antiretroviral therapies and escape to the host immune system because it is prone to recombine via strand transfers occurring during the reverse transcription process. Reverse transcription of the HIV-1 genome consists in a succession of steps allowing the conversion of the single-stranded RNA genome into a double-stranded DNA molecule. An obligatory step of reverse transcription is the first strand transfer. Characterization the molecular mechanisms governing the first strand transfer would contribute to a better understanding of the reverse transcription process and of the strand transfers that are responsible for HIV-1 recombination. The first strand transfer relies on the annealing of the minus-strand strong-stop DNA (ssDNA) (the first DNA's part synthesized during reverse transcription) to the 3′ end of the viral RNA (3′ UTR RNA). This annealing reaction is facilitated by the HIV-1 nucleocapsid protein (NC) that destabilizes the nucleic acids secondary structures and promotes their association. Note that the annealing process of the full-length ssDNA to the 3′ UTR RNA is not known at the molecular and structural level. The main aim of this study is to better understand the NC-mediated annealing process. First, we use structural probes (potassium permanganate, kethoxal, dimethyl sulfate, DNase I, mung bean nuclease and RNase V1), to determine the secondary structures of the full-length ssDNA and the 3′ UTR RNA in the absence or presence of NC. Second, we investigate the structural changes in ssDNA and 3′ UTR RNA during the annealing process in order to identify the initiation and destabilization sites of hybridization. Finally, our results will be confirmed by site-directed mutagenesis.

Keywords: HIV-1, Secondary structure, Strand transfer.

WED-430

Structure-functional study of PHL lectin from *Photorabdus asymbiotica*

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Lectins are a very important group of proteins and glycoproteins, which specifically recognize and reversibly bind glycoconjugates. Due to this interaction, lectins play a crucial role in many physiological and pathophysiological processes including immunological reactions or interactions between tissue's cells. They also play a very important role in interactions between a pathogen and its host as they can mediate the first step of an expansion of infection. Currently, lectins become essential tools in the biological and medical research, i.e. to follow agglutination of erythrocytes, detection and characterization of different saccharide composition of glycoconjugates, mitogenic stimulation of lymphocytes, inhibition of fungal, bacterial and viral growth, etc. [1].

Our project is focused on study of a new lectin from the *Photorabdus asymbiotica* bacterium. It belongs to the *Photorabdus* genus together with *Photorabdus luminiscens* and *Photorabdus temperata*. The latter 2 species are characterized as strictly virulent insect pathogens. In contrast, *P. asymbiotica* is also a human pathogen, which causes locally invasive soft tissue and disseminated bacterimic infections. We have identified a new putative lectin in the *P. asymbiotica* genome with the predicted structure similarity to the lectins from so-called AAL family, which are specific for fucosylated oligosaccharide. The AAL family contains AAL from *Aleuria aurantia* which inhibits a repair of epithelia [2] or the RSL lectin from *Ralstonia solanaccearum* predicted as one of the key virulent factors of this plant pathogen [3].

A wide range of methods was used for structural a functional studies of PHL including surface plasmon resonance, isothermal titration calorimetry, analytic ultracentrifugation, hemagglutination, and X-ray structural analysis.

Analytical ultracentrifugation confirmed that PHL forms a dimer. The crystal structure of PHL revealed a presence of fourteen potential binding sites per monomer. PHL lectin belongs to seven beta-propellers, which makes this protein unique compared to proteins from AAL family, which share the six beta-propeller fold. Crystal structures of PHL with different saccharides demonstrated two types of binding sites, which could bind ligands with different polarity. PHL lectin showed highest affinity to fucose among studied monosaccharides. SPR revealed a strong multivalent effect when binding to the high-density fucosylated surface on a chip. Binding properties of PHL will be further studied with more complex saccharides.

Keywords: lectin, *Photorabdus asymbiotica*.

WED-431

Study of the interaction of proline-rich antimicrobial peptides of caprine leukocytes with bacterial and mammalian cells

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Antimicrobial peptides (AMPs) of the innate immune system play an important role in anti-infective host defense. Proline-rich peptides (PRPs) comprise a special group of AMPs whose members have a potent antimicrobial activity predominantly towards Gram-negative bacteria and negligible toxicity towards host cells

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and therefore can be considered as promising templates for development of new antibiotic drugs.

We explored an interaction of two proline-rich peptides ChBac3.4 and ChBac5, that we previously have isolated from goat leukocytes, with bacterial and mammalian cells. It was found that despite these peptides belonged to the same structural family of bactenecins, they displayed differences in the biological activity. Both peptides exhibited broad-spectrum antimicrobial action under low salt conditions and possessed similar lipopolysaccharide-neutralizing activity. In the presence of physiological concentration of NaCl the peptides retained the high activity towards Gram-negative bacteria including drug-resistant strains of *Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumonia*. While the activity of ChBac5 against staphylococci upon this condition was significantly decreased, the antibacterial action of ChBac3.4 was inhibited to a much lesser degree. Typically for most PRPs, antimicrobial action of ChBac5 was not accompanied by marked damaging of bacterial membranes; in contrast, ChBac3.4 caused relatively increased outer and inner membrane permeability of *E. coli* ML35p.

ChBac5 demonstrated the low toxicity for mammalian cells; ChBac3.4 exerted an unusual for PRPs cytotoxic activity towards cultured cells (human erythroleukemia cells K562, hystiocytic lymphoma cells U937, promyelocytic leukemia HL60 but not for normal human skin fibroblasts). The cells K562 or U937 treated with the peptide (10-20 μM) demonstrated the features of apoptosis; upon the higher peptide concentration (>40 μM) – necrosis. The differences in biological activity of two studied peptides might be partly due to higher hydrophobicity of ChBac3.4 in comparison with ChBac5 and other peculiarities of ChBac3.4 primary structure.

The obtained data contribute to understanding of the molecular mechanisms of the interaction of proline-rich peptides with bacterial and host cells and provide valuable information for the future structural-activity relationship study of PRPs synthetic variants directed to a design of novel anti-infective pharmaceuticals.

The work was supported by the RFBR grants No 13-04-02102; 12-04-01573; 12-04-01498.
Keywords: None.

WED-432
Study on S-nitrosoglutathione reductase from plant pathogens

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Nitric oxide (NO) and NO-related molecules such as *S*-nitrosothiols (*S*-NOs) play a central role in the regulation of plant physiological processes and host defence. The enzyme *S*-nitrosoglutathione reductase (GSNOR) participates in the control of *S*-NOs cellular homeostasis and in the metabolism of reactive nitrogen species. GSNOR has emerged as a key regulator of protein denitrosylation in plant defence to pathogens [1]. GSNOR has been recently characterized from several organisms like human, *Arabidopsis* or *Solanum* spp. [2]. This study represents the first detailed biochemical and structural characterization of GSNOR from plant pathogen, oomycete *Phytophthora infestans*, the causative agent of potato late blight. Corresponding gene (*PiGSNOR*, GenBank XM_002998982, 1160 bp) was sequenced, cloned and expressed in T7 *E. coli* cells as a N-terminal 6xHis tagged recombinant protein. We showed previously that in comparison to hGSNOR plant enzymes exhibit different composition of the anion-binding pocket, which negatively influences the affinity for w-hydroxyfatty acids [2]. GSNOR from *P. infestans* also differs in the anion-binding pocket composition and carries Ser113 in place of Gln112 found in human GSNOR and Gly114 found in plant GSNOR. PiGSNOR shows 63% sequence identity with SlGSNOR (GenBank GU296438) [2] and 67% identity with human (EMBL M30471). Detailed biochemical study of purified PiGSNOR was performed to characterize its stability, substrate affinity, cofactor preferences and sensitivity to known GSNOR inhibitors like a novel, first-in-class drug N6022 [3]. The obtained results will be used to get further insight on the role of S-nitrosylation and GSNOR both in pathogen and host cells during various stages of *Phytophthora* pathogenesis.

This work was supported by Ministry of Education, Youth and Sports of Czech Republic (LH11013), Czech Science Foundation (GACR P501/11/1591) and IGA 2014_020 of Palacký University in Olomouc.

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Keywords: *Phytophthora infestans*, recombinant protein, S-nitrosoglutathione reductase.

WED-433
Substrate Protein interaction plays key role in microbial adhesion

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Biomaterial-associated-infections remain a major cause of failure of biomaterial implants.

Microbial adhesion to a biomaterial surface is determined by ability of a pathogen to form a **biofilm** on it, which is again significantly dependent on the physicochemical properties of the implant surfaces. **Biofilms** are surface-associated microbial communities, which can form on a variety of surfaces in natural conditions and hospital niches.

But what are the controlling factors of **biofilm formation** on the surface of implant materials? That is the primary question that drives my research.

My research is focused on bacteria which are responsible for device-associated infections. Bacterial biofilms arise from microcolonies that become embedded in an extracellular matrix (or extrapolymeric matrix EPS), which is a glue, holding microbial cells together. The biofilm matrix contributes to the overall architecture and the phenotype of biofilms. Uncovering roles played by EPS matrices in biofilm formation will be beneficial in controlling **biofilm formation**. *In vivo*, biomaterials are rapidly covered with layers of adsorbed proteins from plasma, saliva, tear fluid, or other bodily fluids, depending on the implant site. Hence, adsorbed protein films play a vital role in microbial or tissue adhesion to the implant surface.

My experiment initiates with study of adsorption of fibrinogen, fibronectin and albumin from solutions of the respective proteins singly, on a variety of specimens of implant surfaces with varying degrees of hydrophilicity and thrombogenicity. This was studied with the help of a UV-Visible spectrophotometer and the respective data was plotted with requisite software. Adsorption behavior and hence adhesion shows a strong dependence on surface properties. All surfaces show a preference for fibronectin

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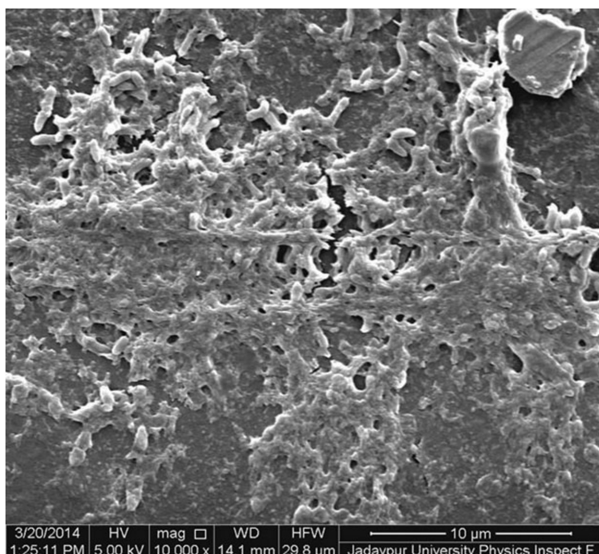

Fig. 1. Biofilm of *Pseudomonas aeruginosa* on polypropylene having fibronectin adsorbed on its surface.

and fibrinogen, and the degree of preference appears to correlate with thrombogenic tendency.

Next, biofilms of Pseudomonas aeruginosa were grown on these specimens of biomaterials by placing them in 24 well microtiter plates with bacterial culture and supplied with nutrient broth. These were kept inside a BOD incubator at 37 °C for 1 week. The biofilms were provided with nutrient broth for the whole period of growth. There was a control experiment in each case to observe the contribution of the adsorbed protein layers.

Finally each specimen of biomaterial was taken out of the microtiter plate, washed with distilled water and PBS. The biofilms were then fixed using glutaraldehyde and all samples were studied under a scanning electron microscope. The variation of the nature of biofilms and the EPS matrices in the differentt cases were worth noting and could be related primarily to the thrombogenicity of the adsorbed proteins and hydrophobicity of the biomaterial surfaces.

Keywords: Biofilms, Biomaterials.

WED-434
The bacterial cytolethal distending toxin: a nuclease inducing indirect DNA double-strand breaks into eukaryotic cells

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The cytolethal distending toxin (CDT) is produced by many pathogenic Gram-minus bacteria like *Escherichia coli, Helicobacter hepaticus, Haemophilus ducreyi, Salmonella typhi* and others. *In vivo*, the production of CDT by *Helicobacter hepaticus* induces the development of dysplastic liver nodules, thus defining CDT as a potential carcinogen. *Ex vivo*, CdtB, the catalytic sub-unit of CDT, is relocated into the eukaryotic cell nucleus. There, CDT induces DNA double-stranded breaks (DSBs), leading to cell cycle arrest and cell death. However, after years of study, the CDT mode of action only begins to be unrevealed and many aspects remain to be studied.

To better characterize the CDT-induced DNA lesions, we are studying the biochemistry of CDT, specifically regarding its catalytic nuclease activity and relate these aspects to the overall cellular effects of the toxin. Based on the literature and on the structural homology of CdtB with the DNase I, we developed several CDT mutants for specific residues involved in the catalytic activity. The DNA binding and the nuclease activities of CdtB are studied by *in vitro* tests, like Supercoiled DNA cleavage and DNA binding assay. *Ex vivo*, CDT-induced DNA damages and the activation of specific DNA damage response (DDR) pathways have been characterized. Thanks to comet assay and immunofluorescence staining, we have shown that, depending on the CDT dose, DSBs (high doses) or SSBs (moderate doses) will be induced, the later degenerating into DSBs following the replication. In fact, after a treatment with moderate doses, CDT-induced DNA damages leading to the activation of the DDR involving the RPA, ATR and CHK1 proteins, characteristic of a replicative stress. The DSBs DDR involving the ATM pathway, occurs later during CDT treatment.

The importance of the S-phase passage for the CDT cytotoxicity suggests that proliferating cells are more sensitive to CDT than quiescent cells. The presence of unrepaired damage can lead to cell death, whereas effective repair will allow cells to resume cell cycle. However, improper repair of DNA damage can induce genetic instability and lead to cancer. Bacterial niches containing CDT producing strains are located at epithelia that are quick renewal tissues. Understanding the effects of CDT at the cellular level is an essential step in order to understand these effects at the tissue and/or organism level as well as CDT's involvement in bacteria pathogenicity.

Keywords: Cytolethal Distending Toxin, DNA damage.

WED-435
The epizootic hemorrhagic disease virus (EHDV) induces and benefits from cell stress, autophagy and apoptosis

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The mode and timing of virally-induced cell death withhold the potential of regulating viral yield, viral transmission and the severity of virally-induced disease. Orbiviruses such as the epizootic hemorrhagic disease virus (EHDV) are nonenveloped and cytolytic. To date, the death of cells infected with EHDV, the signal transduction pathways involved in this process and the consequence of their inhibition have yet to be characterized. Here, we report that the Ibaraki strain of EHDV2 (EHDV2-IBA) induces apoptosis, autophagy, a decrease in protein synthesis and the activation of c-Jun N-terminal kinase (JNK) and the phosphorylation of its substrate c-Jun. Inhibition of: (i) apoptosis with the pan-caspase inhibitor Q-VD-OPH, (ii) autophagy with 3-methyladenine or via the knockout of the autophagy regulator Atg5, and (iii) c-Jun phosphorylation with the JNK inhibitor SP600125 or with the cyclin-dependent-kinase (cdk) inhibitor roscovitine, independently attenuated the increase in viral titer in the course of infection. Moreover, SP600125 and roscovitine attenuated the EHDV2-IBA-mediated induction of autophagy. Taken together, our results imply that EHDV induces and benefits from the activation of signaling pathways involved in cell stress and death.

Keywords: Apoptosis, Autophagy, EHDV.

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WED-437

The impact of phosphate limitation on bacterial pathogenicity of *T. thermophilus* HB8

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Environmental inorganic phosphorus constitutes the growth limiting nutrient for aquatic microorganisms. When starved for phosphate, bacteria adapt to their environment to increase their ability to take up inorganic phosphate. Example of an adaptation is the bacterial morphogenesis ending in the elaboration of long, filiform tubular extensions of the cell envelope of certain aquatic bacteria called stalks. Clinical isolates of *P. aeruginosa* formed stalks induced by phosphate limitation, which contain a DING protein named from the first 14 amino acids. This protein is an alkaline phosphatase. Bacteria, for motility and phosphate acquisition, develop also flagella, where the principal substituent is flagellin. They secrete also rhamnolipids as virulence factors. The formation of flagella is enhanced after prolonged cultivation time where phosphate and other nutrients were depleted; thus it seems likely that these organelles have a role in the virulence of pathogenic organisms.

The non pathogen bacterium *T. thermophilus* when cultivated in low phosphorus showed after dyeing stalks and produced rhamnolipids in contrast to those cultivated in rich medium. The length of the stalks was inversely related to phosphate concentration. Stalks were isolated and an alkaline phospatase activity was found to be localized on stalks. This activity was purified with a gel filtration chromatographic Sephadex G-150 superfine column and a band of 40 kDa molecular weight was immuno-stained in Western blot using antibody against DING protein. To elucidate the possible co-localization on the stalks of flagellin, DING and rhamnolipids, a stalks preparation was also tested by Dot blot immunoassay using three antibodies against these constituents. All antibodies immuno-cross reacted suggesting the presence of these three constituents in the stalks structure and the immunosignal was affected by the phosphate concentration. Moreover, a Western blot analysis of stalks after SDS-PAGE, with the same three pre-referred polyclonal anti-sera, was also performed. This analysis resulted in a band of the same molecular weight with all three antibodies suggesting that DING protein, flagellin and rhamnolipids probably form a complex.

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Keywords: DING, flagellin, rhamnolipds.

WED-438

The intestinal Muc2 mucin can influence on the binding, swarming and chemotaxis processes in *Vibrio cholerae* along the gut

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To successfully infect and colonize the human intestine, *Vibrio cholerae* must penetrate the intestinal mucus layer system and express virulence genes. The first line of defense against the microbes is the intestinal mucus layer system that covers and protects the intestinal epithelial cells. The intestinal mucus system of both the small intestine and colon is organized around the highly glycosylated Muc2 mucin, that forms large net-like polymers that are secreted by the goblet cells. Recent knowledge suggests that mucin-sensing signaling systems are required for the bacterial pathogen colonization of the mammalian intestine. *V. cholerae* activates a colonization mechanism towards the mucus layers which has been correlated with the pathogen′s competence in penetrating the mucus and reaching the small intestine, but the precise role of this process during infection has not been clearly established. We have established an In vitro system where we have purified mouse Muc2 mucin from different regions along the intestinal tract and we expose it to the presence of *V. cholerae* in order to evaluate the growth, binding, the swarming and the chemotaxis process. Our results shows that *V. cholerae* can growth to the presence of intestinal Muc2 mucin obtained from different regions along the gut, and binds preferentially to Muc2 mucin from ileum, proximal and distal colon. The chemotactic responses to the presence of Muc2 mucin are predominantly found from the jejunum, ileum and middle colon. Intriguingly, high *V. cholerae* swarming activity to the presence of Muc2 mucin was found on the jejunum and the whole large intestine. Taken together, these data suggests an interesting scenario where *V. cholerae* might sense the gut environment through the presence of Muc2 mucin.

Keywords: intestinal colonization, Intestinal Muc2 mucin, *Vibrio cholerae*.

WED-439

The periplasmic binding protein AccA from the plant pathogen *Agrobacterium tumefaciens*

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Agrobacterium tumefaciens is a soil bacterium that is pathogenic for several plants causing the crown gall disease, which is characterized by tumour formation at the infection site. The virulence of the bacteria is brought by the presence of the virulence plasmid pTi. The infection mechanism is well known and composed of 4 steps:

1. The wound on the plant activates *A. tumefaciens*' virulence plasmid.
2. T-DNA, a piece of the pTi is then transferred to the plant's cell and integrated to its genome.
3. The plant secretes hormones, triggering the tumour formation, tumours which are colonized by *A. tumefaciens*. The plant also secretes Agrocinopine that is used as energy source by the bacterium.
4. Agrocinopine also activates quorum sensing mechanisms in *A. tumefaciens* leading to spreading of the pTi to non-virulent bacteria.

Agrocinopine is imported in the bacteria by Acc system. Acc is composed of an ABC transporter and a periplasmic binding protein. The PBP AccA that recognize Agrocinopine. Genetic studies showed that AccA also was responsible for the import of Agrocine 84, a lethal toxin, produced by another bacteria A. radiobacter K84. The subject of my thesis is the understanding of the interaction specificity of the PBP AccA with Agrocinopine and Agrocine 84, thus combining structural and biochemical studies of the complexes formed by AccA with its ligands.

In order to understand these interactions, we determined the first 3D structure of a PBP with its opine ligand. We also determined the structure in complex with the toxin Agrocine 84 allowing us to characterize the interaction with each ligand. Combined with biochemical studies, our findings revealed the interactions involved in the binding of the two antagonist ligands leading to their import by the corresponding Acc ABC transporter.

Keywords: *Agrobacterium tumefaciens*, Crown Gall desease, Periplasmic Binding Protein.

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WED-440
The prevalence of *Leucocytozoon toddi* in bird blood samples in Aras-Iğdır and evaluation of its phylogenetic relationships

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Today biological diversity is faced with high risks of extinction due to the overuse of natural resources. Studies of bird species constitutes a central theme in ecological investigations and for conservation of biological diversity. The identification of parasitic infections encountered in birds provide contributions to ecological studies with regards to the persistence of species. The aim of this study is the detection of the prevalance of *Leucocytozoon toddi* infection in birds of Aras-Iğdır region, using genetic methods. 401 blood samples belonging to 58 bird species of 25 different families were investigated. *L. toddi* infection was detected in 41 samples and five distinct haplotypes were obtained from six sequences. Phylogenetic trees were constructed using these five haplotypes along with 265 sequences of 76 species taken from GenBank and MalAvi databases. Four out of five haplotypes of Aras-Iğdır positive samples were distinct from those in literature. Again four of the five Aras-Iğdır haplotypes clustered very closely together, potentially suggesting some genetic isolation in this migratory pathway. The phylogenetic comparisons made using all sequences also support the idea of the presence of two cryptic species of *L. toddi*.

Keywords: Avian haemosporidians, Host-switching, *Leucocytozoon toddi*.

WED-441
The RfaH in *Yersinia enterocolitica* O:3 is a high specificity regulator

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RfaH is a regulatory protein functioning as antiterminator for long operons. In some bacterial species like *Escherichia coli* and *Salmonella enterica* it promotes the expression of selected operons encoding extracytoplasmic cell components, such as lipopolysaccharide (LPS), capsule, hemolysin, exotoxin, hemin uptake receptor and F pilus. Due to the fact that these structures are required for bacterial virulence, loss of RfaH usually attenuates virulence. We report that in *Yersinia enterocolitica* O:3 it acts as a highly specific regulator that enhances the transcription of O-antigen and outer core encoding operons, yet does not affect the expression of enterobacterial common antigen (ECA). The luciferase promoter reporter analysis confirmed the decrease in transcription of these two oprerons upon the inactivation of RfaH. Furthermore, the transcriptomic analysis of $\Delta rfaH$ strain and a spontaneous rough mutant revealed similarities in the gene expression patterns indicating that the $\Delta rfaH$ mutation triggers via the loss of O-antigen indirect responses, including changes in the outer membrane and Cpx pathway stress response. Moreover, decrease in the amount of LPS on the cell surface results in stress response and lower resistance to such compounds as sodium dodecyl sulfate and polymyxin B. Interestingly, loss of RfaH resulted in marginally higher resistance to normal human serum. It may be speculated that RfaH might have *in vivo* role in controlling tissue-specific expression of bacterial surface oligo/polysaccharides.

Keywords: lipopolysaccharide, RfaH, *Yersinia enterocolitica*.

WED-442
The RNA chaperonne activity of Vif is mainly contained in its C-terminal domain

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The viral infectivity factor (Vif) is essential for the productive infection and dissemination of HIV-1 in non-permissive cells, containing the cellular anti-HIV defense cytosine deaminases APOBEC3 (A3G and A3F). Vif neutralizes the antiviral activities of the APOBEC3G/F by diverse mechanisms including their degradation through the ubiquitin/proteasome pathway and their translational inhibition. In addition, Vif appears to be an active partner of the late steps of viral replication by interacting with $Pr55^{Gag}$, reverse transcriptase and genomic RNA. In this study, we expressed and purified full-length and truncated Vif proteins, and analyzed their RNA binding and chaperone properties. First, we showed by CD and NMR spectroscopies that the N-terminal domain of Vif is highly structured in solution, whereas the C-terminal domain remains mainly unfolded. Both domains exhibited substantial RNA binding capacities with dissociation constants in the nanomolar range, whereas the basic unfolded C-terminal domain of Vif was responsible in part for its RNA chaperone activity. Second, we showed by NMR chemical shift mapping that Vif and NCp7 share the same binding sites on $tRNA^{Lys}{}_3$, the primer of HIV-1 reverse transcriptase. Finally, our results indicate that Vif has potent RNA chaperone activity and provide direct evidence for an important role of the unstructured C-terminal domain of Vif in this capacity.

Keywords: HIV-1, RNA, vif.

WED-443
The *Salmonella* effector SteA contributes to the control of membrane dynamics of *Salmonella*-containing vacuoles

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Salmonella enterica serovar Typhimurium (*S.* Typhimurium) is a bacterial pathogen causing gastroenteritis in humans and a typhoid-like systemic disease in mice. *S.* Typhimurium virulence is related to its capacity to multiply intracellularly within a membrane-bound compartment, the *Salmonella*-containing vacuole (SCV), and depends on type III secretion systems that deliver bacterial effector proteins into host cells. Here, we analyzed the cellular function of the *Salmonella* effector SteA. We show that, compared to cells infected by wild-type *S.* Typhimurium, cells infected by $\Delta steA$ mutant bacteria displayed less *Salmonella*-induced filaments (SIFs), an increased clustering of SCVs, and morphologically abnormal vacuoles containing more than one bacterium. The increased clustering of SCVs and the appearance of vacuoles containing more than one bacterium were suppressed by inhibition of the activity of the microtubule motors dynein or

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kinesin-1. Clustering and positioning of SCVs are controlled by the effectors SseF and SseG, possibly by helping to maintain a balanced activity of microtubule motors on the bacterial vacuoles. Deletion of *steA* in *S.* Typhimurium Δ*sseF* or Δ*sseG* mutants revealed that SteA contributes to the characteristic scattered distribution of Δ*sseF* or Δ*sseG* mutant SCVs in infected cells. Overall, this shows that SteA participates in the control of SCV membrane dynamics. Moreover, it indicates that SteA is functionally linked to SseF and SseG, and suggests that it might contribute directly or indirectly to the regulation of microtubule motors on the bacterial vacuoles.

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Keywords: Bacterial pathogenesis, *Salmonella*, Type III secretion effectors.

WED-444
The three Serine Rich proteins encoded in the secA2Y2 system of the human commensal bacterium *Streptococcus salivarius* promotes binding to different targets of the host

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Bacterial adhesion to host and bacterial surfaces is a crucial step for colonization, development of microbial communities and the establishment of a bacterial-host cross-talk. To characterize molecular mechanisms underlying adhesion of commensal bacteria, we used *Streptococcus salivarius* (SSAL) as a model. SSAL is among the most important pioneer colonizers of neonatal oral mucosal surfaces, and later becomes a predominant component of the human adult oral microbiota with pre-eminent ecological role and a sub-dominant bacterium of the human gastro-intestinal tract.

We identified, through phenotypic screening assays, genes involved in SSAL adhesion to host surfaces. In particular, we showed that the SecA2Y2 system, which comprises genes devoted to glycosylation and export of surface Serine Rich repeat Proteins (SRPs), participates to bacterial aggregation, biofilm formation, *in vitro* adhesion and potential colonization of mice. While all bacteria containing a similar system possess only one SRP, the SSAL *secA2Y2* locus comprises three SRPs with complementary role in line with the previous phenotypes. Interestingly, SrpB is specifically involved in the binding to epithelial cells, while SrpC to the extracellular matrix proteins. We showed that these interactions require glycosylation of both bacterial SRPs and host surfaces.

This work is the first report showing the presence in a bacterium of three SRPs, which display complementary roles in bacterial-host interaction. It also underlines the role of glycosylation of both host and bacterial components in these processes. Finally, while the SecA2Y2 system is mostly associated to virulence in pathogenic bacteria, it appears to be involved in the expression of commensal traits in SSAL, such as its colonization and its resilience to oral and intestinal niches. This work may offer new insights into the mechanisms of niche establishment (host, microbial communities) of commensal bacteria.

Keywords: adhesion, commensal-host, interaction.

WED-445
Transcript and metabolite analysis of *Vitis vinifera* cv. Trincadeira berries infected with *Botrytis cinerea* reveals a suppression of developmentally activated defenses

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Vitis vinifera berries are sensitive towards infection by the necrotrophic pathogen *Botrytis cinerea* leading to important economic losses worldwide. The combined analysis of the transcriptome and metabolome associated with fungi infection has not been previously performed in grapes or in another fleshy fruit.

In an attempt to identify the molecular and metabolic mechanisms associated with the infection, pepper-corn size fruits were infected in-field. Green berries and *véraison* berries were collected following infection for microarray analysis. These transcriptome analyses were complemented with metabolic profiling of primary and other soluble metabolites and of volatile emissions.

The results provide evidence of a reprogramming of carbohydrate and lipid metabolisms towards increased synthesis of secondary metabolites involved in plant defense, such as *trans*-resveratrol and gallic acid. The response is already activated in infected green berries with the putative involvement of jasmonic acid, ethylene, polyamines and auxins whereas salicylic acid does not seem to be involved. Genes encoding protein kinases, WRKY transcription factors, pathogenesis-related proteins, glutathione S-transferase, stilbene synthase and phenylalanine ammonia-lyase were up-regulated in infected berries. However, salicylic acid signaling is activated in healthy ripening berries along with the expression of proteins of NBS-LRR superfamily suggesting that ripening berries have defenses that are absent in infected berries. Furthermore, this study provided metabolic biomarkers of infection such as azelaic acid, a substance known to prime plant defense responses, arabitol, ribitol, 4-amino butanoic acid, 1-O-methyl- glucopyranoside and several fatty acids that alone or in

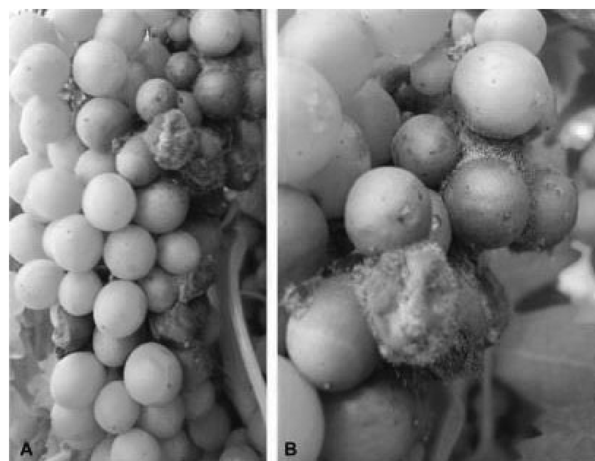

Fig. 1.

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combination can be used to monitor *Botrytis* infection early in the vineyard.

Keywords: host-pathogen interaction, metabolomics and transcriptomics, plant defense.

WED-446

Transcription activator-like effectors (TALEs) are effective modulators of transcription in *Leptospira* spp.

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Objective: Leptospirosis is a neglected zoonotic disease that affects approximately one million people annually with a mortality rate greater than ten percent. While an important global disease, there is a deficiency of effective genetic manipulation tools available for targeted mutagenesis in pathogenic *Leptospira* spp. Transcription Activator-Like Effectors (TALEs) are a recently described group of proteins which modify transcriptional activity in prokaryotic and eukaryotic cells by directly binding to a targeted sequence of repeat variable di-residues responsible for transcriptional control. This study aims to elucidate the efficiency of TALEs within *Leptospira* spp. for targeted repression.

Methods: To determine the function of TALE within *Leptospira* spp., three distinct TALE constructs were designed: a *tale* specific for the *lacO*–like region in the saprophyte *Leptospira biflexa* (pTALE$_{\beta\text{-}gal}$); a *tale* specific for the promoter for *ligA* and *ligB* in the pathogenic strain *Leptospira interrogans* (pTALE$_{lig}$); and a *tale* specific for the promoter of *loa22* in the pathogenic strain *Leptospira interrogans* (pTALE$_{loa22}$). Prior to *tale* insertion in each genome, respectively, the TALE repressors were placed distal to *flgB*, a constitutively expressed promoter, and inserted into suicide vector pSC189ColE1 carrying the *Himar1* mariner transposon system, which then randomly inserts the *flgB-tale* construct into the bacterium's genome.

Results: RT-PCR analysis demonstrated β-galactosidase was not transcribed in pTALE$_{\beta\text{-}gal}$ in vitro; Miller ONPG enzymatic assay revealed pTALE$_{\beta\text{-}gal}$ produced 0 mU of β-galactosidase, while wild type produced 3.36 mU β-galactosidase. Cells grown on solid media supplemented with 100 μM IPTG and 80 μg/ml X-gal revealed wild type produced blue colonies, while pTALE$_{\beta\text{-}gal}$ produced white colonies. pTALE$_{lig}$ showed a 6-8 fold reduction in expression of LigA and LigB by Western blot analysis when grown under osmotic stress (120 mM NaCl). pTALE$_{loa22}$ showed a 3 fold reduction in expression of Loa22 by qRT-PCR and Western blot analysis.

Conclusion: TALE effectively reduces expression of targeted genes within saprophytic and pathogenic strains of *Leptospira* spp., which provides an additional genetic manipulation tool for this genus. Future work includes testing pTALE$_{lig}$ and pTALE$_{loa22}$ for reduced virulence in vivo, and testing additional TALE constructs that have been designed for attachment to distinct promoter regions of *ligA*/*ligB*, and *loa22* to determine if TALE can further repress expression of these genes.

This work was supported in part by National Science Foundation grant IIA-1159099.

Keywords: *Leptospira*, TALE, Transcription Repression.

WED-447

Understanding the cellular function of SAMHD1: from HIV restriction to tumor suppression

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SAMHD1 was identified as a deoxynucleoside triphosphate (dNTP) triphosphohydrolase and a potential nuclease that acts as an HIV-1 restriction factor in noncycling cells. Germline mutations in the corresponding gene have been described in 17% of the patients suffering from Aicardi-Goutières syndrome, a disease mimicking congenital infections.

We investigated the role of SAMHD1 outside the context of HIV-1 infection and we showed that (i) This protein is expressed at low levels in proliferating transformed cell lines. (ii) Overexpression of wild-type SAMHD1 slows down cell proliferation and promotes cell death after induction of DNA double strand breaks (DSB). (iii) SAMHD1 was specifically regulated and recruited to DSB sites after DNA damaging treatment.

Considering the importance of cell proliferation, DNA damage response and dNTP metabolism in tumorigenesis, we looked at SAMHD1 expression in several cancer types. We provided evidence that SAMHD1 expression is decreased in patients suffering from various hematopoietic cancers and breast cancers. Moreover, in the specific case of Chronic Lymphocytic Leukemia, in addition to reduced SAMHD1 expression at both mRNA and protein levels, we observed mutations in the corresponding gene. This mutation rate is increased in treatment-resistant tumors.

Taken together, this data strongly suggest that SAMHD1 loss during tumorigenesis may not only favor cancer development by promoting DNA instability and cell proliferation, but also confer a drug-resistant phenotype to tumor cells. More studies will be needed to further characterize SAMHD1 roles in resistance to chemotherapy and DNA damage repair.

Keywords: Cancers, DNA damage, HIV-1.

WED-448

Virulence and resistance markers in foodborne bacteria isolated in Bucharest, Romania

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Objective: The aim of this work was the assessment of the microbiological risk for consumers health, by evaluating virulence and resistance determinants of food borne bacteria isolated from food products in Bucharest, Romania.

Methods: A number of 144 bacterial strains belonging to *Enterobacteriaceae*, *Listeriaceae* and *Micrococcaceae* families were isolated and identified, according to the updated ISO standards concerning food safety. Phenotypic assays: (i) adherence to eukaryotic HEp-2 cells (Cravioto's adapted method); (ii) biofilm development on the inert substratum (slime production assay);

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(iii) soluble enzymatic factors expression; (iv) antibiotic susceptibility testing (disk diffusion method). PCR detection of virulence and resistance genes: (i) virulence associated genes in enterobacteria (eaea, bfpA, eaf, AggR, EAggE, afa, pap, sfa, VT1,VT2, EAST1, pldA, helD, spvC, invA); in *Listeria* sp. (hlyA, prfA); and in strains from the genus *Staphylococcus* (cna, icaA, tst, eta, fnbB, fib, clfA, clfB) (ii) antibiotic resistance genes (fox, mox, blaTEM, blaCTX-M, cit, dha, accm, ebcm, tetA, tetB, tetC, tetD, dfrD).

Results: Most of the analyzed strains showed the ability to adhere to both eukaryotic cells and inert substratum. Also, many of the analyzed strains produced proteases, glucidases and iron-chelating agents. We identified 9 multidrug resistant strains (beta-lactams, tetracyclines, folate pathway inhibitors/aminoglycosides), ESBL and AmpC producing strains being also detected. The Gram-positive strains belonging to *Staphylococcus* and *Listeria* genera were susceptible to most of the tested antibiotics. The molecular assay revealed the presence of multiple virulence and/or resistance genes in the analyzed strains.

Conclusions: The ability of food borne bacteria to adhere to eukaryotic cells is indicating the potential of these strains to initiate an infection in the human or animal host, while adherence to the inert substratum and biofilm formation on the food processing surfaces is the main method through which strains of different genera persist in the food chain. The presence of ESBL and AmpC producing strains in food products is a cause of concern, because these phenotypes are usually accompanied by a low susceptibility to other classes of antibiotics. These results are of major importance not only for the health of Romanian consumers, but also for those in other countries that import food products from Romania.

Keywords: foodborne bacteria, resistance, virulence.

WED-449

Yeast as a tool to unravel the mode of action of bacterial effectors from *Bartonella* spp. and *Brucella* spp.

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Many intracellular bacteria ensure their survival and proliferation within host cells by secreting effector proteins that modulate various cellular functions. Among these, we focus on two closely related alpha-proteobacterial pathogens: *Bartonella* spp. and *Brucella* spp. Both pathogens cause chronic infections in mammals including humans using distinct strategies. Several virulence factors allow them to adhere, invade, proliferate and persist within various host-cell types. Among these virulence factors, type IV secretion systems (T4SS) are essential to these bacteria by injecting effector proteins inside host cells. Once into the cells, these proteins modulate cellular processes to the benefit of the bacteria. Thus, defining the role of these Type IV effector proteins is a key to understand pathogenesis. The use of *S. cerevisiae* as a surrogate host is emerging as an efficient strategy to study bacterial effectors. As a first approach, we used the yeast model to establish a genetic screen and look for mutants hypersensitive or suppressor to the expression of effector proteins from *Bartonella rochalimae* and *Brucella abortus*. This genetic screen led to the identification of mutants affected in transport from endosome to vacuole, for several bacterial effectors tested and mutants affected in actin organization and regulation, for one effector tested from *B. rochalimae*. Now, we would like to validate the results of the genetic screen by expressing these bacterial effectors in human cells.

Keywords: Type IV effectors, pathogenesis, yeast.

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CSV-05 – RNA Transport, Trafficking and Processing

WED-451
Biosynthesis of wyosine derivatives in tRNAPhe of Archaea: role of a remarkable bifunctional tRNAPhe:m1G/imG2 methyltransferase

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The presence of tricyclic wyosine derivatives 3′-adjacent to anticodon is a hallmark of $tRNA^{Phe}$ in eukaryotes and archaea. In yeast *Saccharomyces cerevisiae*, formation of wybutosine (yW) results from five enzymes acting in a strict sequential order. In archaea, the intermediate compound imG-14 (4-demethylwyosine) is a target of three different enzymes, leading to the formation of distinct wyosine derivatives (yW-86, imG, and imG2). Based on our previous experimental data (de Crecy-Lagard et al., Mol.Biol.Evol., 2010) and present comparative genomics analysis, we predicted the existence of a peculiar methyltransferase displaying a dual-specificity (aTrm5a) in several archaeal species. Combining a TLC and HPLC/MS analysis, we confirmed that Trm5a enzyme of *Pyrococcus abyssi* catalyzes two distinct reactions: N1-methylation of guanosine and C7-methylation of imG-14, whose function is to allow the production of isowyosine (imG2), an intermediate of the 7-methylwyosine (mimG) biosynthetic pathway. Based on the formation of mesomeric forms of imG-14, a rationale for such dual enzymatic activities is proposed. This bifunctional tRNA:m1G/imG2 methyltransferase, acting on two chemically distinct guanosine derivatives located at the same position of $tRNA^{Phe}$, is unique to certain archaea and has no homologues in eukaryotes. This enzyme here referred to as Taw22, probably played an important role in the emergence of the multistep biosynthetic pathway of wyosine derivatives in archaea and eukaryotes.

Keywords: Archaea, tRNA, wyosine.

WED-452
Comprehensive analysis of single-nucleotide polymorphisms in major polyadenylation signal (AATAAA) in human genome

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Polyadenylation is an important step of mRNA processing and crucial for mRNA stability and expression. More than a half of human genes have more than one polyadenylation site, resulting in great transcript diversity. These transcript isoforms, generated by a process called "alternative polyadenylation", differ in their stability, translational activity, and even in coding region structure. It is known that cell differentiation and malignant transformation affect the pattern of mRNA polyadenylation. The major polyadenylation signal comprises the AATAAA-hexamer that is recognized by CPSF (Cleavage and polyadenylation specifity factor) multisubunit complex. In this work, we for the first time investigate single nucleotide polymorphisms (SNPs) in the AATAAA-polyadenylation signals (and its variants) in human genome using the data from The 1000 Genomes Project. In about 54000 investigated hexamers we observed about 2400 SNPs. SNPs are depleted in two common hexamer variants (AATAAA and ATTAAA), and enriched in rare signal variants (such as AATATA, AATACA and others). Polyadenylation signals contained SNPs are less likely to be conserved in mouse. Furthemore, SNPs are depleted in signals near "unique" polyadenylation site (one site per gene). Interestingly, we observed that alternative polyadenylation sites (proximal and distal) do not statistically differ in amount of SNPs as well as in its allelic frequencies. Polyadenylation signals carrying SNPs are enriched in mRNA coding regions and depleted in 3′-untranslated regions (3′UTR), suggesting the important role of these signals in 3′UTR alternative polyadenylation and weak participation of AATAAA-hexamers in coding-region alternative polyadenylation. Despite these tendencies, some of the observed SNPs in polyadenylation signals may affect the function of corresponding genes. We found a limited number of investigated SNPs among the pathologic alleles described in OMIM and ClinVar databases. In sum, our work established the trends of SNPs' distribution in polyadenylation signals according to its functional features.

Keywords: mRNA processing, Polyadenylation, single nucleotide polymorphisms (SNP).

WED-453
Deciphering the nuclear export of Intron-containing mRNA: the model of the murine Leukemia virus

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Eukaryotic cells have evolved stringent proofreading mechanisms to ensure that intron-containing mRNAs do not leave the nucleus. However, all retroviruses must bypass this checkpoint for replication. Indeed, their primary polycistronic transcript

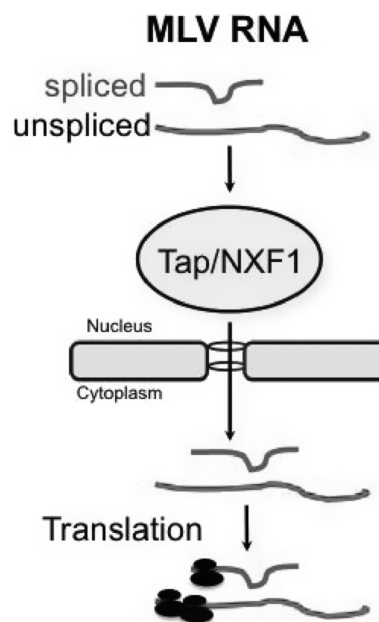

Fig. 1.

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(Full-Length) must reach the cytoplasm to be either translated or packaged as genomic RNA in progeny viruses.

Murine leukemia virus (MLV) is a prototype of simple retroviruses with only two well-regulated splicing events that directly influence viral leukemogenic properties in mice. Several *cis*-elements have been identified in the FL RNA that regulate its cytoplasmic accumulation. However, their connection with an export mechanism is yet unknown. Our goal was to identify the cellular pathway used by MLV to export its intron-containing RNA into the cytoplasm of the host cells.

Since other retroviruses use the CRM1 and/or the Tap/NXF1 pathways to export their unspliced RNA from the nucleus, we investigated the role of these two pathways in MLV replication by using specific inhibitors. The effects of export inhibition on MLV protein synthesis, RNA levels and RNA localization were studied by Western blotting, RT-qPCR, fluorescence microscopy and ribonucleoprotein immunoprecipitation assays. Taken together, our results show for the first time that MLV requires the Tap/NXF1-mediated export pathway, and not the CRM1 pathway, for the expression of its spliced and unspliced RNAs and for FL RNA nuclear export.

By contrast to HIV-1, MLV recruits the same pathway for the cytoplasmic expression of its spliced and unspliced RNAs. Thus, MLV RNA expression depends upon coordinated splicing/export processes. In addition, FL RNA translation relies on Tap/NXF1-dependent export, raising the critical question of whether the pool of FL RNA to be packaged is also exported by Tap/NXF1.

Keywords: Intron-containing RNA, Nuclear export, Tap/Nxf1.

WED-454
Deficiency in Gle1, an mRNA export mediator, inhibits Schwann cell development in the zebrafish embryo

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Background: *GLE1* mutations are associated with Lethal Congenital Contracture Syndrome Type 1 (LCCS1), a severe autosomal recessive foetal motor neuron disease. The gene encodes a highly conserved protein with an essential role in mRNA export. The mechanism linking *GLE1* function to motor neuron degeneration in the human syndrome has not been elucidated, but increasing evidence implicates abnormal RNA processing as a key event in the pathogenesis of several motor neuron diseases. Homozygous *gle1*$^{-/-}$ mutant zebrafish model various aspects of LCCS, displaying multiple developmental abnormalities and embryonic lethality. However, these embryos only show moderate defects in motor neuron specification. A previous microarray study of human LCCS foetuses suggested oligodendrocyte abnormalities may be a feature of LCCS. We have therefore examined the development of myelinating glia in *gle1*$^{-/-}$ mutant zebrafish.

Methods: To analyse stages in the specification of myelinating glia (both oligodendrocytes and Schwann cells), we performed whole mount *in situ* hybridization for *olig2*, *sox10*, *krox20* and *mbp*. Transmission electron microscopy (TEM) was used to examine the differentiation of Schwann cells in the posterior lateral line nerve (PLLn).

Results: We found that *gle1*$^{-/-}$ mutants had a dramatic reduction in *mbp* expression along the PLLn, whereas the expression of *mbp* in hindbrain oligodendrocytes was relatively normal, indicating a specific defect in Schwann cell development. Analysis of *krox20* and *sox10* expression showed a moderate reduction in their expression along the PLLn in *gle1*$^{-/-}$ mutants, suggesting reduced numbers of Schwann cell precursors. TEM revealed a near total absence of wraps of myelinating membranes around PLLn axons in mutant embryos compared with sibling controls. These results suggest a failure in the differentiation of Schwann cell precursors in *gle1*$^{-/-}$ mutants.

Conclusions: These studies suggest an essential role for *gle1* in Schwann cell development. Interestingly, LCCS can also be caused by *ERBB3* mutations, which is essential for Schwann cell development in mice and zebrafish. Schwann cell deficits may therefore be a key factor undermining motor neuron survival in multiple forms of LCCS. Our findings highlight the potential role of myelinating glial cells in the growing number of motor neuron diseases linked to RNA processing defects.

Keywords: Animal Model, mRNA export.

WED-455
Differential targeting of VDAC3 mRNA isoforms influences mitochondria morphology

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Intracellular targeting of mRNAs has recently emerged as a prevalent mechanism to control protein localization. For mitochondria, a co-translational mode of protein import is now proposed in parallel to the conventional post-translational model, and mitochondrial targeting of mRNAs has been demonstrated in various organisms. Voltage-dependent anion channels (VDACs) are the most abundant proteins in the outer mitochondrial membrane (OM) and the major transport pathway for numerous metabolites. Four nucleus-encoded VDACs have been identified in *A. thaliana*. Alternative cleavage and polyadenylation generate two VDAC3 mRNA isoforms differing by their 3′ UTR. By RT-qPCR and *in vivo* mRNA visualization approaches, the two mRNA variants were shown differentially associated with mitochondria. The longest mRNA presents a 3′ extension named “alternative UTR” (aUTR) that is necessary and sufficient to target VDAC3 mRNA to the mitochondrial surface. Moreover aUTR is sufficient for the mitochondrial targeting of a reporter transcript, and can be used as a tool to target an unrelated mRNA to the mitochondrial surface. Finally VDAC3-aUTR mRNA variant impacts mitochondria morphology and size, demonstrating the role of mRNA targeting in mitochondria biogenesis.

Keywords: 3′ UTR, mitochondria, mRNA localization.

WED-456
Exploring synthetic biology in Leishmania through auto regulatory RNA elements: a mechanistic perspective

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Leishmaniasis is a major neglected tropical world disease. Various forms of Leishmaniasis affects nearly 12 Million people worldwide. Genome Sequence has revealed that the genomes of eukaryotes are nearly totally transcribed and generating a vast number of non-coding RNAs having important regulatory functions. Eukaryotic parasite *Leishmania* induces non coding RNA molecules in macrophages upon infection. These Non-coding RNA require a specific secondary and tertiary structure to accomplish the specific function and predicted structure of these RNA elements understudy can be compared with functionally

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annotated structural signatures. In this current study we have identified putative regulatory elements from 3′ Untranslated regions (UTR) of several *Leishmania major* genes which can fold to form a Ribozyme like structure. *L. major* transcriptome wide analysis of these 3′UTR was performed. It was predicted based on the observations of these regulatory elements when subjected to secondary and tertiary structure prediction. The tertiary structure validation was done using Interactive Network Fidelity (INF).stability and thermodynamic properties of these predicted ribozymes were calculated using Molecular Dynamic simulations. These MD Simulations helped us to understand the catalytic functions of these modeled synthetic ribozyme. MDS revealed that these ribozymes have a specific stem-loop movements which incorporates a well-defined catalytic site and is highly required for their catalytic activity upon binding to its target RNA sequences.Thus they are essential for possible post transcriptional gene regulation. *In silico* study revealed that almost 70 genes of *L. major* contains these 3′ UTR sequences which may have a possible role in its target gene regulation. Further 2D and 3D structure predictions revealed that 3 critical genes play a vital role in the parasites life cycle viz., DNA dependent RNA polymerase III subunit(LMJF_09_1060),ATP dependent DNA helicase(LMJF_09_0590),pre mRNA Tran splicing factor (LMJF_33_0130).These genes 3′ UTR sequences with higher propensity to form a possible ribozyme structures upon folding. Further these putative sequences can be synthesized *in vitro* using Invitro transcription and validated for their catalytic activity against specific target RNA.Their post transcriptional regulation mechanism can be deciphered using various biophysical and biochemical techniques. Further structure determination can reveal the possible mechanism behind their catalytic activity.These 3' UTRs can even be used along with small molecule metabolites to act as Riboswitches that can be used to knock down the specific gene in post transcriptional manner. Ribozymes and Riboswitches can play critical role in regulating the virulence of leishmanial parasite and may serve as therapeutic agents.

Keywords: Auto Regulation, Leishmaniasis, RNA Modeling.

WED-457

High spatio-temporal resolution study of nuclear mRNPs, from biogenesis to export

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Transcripts generated by RNA polymerase II (RNAPII) undergo a precisely orchestrated cascade of processing steps, before being transported to the cytoplasm as export-competent mRNA ribonucleoprotein particles (mRNPs). Although we likely know most of the actors required for the formation of export-competent mRNPs, and some of their roles in separate scenes, we still lack an integrated storyboard of the precise chronology of mRNA biogenesis and its spatiotemporal organization. So far, the results obtained by standard technics used to study mRNA localization, either in fixed conditions (with RNA-FISH or single-FISH) or in live conditions (MS2), have described localization at time and spatial scales which are not compatible with the study of the dynamics of mRNPs export. To understand the coordinated roles of the different machineries involved at the different steps of mRNP formation as well as to decipher their spatiotemporal occurrence, advanced imaging has now become essential. We propose an alternative approach to detect mRNAs in yeast living cells, to analyze the spatiotemporal coordinated cascade leading mRNPs from their site of transcription to their site of nuclear exit. For this purpose, we adapted a recently described system (Paige et al., 2011) where a sequence corresponding to a short RNA aptamer (Spinach) that emits green fluorescence upon binding with a chemical compound (DFHBI) is used to tag the transcript of interest in the yeast *S. cerevisiae*. We focused on both inducible gene transcripts (GAL1, STL1), and genes whose mRNAs display a polarized localization during cell cycle progression (ASH1). Using advanced microscopy and bioimage analysis approaches, we were able to follow the spatiotemporal localization of those mRNAs. As previously described, we found that ASH1 mRNA localization was affected upon deletion of the nuclear pore complex protein Nup60 but also in mutants for post-translational modifications of this Nup. We are also able to follow the expression of STL1 mRNA in timescale consistent with previous data. Interestingly, a specific dot signal corresponding to STL1 transcript was detected within minutes upon osmotic stress, in close relation with the nuclear periphery. To our knowledge, none of the current super-resolution methods in light microscopy has yet been applied to record multiple step transcriptional dynamics and NPC functional imaging in living yeast cell.

Keywords: Live cell imaging, mRNA dynamics.

WED-458

Intrinsic disorder mediates secretory protein sorting and targeting

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Most secretory proteins are synthesized as preforms composed of signal peptides and mature domains. They cross the bacterial inner membrane mainly post-translationally, through the “translocase” that comprises the ATPase motor SecA and the membrane channel SecYEG. Sorting of secretory from cytoplasmic proteins and membrane-targeting of the former is believed to be mediated by signal peptides and facilitated by chaperones, like SecB, that prevent their aggregation and bind to the translocase. Mature domains also possess elusive targeting signals. Signal Peptides and mature domains bind on distinct SecA sites. We now show that most secretory mature domains are *i*ntrinsically *d*isordered *p*roteins (IDPs). They are stabilized in soluble, translocation-competent, non-native states in the absence of any chaperone or signal peptide. Multiple minor evolutionary adaptations establish ID and distinguish secretory mature domain sequences from those of cytoplasmic proteins. A minority of non-native secretory proteins are aggregation-prone due to their mature domains or signal peptides, can associate with chaperones to remain soluble. The ID state also allows targeting signals to remain exposed and guide preproteins to the translocase SecA receptor even in the absence of signal peptides or chaperones. These are short, continuous or discontinuous hydrophobic patches, that can be multiple and interchangeable and are buried in the hydrophobic core after translocation and upon periplasmic folding. This novel mechanism inevitably sorts secretory from fast-folding cytoplasmic proteins and provides molecular understanding of mature domain targeting. Its ramifications are wide, particularly given the increasing evidence of post-translational secretion in eukaryotes.

Keywords: preproteins, targeting, translocase.

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WED-459
Mechanisms of mobile ncRNAs in neural stem/precursor cells

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Neural stem/precursor cell (NPC) transplantation protects the central nervous system from inflammatory damage. While it was first assumed that stem cells directly replace lost/damaged cells, it is now becoming evident that they are able to protect the damaged nervous system also through cell-to-cell communication mechanisms. Importantly, recent reports indicate that the secretion of exosomes is a key player in facilitating horizontal cell signalling. Exosomes secreted from donor cells can capture bioactive molecules, such as ncRNAs, able to affect the biology of recipient cells in various ways from inducing/repressing the immune response, to promoting tissue repair and cancer progression. Employing Next Generation Sequencing on NPCs and NPC-derived exosomes, we have identified a set of miRNAs whose abundance is significantly increased in exosomes, compared to parental cells. This finding suggests the existence of dedicated and selective (but still poorly characterized) cellular trafficking mechanism that selectively exports miRNAs towards exosomes. This observation led us to hypothesise that – similarly to what has been found in other contexts – specific carrier proteins could recognize a 'secretion motif' within the miRNA sequence, bind to it and mediate its export towards exosomes. Therefore, we attempted to identify components of such machinery by analysing the sequence of secreted miRNAs in search for an enriched short motif that could serve as a binding site for a carrier protein. Using a variety of motif enrichment tools available in R/Bioconductor we identified two candidate motifs that are significantly over-represented in secreted miRNAs compared to miRNAs retained in the cell. One of the two motifs matches the binding sequence of the heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNPA2B1), which previous works have already shown to be involved in the trafficking of secreted miRNAs towards vesicles. However, by western blot we found that hnRNPA2B1 is not present within exosomes, suggesting that other proteins could be involved. We are currently investigating the possibility that other proteins and/or other miRNA features (such as the secondary structure of miRNA precursors) might be responsible miRNA secretion in NPCs. Altogether, this work will help to shed light on the molecular mechanism behind inter-cellular miRNA trafficking and on its implication on the therapeutic effect of transplanted NPCs.

Keywords: exosomes, miRNAs, Neural Stem Cells.

WED-460
Molecular and cellular mechanisms of the Cold-inducible RNA-binding protein (CIRP) nuclear-cytoplasmic shuttling

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Motifs rich in arginine and glycine residues were recognized several decades ago to play functional roles and were termed RGG motifs. More than 1000 proteins harbor the RGG motif, and these proteins play essential roles in a plethora of physiological processes such as transcription, pre-mRNA splicing, DNA damage signaling, mRNA translation, and the regulation of apoptosis (i). The physiological relevance of the RGG motif is highlighted by its association with several diseases including neurological and neuromuscular diseases and cancer (ii). The Madl group has recently discovered for the protein FUS (fused in sarcoma) that this RGG motif is involved in the regulation of protein nuclear import and that arginine methylation of these motifs plays a key role in the regulation (iii). Misregulation of this novel nuclear import mechanism leads to amyotrophic lateral sclerosis, a severe neurodegenerative disease. Nevertheless, the exact function of RGG motifs in nuclear import and disease is still poorly understood because there is no molecular data allowing to visualize at an atomic level its binding properties with the nuclear import/export machinery. To study these mechanisms the Cold-inducible RNA-binding protein (CIRP) seems to be an excellently suited candidate. Indeed, it's already known that the RGG motifs of CIRP are involved in its cellular localization and contrary to most of all the RGG-containing proteins CIRP possesses only one RGG domain which facilitates this study. By that, CIRP is the best suited model system to study the mechanisms of RGG-mediated nuclear import, its regulation by arginine methylation and link to human disease. For the first time, I show using ITC and NMR that Transportin-1 directly interacts with the RGG region of CIRP with high nanomolar affinity. Thus, the CIRP RGG is sufficient for CIRP nuclear import. I will present preliminary data allowing a better understanding of the molecular mechanism of RGG recognition by Transportin-1 but also its regulation by arginine methylation.

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Keywords: neurodegenerative diseases, nuclear import, RGG motifs.

WED-461
Nuclear mRNA export factor 1 (NXF1) in *Drosophila*: unconventional functions

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In eukaryotes mRNAs produced in the nucleus are transported to the cytoplasm by proteins of the nuclear mRNA export factors (*nxf*) family. Dm NXF1 (also known as SBR) mediates the export of mRNAs in *Drosophila*. Previously four *Dm nxf1* transcripts and a protein isoform were identified. Some of them was specific for testes or brain. The determined structures of additional (not ubiquitous) transcripts fail to ensure mRNA export function of the protein coded (Ivankova *et al.*, 2010 // Gene). These data together with various phenotypes of mutant flies let us suggest the other functions of *Dm nxf1* in the cell not reduced to mRNA nuclear export. We analyzed the *Dm nxf1* transcription in testes and heads of the adult flies by qPCR to reveal the transcripts repertoire in these tissues. Both organs contained the transcripts with the retained intron 5-6 and transcripts which included the central region of the same intron. The similar *nxf1* transcripts with corresponding intron retention also exist in *Homo sapiens* and *Mus musculus* and thus this is an evolutional feature (Mamon *et al.*, 2013 // Open Journal of Genetics). Therefore, we propose a regulatory role of these intron-containing transcripts for mRNA metabolism. We further analyzed *Dm nxf1*

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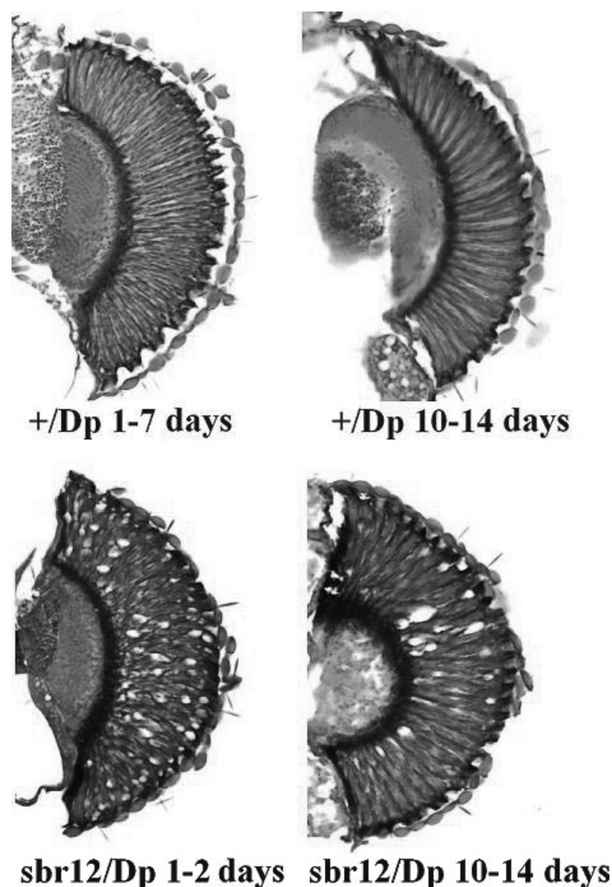

Fig. 1. Retina degeneration in *sbr*^{12}*/Dp* mutant of Drosophila comparing to control (+/Dp) is connected with Dm nxf1 (sbr) function in neurogenesis.

sterile mutant heterozygous males *sbr*^{12}*/Dp* and revealed the vacuoles in the retinal sections which are the common feature for *Drosophila* neurodegeneration. Together with morphologic abnormalities in the brain of *sbr*^{12}*/Dp* males and their sexual behavior disturbances our data suggest *Dm nxf1* involvement in neurogenesis of *Drosophila*. We hypothesize that nervous and reproductive anomalies in *sbr*^{12}*/Dp* mutants are connected through *Dm nxf1* function in mRNA transport and post-transcriptional regulation.
Keywords: mRNA nuclear export, intron retention, alternative transcription.

WED-462
Post-translational modifications of Nuclear Pore Complex protein Nup60: mechanisms and functional consequences

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The nuclear pore complex (NPC) is a multiproteic channel that permits the bidirectional and regulated transport of macromolecules between the nucleoplasm and cytoplasm. With a size of ~50 MDa and composed of ~30 different nucleoporins (Nups), the NPC is one of the largest assemblies in the cell and presents a structural organization highly conserved from yeast to humans. Although extensive research has revealed the molecular architecture and roles of the NPC subcomplexes, little is known about the regulation of NPC functions by post-translational modifications (PTMs). We hypothesized that PTMs such as ubiquitylation and Sumoylation, could participate in the regulation of NPC plasticity and functions. Here we focused on the yeast nucleoporin Nup60, a component of the NPC's nuclear basket subcomplex localized at the nuclear side of the NPC. Our analysis revealed that Nup60 is both mono-ubiquitylated and Sumoylated. Mono-ubiquitylation of Nup60 occurs all along the cell cycle whereas its sumoylation is restricted to G1 and S phases. To carry out functional analyses, we determined the enzymes responsible for these PTM as well as the specific lysine residues that are targets for Ub and SUMO modification. Corresponding mutations (Lys to Arg) were introduced genomically in order to generate Ubiquitin or SUMO-deficient Nup60 proteins. Preventing these PTM did not alter the localization of Nup60 at the nuclear pore. As Nup60 is important for the anchoring of others nuclear basket components (and in particular the Mlps and the SUMO protease Ulp1), we are currently analyzing the role of these PTM in the integrity of the nuclear basket and its associated factors. Nup60 PTM mutants do not present any nuclear protein import and export defect. In contrast, transport of polarized mRNAs to the cytoplasm is significantly affected upon mutation of the modified lysine residues indicating that these Nup60 PTM might regulate specific steps of the export-competent mRNP formation.
Keywords: Nuclear pore complex, Post-translational modifications.

WED-463
Role of RNA-binding protein in MAPK signaling and cell fate regulation

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Mitogen-activated protein kinases (MAPKs), which are found in all eukaryotes, are signal transducing enzymes playing a central role in diverse biological processes, such as cell proliferation, sexual differentiation, and apoptosis. The MAPK signaling pathway plays a key role in the regulation of gene expression through the phosphorylation of transcription factors. We have utilized the fission yeast, *Schizosaccharomyces pombe* (*S. pombe*) as a model system to elucidate the role of MAPK signaling pathway in cell fate regulation.

Our recent studies based on the functional interaction between protein phosphatase 2B (calcineurin) and Pmk1 MAPK, the *S. pombe* homologue of ERK1/2, identified several RNA-binding proteins (RBPs) as regulators of MAPK signaling. These include *rnc1*$^{+}$ encoding a KH-type RNA-binding protein and *nrd1*$^{+}$ encoding an RRM-type RNA-binding protein. Rnc1 binds and stabilizes the Pmp1 mRNA that encodes a MAPK phosphatase for Pmk1 thus negatively regulates the Pmk1 signaling (1). Nrd1 binds and stabilizes the essential myosin II light chain Cdc4 mRNA in a cell-cycle dependent manner, thereby suppressing the cytokinesis defects of the *cdc4* mutant cells (2,3). Moreover, Rnc1 and Nrd1 also serve as targets of MAPKs because MAPK phosphorylate and regulate the ability of these RBPs to bind and stabilize target mRNAs thus controlling various cellular functions, including cell integrity, cytokinesis and differentiation (1,2,4).

We also found that these RBPs can localize to stress granules in response to various stimuli, including heat shock, oxidative stress and osmotic stress. Notably, deletion of these RBPs leads to defects in stress granule formation, whereas the overproduction promote stress granule assembly, indicating that these RBPs play an important role in stress granules assembly/formation (5).

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Moreover, Nrd1 translocates to the stress granule in a phosphorylation- and/or Cpc2/RACK-dependent manner. Combined with our previous findings, these results suggest that these phospho-regulated RBPs are bifunctional proteins that can affect both mRNA stability and stress granule formation.

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Keywords: Fission Yeast, MAPK, RNA Binding Protein (RBP).

WED-464
The binding of TIA-1 to RNA C-rich sequences is driven by its pH dependent C-terminal RRM domain

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T-cell intracellular antigen-1 (TIA-1) is a key DNA/RNA binding protein that regulates critical events in cell physiology by the regulation of pre-mRNA splicing and mRNA translation [1, 2]. It possesses three RNA recognition motifs (RRM) along with a glutamine-rich domain, with the central domains (RRM2 and RRM3) acting as RNA binding platforms. While RRM2 domain is primarily responsible for the RNA interaction, the RRM3 contribution to the RNA binding, as well as its targets sequences, are still unknown. Here we combine Nuclear Magnetic Resonance (NMR), Surface Plasmon Resonance (SPR) and pull-down assays to elucidate the sequence specificity of TIA-1 RRM3 [3]. We demonstrate that RRM3 significantly binds to those oligonucleotides enriched with cytosines. Notably, in combination with RRM2 or in the context of the full length protein, the RRM3 domain enhances the binding to C-rich sequences. In addition, the binding of RRM3 to RNA is modified by pH conditions, having a significant effect on the overall interaction of TIA-1 protein [4]. Our findings provide a new insight into the role of RRM3 in regulating TIA-1 binding to C-rich stretches, abundant at the 5' TOPs (5' Terminal Oligopyrimidine Tracts) of translationally-repressed mRNAs under stress situations [5].

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Keywords: RNA Binding Protein (RBP), RNA Recognition Motif (RRM), TIA-1 protein.

WED-465
The human SURF6 protein participates in pre-rRNA processing and HeLa cells proliferation

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SURF6 is a specific nucleolar protein that is evolutionarily conserved from human to yeast. The yeast homolog of SURF6, the Rrp14p, takes part in processing of rRNA, ribosome biogenesis and cell budding. However, roles of human SURF6, including its potential implication in processing of rRNAs and proliferation of cells remains still unstudied.

In the current study, we have shown for the first time that experimental knockdown of SURF6 in HeLa cells is not toxic for the cells, but accelerates their proliferation and increases the mitotic index and the number of cells in S period. Furthermore, SURF6 knockdown decreases the number of cells in G1/G0 phase and diminishes the number of apoptotic cells. Taken together, these results demonstrate that depletion of the SURF6 pool renders positive effects on the proliferative status of HeLa cells.

We also examined whether SURF6 knockdown would effect rRNA processing in HeLa cells using Northern blotting and RT-qPCR methods. Both approaches show that diminution of the SURF6 amount results in up-regulation of the synthesis of 47S-45S pre-rRNA and don't decrease the content of 18S, 5,8S and 28S rRNAs. We also observed accumulation of 45S, 41S pre-rRNA, whereas the amount of 26S, 32S, 18S-E, 17S, 12S rRNA intermediates declined. Based on these observations, we concluded that human SURF6, like yeast Rrp14p, is involved in rRNA processing, where the particular roles of SURF6 are participation in degradation of the pre-rRNA transcribed spacers and in regulation of the rRNA processing pathways. In addition to rRNA processing, human SURF6 plays a role in regulation of rDNA transcription, activation of which may stimulate proliferation of HeLa cells in the SURF6 knockdown conditions.

The study has been financed by a grant issued by the President of the Russian Federation (MK-6426.2013.4).

Keywords: nucleolus, pre-rRNA processing.

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CSV-06 – The Niches: Stem cells and Metastasis

WED-467

BC44 carcinoma-associated fibroblasts promote stemness of urothelial carcinoma cells in coculture

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According to the cancer stem cells hypothesis tumours like normal tissues are organized in a hierarchical manner. At the top of this intrinsic cellular hierarchy are cancer stem cells (CSC), that are largely responsible for continuous tumour growth and progression, as well as for the development of therapeutic resistance. Consequently, their eradication could lead to the development of novel, curative cancer therapies. We have recently established and characterized an experimental system consisting of a pair of carcinoma (BC44) and carcinoma-associated fibroblast (BC44Fibr) – cell lines derived from the same urothelial tumour [1].

Currently we aimed to understand how carcinoma-associated fibroblast influence stemness of cocultured urothelial carcinoma cells and their sensitivity to anticancer therapies.

Primarily we are focusing on tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). This death ligand belonging to the tumor necrosis factor (TNF) superfamily of cytokines, is known to have the ability to selectively activate apoptosis in cancer cells but not in normal tissues. Thus, TRAIL as well as components of its signalling pathways is considered as promising targets of antitumour therapy. Comparing TRAIL sensitivity of pure culture and coculture with carcinoma-associated fibroblasts indicate a dramatically increased resistence of othewise sensitive SW780 bladder cancer cells. Furthermore co-culture of BC44Fibr and several bladder carcinoma cell lines (BC44, SW780, HT1197) revealed that carcinoma cells strongly positive for putative CSC markers (CK-17, 67 KDa Laminin Receptor, CD44v6) were located in direct contact with co-cultured carcinoma-associated fibroblasts. Therefore we conclude that BC44 carcinoma fibroblasts highly likely promote stemness of urothelial carcinoma cells.

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Keywords: cancer stem cells, carcinoma-associated fibroblasts, coculture.

WED-468

Cells with expression of pluripotency marker SSEA-4 are present in adult human skin

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The epidermis is continuously renewed throughout life. Different populations of stem cells can be distinguished that maintain tissue homeostasis by providing a pool of new cells to replace those lost during skin turnover or after injury. Furthermore, several previous studies demonstrated that cells with multipotent character reside in the bulge region of hair follicles and contribute not only to interfollicular epidermis but also to hair follicle and sebaceous gland. Nowadays, keratin 15 and CD200 are considered as the most specific biomarkers for identifying human hair follicular stem cells.

The stage-specific embryonic antigen 4 (SSEA-4) is an epitope on glycosphingolipid widely used as a marker for pluripotent embryonic stem cells, which is down-regulated during their differentiation. Furthermore, it was shown that in human, SSEA-4 is expressed by neural cells and by non-neural cells such as erythrocytes.

In the present study, we show that SSEA-4 positive cells are present in adult human skin. While we were looking for a rare population of pluripotent stem cells in adults called very small embryonic stem cells (VSELs) characterized by small size and expression of specific markers (CD133, SSEA-4, Oct-4), we found distinct population of cells which were SSEA-4 positive, but they did not co-express stem/progenitor marker CD133. Using flow cytometry, we estimated that an average number of these cells constitutes 0.06% of analyzed small nuclear cells both in epidermis and dermis of adult humans. With confocal microscopy we searched for SSEA-4 expressing cells in the commonly known niche for epidermal stem cells - the bulge region of a hair follicle. In our experiments we detected single cells expressing SSEA-4 on their surface (about 20 μm in diameter) localized in the inner root sheath (IRS) of hair follicles. Next, we used immunohistochemical staining to analyze skin tissue sections. We confirmed localization of SSEA-4 positive cells in hair follicles, however, we could also observe these cells within the basal cell layer of the epidermis, with a marked tendency to be localized at the tips of rete ridges, a protective niches of interfollicular epidermal stem cells. Additionally, scattered single SSEA-4 immunoreactive cells were detected within the dermis, especially in the papillary layer.

The obtained data indicate that there is a rare population of cells deposited in human skin demonstrating one of characteristic features of embryonic stem cells. However, further characterization of these cells is needed to establish whether expression of SSEA-4 marker in adult human skin is associated with pluripotency, unipotent epidermal stem cells or neither of them.

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Keywords: skin, SSEA-4, stem cells.

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WED-469

Characterization of cancer stem cells derived from pancreatic ductal adenocarcinoma cell lines and generation of three-dimensional culture models

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Pancreatic ductal adenocarcinoma (PDAC) is a malignant neoplasm with a 5-years survival rate of 3%–5%. Recently, much attention has been addressed to cancer stem cells (CSCs), which possess enhanced tumour-initiation and self-renewal properties, capacity to differentiate into different cellular types, and chemo- and radio-resistance. Their phenotype is generally associated with epithelial-to-mesenchimal transition (EMT) in which epithelial cells lose their characteristics, acquiring stem-like features.

In this study, we cultured different PDAC cell lines, defined parental, in selective growth conditions and derived CSCs forming spheres. Panc1 CSCs were characterized for the expression of the stem and EMT markers E-Cadherin, CD44v6 and EpCAM. *In vivo* studies show that, at the ninth week after injection, 1×10^6 cells/mouse of Panc1 CSCs are significantly more tumorigenic than the correspondent parental cells at the same cell concentration.

Since PDAC is characterized by an intense stromal reaction with a microenvironment rich in extracellular matrix (ECM), the development of three-dimensional cultures appears to be relevant to simulate the *in vivo* conditions. Panc1 and Panc1 CSCs were cultured in matrices composed by collagen I, the major component of ECM, and Matrigel and the differences in morphology have been analysed. In Matrigel, Panc1 CSCs show a tube-like structure, resembling vasculogenic mimicry (VM), while the parental cell line maintained its typical spheroidal-like morphology observed also in 100% collagen I. The VM phenotype is a feature associated with aggressiveness, survival ability and metastatic potential. At the present, we are studying the changes in morphology of cells cultured in a mixture of collagen I and Matrigel.

The microscopy fluorescence analysis of the invasive potential of cells through the formation of invadopodia shows that, unlike the parental cells, Panc1 CSCs did not digest Matrigel, a marker of invasiveness. Since our hypothesis is that low invasive capacity is correlated with high cell migration, we will study the type and migratory ability of CSCs in comparison to the parental cells.

Future experiments will aim to analyse the differences in proteome between CSCs and the parental cells in order to identify specific CSC markers and, then, to selectively target them by using liposome formulations containing anti-proliferative drugs.

Keywords: 3D cell culture, cancer stem cells, migration.

WED-470

Controlling the communication between brain endothelium and tumor initiating cells as a new therapeutic approach against glioblastoma

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Glioblastoma multiforme (GBM), is the most common and most aggressive brain tumor in adults. Despite intensive treatment GBM always relapses as an incurable lesion. Recurrence is tightly coupled to increased resistance to radiation and chemotherapy, hallmark features of stem-like glioma cells (GSC). GSC are characterized by self-renewal ability, stem-cell marker expression and tumor initiation capability. Their fate, plasticity and survival are in part regulated by external cues from the immediate adjacent differentiated cell types within the vascular niche, among which are brain endothelial cells (EC). Thus, understanding how niche factors are involved in maintaining aggressive glioma cell phenotypes may help identify novel potential targets for enhancing the efficacy of cancer therapeutics.

We recently demonstrated that the endothelial secretome sustain GSC self-renewal and survival through the Akt/mTor pathway. To further explore the nature of such angiocrine signals, human brain endothelial cell-released factors were identified through an unbiased proteomic approach. Mass spectrometry analysis uncovered 22 endothelial-produced peptides and proteins (8 growth factors, 7 extracellular proteases and 7 extracellular matrix proteins), whose expression was further confirmed by RT-PCR. Interestingly, the use of recombinant peptides was sufficient to support self-renewal and survival, as evidenced by secondary neurosphere formation, Sox2/Nestin expression and trypan blue exclusion, as well as Akt/mTor activation in a panel of 16 patient-derived GSC. Conversely, blocking either the endothelial production of these factors or the activation or expression of their cognate GSC receptor hampered EC-mediated GSC maintenance and signaling. To evaluate the therapeutic potential of our findings *in vivo*, GSC stably expressing interference RNAs targeting angiocrine factors-associated receptors were injected subcutaneously in mice. After 4 weeks, only 30–55% of the shRNA expressing-GSC-derived tumors were palpable in sharp contrast with control tumors, detectable in 100% of the cases. Addition-

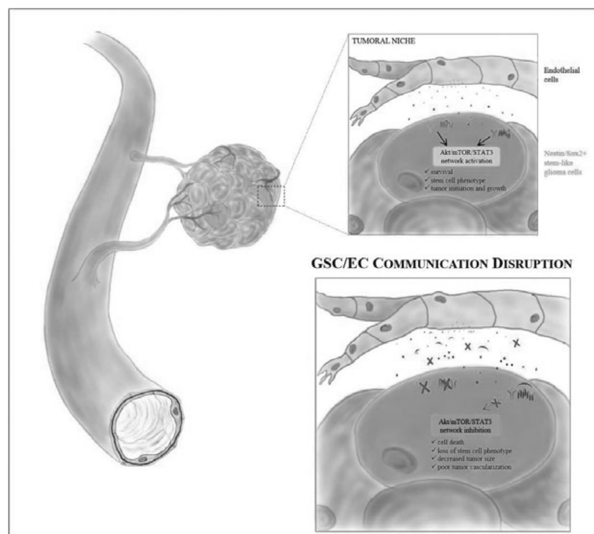

Fig. 1.

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ally, receptor-aimed pharmacologic treatment of mice bearing GSC tumors during 6–8 weeks resulted in decreased tumor size, poorer vascularization, lesser proliferation rate and reduced GSC fraction.

Altogether our results reveal that the endothelial-derived factors have an instrumental role in the maintenance of GSC population, controlling self-renewal, tumor-initiating capability and tumor growth. Thus, we propose interfering with the endothelium/GSC dialogue, critical for the integrity of the GSC fraction, as potential therapeutic target to obstruct glioblastoma progression.

Keywords: Cancer niche, Glioblastoma therapy, Glioma stem-like cells.

WED-471
Dissecting the role of histone deacetylases 1, 2 and 6 in Eμ-myc driven B cell lymphoma

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Histone deacetylases (HDACs) belong to a family of 18 enzymes that exert their function by deacetylating histones and non-histone proteins. HDACs are predominantly known to play a key role in the regulation of gene expression and abnormal regulation of HDACs is often associated with carcinogenesis and tumor progression. The two major class I; HDAC1 and HDAC2 as well as the cytoplasmic class II; HDAC6 were shown to play important roles in several cancer settings. A variety of broad-spectrum HDAC inhibitors have been developed to overcome the carcinogenic activity of HDACs. However, the function of single HDACs in normal physiology or pathological settings such as cancer remains elusive. In this study, using targeted or conditional deletion of individual HDACs, we have investigated the functional role of these enzymes in the Eμ-myc murine B-cell lymphoma model. Eμ-myc mice overexpress the c-myc oncogene in B-cells and develop aggressive leukemia with circulating leukemic cells in blood and extensive infiltration in lymphoid organs. In order to examine the effect on tumor formation we conditionally deleted HDAC1 and/or HDAC2 specifically in B-cells (using *mb1-cre*) and crossed them with Eμ-myc mice. We found that only dual, but not single loss of HDAC1 and HDAC2 prevents lymphoma development. Likewise, we crossed mice lacking HDAC6 (germ line KO and B-cell KO) as well as mice overexpressing HDAC6 (HDAC6 BAC) with Eμ-myc mice. Interestingly, Hdac6 germ line KO and possibly also B cell specific KO prolong tumor-free development. In contrast, HDAC6 overexpression accelerates lymphoma development. Our findings demonstrate that HDAC1, 2 and 6 play important roles in tumor development of Eu-myc mice.

Keywords: cancer epigenetics, Cell cycle, histone deacetylase.

WED-472
Gene signature of invading human melanoma cells

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Due to its extremely high metastatic capacity, cutaneous malignant melanoma represents the most fatal skin tumor in industrialized countries. Up to date the only possible therapy to cure melanoma patients is surgical excision of localized, non-metastatic primary tumors. Unfortunately, many patients already present micrometastatic disease at the time of diagnosis resulting in a poor 5-year survival probability. Hope for a future therapy might therefore lie in the early identification of metastasizing melanoma cells and the elucidation of the mechanisms governing their dissemination. Melanoma arises in the epidermis from transformation of cells of the melanocytic lineage. Melanocytes originate from neural crest cells (NCCs). During development NCCs delaminate from the neural tube by undergoing an epithelial-to-mesenchymal transition (EMT). Upon EMT NCCs adopt a remarkable migratory capacity, which allows them to disseminate throughout the embryo and to colonize distant sites where they differentiate into specialized cell types. Intriguingly this process is highly reminiscent of metastasis formation during which tumor cells disseminate from the primary neoplasm to establish secondary tumors in distant organs. This raises the question of whether the strong propensity of melanoma to metastasize reflects an intrinsic property of melanoma cells to disseminate by exploiting signaling cues normally active in migratory NCCs. To address this point we performed a microarray analysis of invading melanoma cells using a humanized *in vivo* model able to recapitulate the first steps of melanoma metastasis (Kiowski et al. 2012). With this approach we compared two populations, one positive for a known NCCs marker expressed during neural crest delamination, CD271, and the other negative for this marker. The analysis revealed that genes mostly involved in EMT were differentially modulated in the two populations. Currently we are investigating the mechanisms linking CD271 to melanoma invasion.

Keywords: melanoma, metastasis, Neural Crest Cells.

WED-473
Identifying compounds to selectively target glioblastoma stem like cells

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Glioblastoma multiforme (GBM) is the most aggressive cancer of the central nervous system, and is associated with extremely poor prognosis due to local recurrence. A subpopulation of cells within GBM has been identified that possess the characteristics of progenitor cells such as self-renewal and multipotency. Glioblastoma stem like cells (GSCs) are a relatively quiescent population of cells that have the potential to evade current treatments, as conventional therapies predominately target rapidly dividing cells. Consequently, it has been suggested that GSCs are responsible for therapeutic resistance and tumour recurrence in GBM. As such, identifying potential therapies that target this resistant population of cells is of great importance.

A chemical screen of 1280 FDA and EMEA approved drugs was performed on patient derived GSCs and to identify potential therapies for the use against GSCs. To assess the efficacy of these compounds cell viability and neurosphere formation capabilities were employed. The neural stem cell line HFT13 served as controls. The most effective candidate was selected for further characterization in a panel of human GSCs.

The initial screen of 1280 compounds resulted in 9 hits with >40% inhibition of GSC viability in two patient derived GSC lines. The hit with the highest inhibition of cell viability was chosen for further assessment on a larger panel of patient derived cells. This natural compound resulted in a significant ($p < 0.001$) reduction in GSC viability at 10uM, with this dose corresponding with impaired neurosphere formation in treated GSC compared to controls.

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These preliminary findings indicate that this natural compound has the ability to inhibit GSC growth as well as neurosphere formation, a predictor of rapid tumour progression. Further studies are required to fully elucidate the molecular mechanisms by which this compound is acting to reduce GSC growth. However, our study suggests this compound may represent a novel therapeutic approach to targeting GSC.

Keywords: Brain tumors, cancer stem cell, Cancer therapy.

WED-474

Interfering with stem cell-specific p53 gatekeeper functions controls the DNA damage response of hair follicle bulge stem cells and initiates sebaceous skin tumors

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Tumour growth is driven by tumour-initiating cells (TICs) carrying mutations that favor cell expansion by allowing the cells to escape normal growth control crucial for tissue homeostasis. Recently, multiple epidermal cell populations have been tested for their potential to initiate tumour growth in mouse models for different types of skin cancer including squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). These studies have identified the hair follicle (HF) bulge region as one cellular source for SCC and potentially BCC formation. However, a "cell-of-origin" for sebaceous types of skin cancer as well as mechanisms of initiation and progression of sebaceous tumours are not known.

Generating an inducible skin tumour mouse model, which mimics mutations and phenotype of human sebaceous tumours, we have shown that expression of a mutant form of the transcription factor Lef1 leads to sebaceous tumours. *In vivo* lineage-tracing in tumour-whole-mounts demonstrated that bulge stem cells (SCs) contribute to sebaceous tumours and are a "cell-of-origin".

Mechanistically, our data demonstrate that mutant Lef1 interferes with stem cell-specific surveillance mechanisms, including attenuated p53 activation as a consequence of faster DNA repair activity and increased expression of the pro-survival factor Bcl2, protecting SCs from DNA damage-induced apoptosis. Particularly, mutant Lef1 abolishes p53 activity by interfering with important regulators of the DNA damage-induced signaling cascade, like ATM and Chk2, resulting in increased DNA damage. Consequently, DNA damage induces apoptosis of HF bulge stem cells, since mutant Lef1 blocked the Bcl-2 response in HF bulge stem cells. To compensate loss of SCs, proliferation was stimulated within the bulge stem cell compartment, resulting in propagation of cells that escape normal cell cycle control, thereby supporting the accumulation of tumour-initiating mutations within HF bulge SCs.

Thus, normal control of SC proliferation is disrupted by mutant Lef1, representing a new mechanism of tumor-initiating events in tissue SCs and showing the importance of a tight control of these crucial stem cell-specific surveillance mechanisms to prevent tumourigenesis.

Keywords: None.

WED-475

Microvesicles secreted from the colorectal cancer cell line HT29 can transfer into cells constituting metastatic niche

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Malignant tumors are complex structures that consist of cancer cells and the surrounding tumor stroma. A continuous cross-talk between cancer cells and local/systemic environment is required for effective tumor growth and dissemination. Studies show that microvesicles derived from activated tumor cells (oncosomes) may participate in cell-to-cell communication and mediate the formation of metastatic niches. Moreover, epithelial to mesenchymal transition (EMT) is crucial in carcinoma metastasis. A key transcription factor involved in EMT is Snail that enhances proliferation, cell adhesion and migration of colorectal cancer cells (CRC).

Important question in above process is the role of CRC microvesicles that may transfer into metastasis niches. Further we would like to clarify the role of Snail in EMT of CRC. In this study we performed the analysis of microvesicles derived from CRC line HT29 and their incorporation into endothelial cells (HUVEC) and monocytes/macrophages cell line (THP-1). We expressed Snail in HT29 line by stable transfection using pcDNA 3.1 vector (HT29/Snail). In this report we show that both HT29 and HT29/Snail produce microvesicles, which can be transferred to the cells constituting metastatic niche (HUVEC and THP-1). We developed protocol and performed isolation of microvesicles from the supernatants of HT29/Snail. The incubation of HT29/Snail in experimental medium did not induce apoptosis and necrosis of the cells. The characterization of the microvesicles was performed by SDS-PAGE and Western blotting. In the purified microvesicles we detected CD63 and CD81, markers of microvesicles, whereas cytochrome c was not detected. Further the microvesicles were labeled using PKH67 Fluorescent Cell Linker kit to examine the uptake into HUVEC and THP-1. The cells with incorporated microvesicles were visualized under a confocal microscopy (Nikon D-Eclipse C1). Localization od microvesicles were confirmed by merging PKH67 fluorescence with Texas Red-labeled antibody against beta-actin and Hoechst dye.

Our study show that microvesicles released by the HT29 and HT29/Snail are internalized by HUVEC and THP-1. This observation is a starting point for our next investigation of the effect of the microvesicles and Snail on the response of cells constituting metastatic niche. It could be important for understanding the induction of immunosuppressive mechanism during cancer progression and inflammatory diseases and also may help to develop the future strategies to block metastasis.

This research was supported by Project DEC-2011/02/A/NZ3/00068 form National Science Centre.

Keywords: colorectal cancer, metastatic niche, microvesicles.

WED-476

Migrating breast cancer stem-like cells are slowed down by zoledronic acid

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Rationale: Various effects on tumor cells have been described for zoledronic acid (ZOL). However, only limited data exist

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regarding its influence on the motility of tumor cells. Since migration is a decisive step in metastasis, we examined whether ZOL reduces the motility of tumor cells, which may lead to less aggressive metastasis. The hypothesis of tumor stem cells postulates that recurrences may occur from cancer stem cells rather than from "normal," somatic tumor cells. We therefore investigated the effects of zoledronic acid on stem-like progenitor cells obtained from the human breast cancer cell line MDA-MB 231.

Methods: Breast cancer stem-like progenitor cells were obtained via the formation of spheroids from the human breast cancer cell line MDA-MB 231. Stem cell properties were confirmed immunohistochemically by high CD44 and low/no CD24 expression. The motility of cells in the presence of 0, 1, or 10 μM zoledronic acid was documented via time-laps videography over 24 h. The image-stacks were analyzed with the ibidi "chemotaxis and migration tool" as to the motility markers velocity, accumulated and Euclidean distance. The effect of a ZOL gradient on the direction of migration was determined in ibidi μ-slides.

Results: Time-resolved videography showed that zoledronic acid strongly reduced the migration of cancer stem cells. Cellular velocity was reduced by 61% following exposure to 1 μM zoledronic acid, and by 82% after exposure to 10 μM zoledronic acid. Accumulated distance traveled by the cells was reduced by 60% and by 79% after exposure to 1 μM and 10 μM zoledronic acid, respectively. The Euclidean distance was strongly reduced by 48% or by 73% in the presence of 1 μM or 10 μM ZOL respectively. The remaining cellular motility led to very little change in distance, with cellular activity appearing more as "stepping on the spot." A ZOL gradient dislocated the center of mass of migrating cells towards lower concentrations.

Conclusions: The suppression of cancer stem cell motility could potentially contribute to the clinical benefits following the adjuvant administration of zoledronic acid in breast cancer as recently described in clinical studies.

Keywords: breast cancer stem cells, motility, zoledronic acid.

WED-477
Pancreatic cancer stem cells characterization and secretome analysis

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Pancreatic ductal adenocarcinoma (PDAC) is generally asymptomatic until the late stage of the disease and it often metastasizes. Cellular dissemination leading to metastasis occurs prior to the formation of an identifiable primary tumour and this event strictly correlates with the presence of cancer stem cells (CSC). These observations render imperative the identification of the specific biological features of CSC, in order to improve PDAC diagnosis and prognosis. We obtained *in vitro* CSC from different pancreatic ductal adenocarcinoma (PDAC) cell lines, named parental cell lines (P), using a selective medium. After the formation of spheres, which represent the first evidence of the staminal traits, cells have been characterized for the expression profile of different markers, e.g. CD44v6, Ep-CAM, E-caderin via FACS or Western Blot (WB) analyses. Furthermore, since CSC have been shown to represent the most threatening and resistant portion of the tumour, we tested their aggressiveness in mice subcutaneously injected with Panc1 P or CSC, at different cell concentrations. At the higher cell concentration ($1*10^6$ cells/mouse), mice injected with CSC displayed a significant higher tumour volume, as well as the tumour cellular morphology appeared to be very different, by histochemical analysis, compared to the mice injected with P cells. We are now performing other analyses, in particular immunohistochemistry on the tumour tissues, WB and real-time PCR on the tumour cells, to evaluate differences in morphology and marker expression between the two cell types. Moreover, to test the capacity of CSC to metastasize, we injected Panc1 P or CSC in the spleen, which was then excised after 5 minutes, with the advantage to follow metastasis formation, without the presence of a primary tumour. The invasive capacity of CSC is still under evaluation, through the monitoring of metastasis formation by MRI.

Since it is known that cells secrete proteins for cell-cell communication and that the specificity of the secreted proteins can direct cells to distinct environments, we analysed the difference in protein secretion between Panc1 P and CSC by iTRAQ, an innovative protein quantification technique. The results showed that 71 proteins were secreted by CSC with an average fold change higher than 1.5 ($p < 0.001$) relative to P cells, and 9 proteins were secreted only by CSC. We are validating these results with other techniques, mainly by WB on the secreted proteins in the culture medium, and by ELISA, using patient serum.

The study of CSC and the analyses of secreted molecules is an approach with the strong potential to improve PDAC biology knowledge and to identify new potential early diagnostic markers.

Keywords: cancer stem cells, Pancreatic ductal adenocarcimoma, secretome.

WED-478
Regulation of actin dynamics by non-muscle tropomyosin and cofilin isoforms

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In many cells, directional migration and formation of membrane protrusions depend on regulation of actin filaments by actin-binding proteins: cofilin (Cfl) and tropomyosin (TM). Cfl severs and depolymerizes actin filaments, but it also can facilitate actin polymerization by increasing the number of polymerization-competent ends. Cfl expression level increases in motile cells such as metastatic tumor cells. In contrast, TM is believed to stabilize actin filaments. Increased expression of some non-muscle TM isoforms suppress invasiveness of tumor cells and inhibit mesenchymal migration of multiple cell lines. TM2 and TM5NM1 were previously reported as suppressors of tumor cells motility, whereas TM5a sorted to stable regions of neuronal cells. The goal of this work was to decipher the molecular mechanisms of actin dynamics regulation by cofilin and TM isoforms.

Non-muscle TM isoforms – TM2, TM5a (products of *TPM1* gene) and TM5NM1(a product of *TPM3* gene) were used along with non-muscle isoform of cofilin 1 (Cfl1). TMs and Cfl1 were expressed in *E.coli* BL21 strain and purified using standard methods. Actin was isolated from chicken skeletal muscle. Regulation of actin filaments dynamics by TMs and Cfl1 was analyzed with the use of co-sedimentation and turbidymetric assays.

Binding constant (K_{app}) of Cfl1 to F-actin was reduced about 2-fold by TM2 and TM5a, but was not affected by TM5NM1. Binding of Cfl1 removed TMs from the filament, though with different efficiency. At 1:1 Cfl: actin molar ratio 80% of TM2 and TM5a and only 40% of TM5NM1 were bound to actin. TM2 and TM5NM1 protected the filaments from severing/depolymerization by Cfl1. Surprisingly, TM5a facilitated this activity. The time-course of salt-induced G-actin polymerization was not affected by equimolar concentration of Cfl1. In contrast, all studied TM isoforms slowed down actin polymerization. Cfl1 reversed the inhibiting effect of TM2 and TM5a. However, when polymerization was

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conducted in the presence of TM5NM1 and Cfl1, the inhibition was even larger than in the presence of TM5NM1 alone.

A complex mechanism of the thin filaments regulation by Cfl and TM isoforms emerges from the obtained results. On one hand TM isoforms compete with Cfl for binding to actin, but on the other hand the proteins can act in synergy to facilitate filaments dynamics.

The project was supported by National Science Center grant No. 2012/07/N/NZ1/03094.
Keywords: actin filament, cofilin, tropomyosin.

WED-479
Site specific expression of the Wnt signaling target gene Nkd1 in mouse intestine and liver

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Wnt signalling pathway is crucial for development and adult tissue maintenance in all metazoans. In adult mammals, the Wnt signalling is required especially for intestinal homeostasis and establishment and preservation of the hepatic zonation. Conversely, aberrant activation of the Wnt pathway in these tissues is linked to development of cancer.

To investigate role of the Wnt pathway in the self-renewal of the intestinal epithelium and its malignant transformation, we employed chromatin immunoprecipitation (ChIP) in combination with DNA microarrays (ChIP-on-chip). As one of the most prominent Wnt signalling target genes, we identified the Naked Cuticle Homolog 1 (NKD1); previously described as a Wnt-induced negative regulator of canonical Wnt signalling. Using the BAC recombineering, we generated mice with CreER^{T2} recombinase produced from the Nkd1 locus (Nkd1-CreER^{T2}). Crossbreeding these mice with ROSA-Lacz reporter strain, we obtained animals with traceable Nkd1^{+} cells. In the small intestine, the expression of Nkd1 overlaps with expression of stem cell and Paneth cell markers, but not exclusively. Moreover, Nkd1-CreER^{T2} expression decreases in proximodistal manner with no expression in illeal stem cells. In the liver, Nkd1-CreER^{T2} marks the Wnt stimulated pericentral hepatocytes and so enables sorting of these otherwise indistinguishable hepatocytes.

In conclusion, gradient zonal expression of Nkd1 in the mouse intestine and liver gives insight into different regulation of Wnt signalling in these tissues.

Keywords: Intestine, liver, WNT signalling.

WED-480
Stem cell plasticity and tumorigenesis: regulatory roles of heparan sulphate in the cancer stem cell niche

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Dysregulated stem cell homeostasis is associated with disease and ageing. Sub-populations of cancer cells have been identified in tumours, leading to the cancer stem cell hypothesis, and the potential to target these cells for effective new anti-cancer therapies. Cancer stem cells, also called tumour-initiating cells, are similar to normal stem cells, being capable of self-renewal and differentiation, but the balance between these two processes is highly deregulated in cancer stem cells, leading to tumour formation. Stem cell fate is controlled by intrinsic (genetic information) and extrinsic (microenvironment cues) factors. Changes in either or both these classes of factors could contribute to the carcinogenic process. Heparan sulphate (HS) is a family of multifunctional regulators of different biological processes, including aspects important to cancer cell behaviour such as proliferation and differentiation. HS play important roles in cell growth and development, tissue homeostasis and cell migration, through interactions with a wide variety of proteins such as chemokines, enzymes and growth factors. These activities represent targets for therapeutic or diagnostic approaches. We hypothesise that a targeted modulation of the HS complement in the cancer stem cell niche may lead to reduced cell proliferation, increased apoptosis, and/or loss of malignant properties of the tumour-initiating cells. To study HS involvement in cancer stem cell homeostasis we established an *in vitro* model of cancer stem cells. We used flow cytometry to detect a subpopulation expressing reported markers of breast cancer stem cells (CD44^{+}/CD24$^{-/low}$) in several breast cancer cell lines. Interestingly, only the two most aggressive cell lines, MDA-MB-231 and Hs587-T, contained the CD44^{+}CD24$^{low/-}$ subpopulation described in the literature as a cancer stem cell-like subpopulation. Furthermore, we analysed heparin-treated MDA-MB-231 and Hs587-T cells by flow cytometry and observed that heparin treatment reduced the proportion of CD44^{+}/CD24$^{-/low}$ cells in culture, even at very low doses (1 ng/ml). These preliminary results suggest that HS may play an important role in regulating the homeostasis of breast cancer stem cells. Further investigation is needed to better understand the effects of heparin and other modified heparin compounds on breast cancer cells and their cancer stem cell sub-population and to identify potential therapeutic applications.

Keywords: cancer stem cells, Heparan sulfate, protoglycans.

WED-481
Thymosin β4 enhance adhesion to extracellular matrix proteins and reduced adhesion to endothelial progenitor cells

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After cancer patient's successful treatment the main effort is to inhibit the metastasis. The initial steps of cancer metastasis are: loss of cell polarity in the primary tissue, increased interaction with the extracellular matrix (ECM) through integrins and migration through the ECM towards blood and lymphatic vessels. The paramount significance for anti-metastatic therapy is one of initiating steps - the adhesion of cancer cells to the endothelium.

Thymosin β4 (Tβ4) is a small protein plays an essential role in regulation of actin polymerization. It is also involved in a number of cell functions: adhesion, proliferation, migration, differentiation, directional migration, angiogenesis, wound healing, hair follicle growth, and apoptosis. Tβ4 plays a variety of different roles depending on the cell type and whether it acts extracellularly or intracellularly. Tβ4 peptide, if used after a heart attack, might reactivate cardiac progenitor cells to repair damaged heart tissue but on the other hand Tβ4 is overexpressed in various types of tumors.

We tested the human colon adenocarcinoma cells line HT29 and CX1.1 that differ in integrin β1 level. In our study transient overexpression of Tβ4 in HT29 and CX1.1 cells is correlated with transendothelial cell migration. Tβ4 increased the adhesion of can-

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cer cells to the major ECM proteins (fibronectin, vitronectin, collagen and fibrin). Coincidently, the treatment of HT29 and CX1.1 cells with Tβ4 slightly decreased their adhesion to endothelial cells and endothelial progenitor cells. This effect was not observed for Tβ4-treated HT29 cells those adhered to endothelial cells. These observation may indicate that the potential role of Tβ4 in cancer cell adhesion process to endothelial cells is much more complex. In conclusion, our preliminary data suggest a role for Tβ4 in metastasis colon cancer. Further studies are needed in order to shed light on the intimate significance of the expression of this thymosin not only during adhesion and transendothelial migration, but also during the change of polarity in the primary tissue.

Keywords: None.

WED-482

Unraveling the mechanism of chronic myeloid leukemia progression by next-generation sequencing of leukemic progenitor and stem cells

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Chronic myeloid leukemia (CML) is the first human malignancy in which a genetic abnormality was linked to an oncogenic transformation and a successful targeted treatment was developed. The BCR-ABL1 fusion gene formed by a reciprocal translocation, encodes constitutively active tyrosine kinase effectively inhibited by tyrosine kinase inhibitors (TKI). However, the mechanism of CML progression is relatively poorly understood. TKI therapy induces durable remissions in majority of patients, but leukemic stem cells are never fully eradicated and significant number of patients experience relapse.

Research in the field of cancer genetics accelerated after the introduction of next-generation sequencing (NGS), but there have been only few systemic approaches for CML. Therefore, we have started a study to investigate mechanism of CML progression by sequencing paired diagnosis-progression samples using NGS in order to find new genetic abnormalities responsible for disease progression and/or TKI-resistance. Here we report an analysis of a patient who rapidly developed resistance to imatinib and progressed to acute phase.

Fig. 1.

DNA was isolated from CD34 + cells (containing leukemic progenitor and stem cells), immunomagnetically selected from peripheral blood collected at two time points (at diagnosis and 3 months later when patient progressed to accelerated phase). Exome sequences were acquired by solution-based capture and high-throughput sequencing (Illumina HiSeq1500). Mutational differences between samples were analyzed by Mutect. ABL1 exon 4 was resequenced by NGS and Sanger methods in both samples.

19 and 15 variants were detected exclusively in the diagnosis and progression sample, most of which were uncommon, suggesting an ongoing clonal evolution of the leukemic clone. The most significant mutation discovered exclusively in the progression sample was a G>C transversion in ABL1 kinase domain of BCR-ABL1, which results in p. Q252H substitution in the P-loop, causes resistance to imatinib and is a negative prognostic factor. We verified by additional deep sequencing that this mutation was rapidly acquired by CML progenitor/stem cells, as it was completely absent at the time of diagnosis.

Although results obtained in this particular case revealed previously described mutation as an underlying cause of TKI-resistance and disease progression, we were able to determine with high sensitivity if patient already harbored leukemic clone with mutation at the time of diagnosis and thus evaluate the pace of disease progression at the progenitor/stem cell stage.

Keywords: Chronic Myeloid Leukemia, next generation sequencing.

WED-483

Tropomyosin region encoded by alternative exon 2 confers different flexibility to smooth muscle and non-muscle isoforms

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Tropomyosins (TM) are a family of actin regulators, which determine functional diversity of actin filaments in muscle and non-muscle cells. TM molecules are elongated two-chain coiled coils, which polymerize along actin via end-to-end interactions. The crucial role of TM is the regulation of actin-myosin interactions. It is executed through steric blocking and allosteric mechanisms. It is believed that flexibility is a structural feature of tropomyosin important for TM functions. TM isoforms variety is generated by different genes and selection of alternative exons. Our previous studies show that isoform-specific sequence composition of the N- and C-termini determines functionally important orientation of TM chains on the filament (Śliwińska et al., 2011; Śliwińska and Moraczewska, 2013).

The aim of this work was to check whether alternative sequences of internal region encoded by exon 2 affect positions of TM isoforms on actin. Two TM isoforms: the smooth muscle isoform TMSm, and the non-muscle TM2 were analyzed. Both isoforms are products of α-TM gene and share the same sequence except for the internal region encoded either by exon 2a (TMSm) or exon 2b (TM2). Orientations of both regions were assessed by Förster Resonance Energy Transfer (FRET) between AEDANS attached to Cys^{51} and Cys^{73} of each TM isoform and DABMI bound to actin's Cys^{374}. FRET distances were measured in two activation states of actin filaments – closed state represented by TM-actin complex and open state induced by binding of myosin heads (S1) to TM-actin. In order to introduce reactive Cys residues into alternatively expressed region of TM isoforms site-directed mutagenesis was done.

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The obtained results indicated that the analyzed TM isoforms significantly differed in their positions on actin filament. Calculated donor-acceptor distances varied between 36.5 Å and 48.4 Å. Myosin S1 binding to actin filaments induced changes in AEDANS-DABMI distances. It increased FRET distances separating Cys^{51} and Cys^{73} in TM2 from actin by 9.5 and 5.7 Å, respectively. The distance measured from Cys^{73} of TMSm was also increased by 4.3 Å. In contrast, TMSm Cys^{51} was brought closer to actin's Cys^{374} by 2.5 Å. This shows a considerable flexibility of the region encoded by exon 2a. Together the results indicate that alternative TM sequence encoded by exon 2 strongly affects TM orientation on actin filament. Functional importance of the structural differences between TMSm and TM2 are currently under investigation.

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Keywords: flexibility, FRET, tropomyosin.

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