# Huntington's Disease Alters the Dermal Fibroblasts Secretome

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**Abstract**—Huntington's disease is a hereditary incurable neurodegenerative disease caused by expansion of the polyglutamine tract in exon 1 of the huntingtin gene. Huntingtin is a large protein involved in many cellular processes, such as division, transport and secretion. Mutations in the protein lead to disruption of many cellular processes, including secretion, but differences in the composition of the secretome of cells in normal conditions and in Huntington's disease remain poorly studied. Since huntingtin is expressed at high levels in peripheral tissues and in the skin, we have focused our attention on the study of secretome produced by dermal fibroblasts. In order to identify differences in secreted factors caused by the huntingtin mutation we used tandem time-of-flight mass spectrometry. Forty-two differential proteins were identified in the secretomes of dermal fibroblasts from healthy donor and patient with Huntington's disease. We examine several proteins of interest including filamin A, periostin, ACTN4, BASP1, adrenomedullin, HSP70 and 14-3-3, whose expression is associated with processes such as cytoskeletal organization, cell adhesion, proliferation, cell migration, protein binding and regulation of cytoskeletal structure. HSP70 and 14-3-3 have neuroprotective properties, and interestingly, their expression was not detected in the secretome of cells with Huntington's disease. Thus, it was shown that the set of proteins secreted into the extracellular space by dermal fibroblasts with the Huntington's disease genotype differs from healthy cells, and the differences in cellular processes (proliferation, migration) observed in these cells in vitro are probably due to differences in the composition of the extracellular matrix which they synthesize.

**Keywords:** Huntington disease, secretome, extracellular matrix, neurodegeneration, proliferation, neuroprotective

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## 1. INTRODUCTION

Huntington's disease (HD) is an incurable monogenic disorder caused by a mutated huntingtin gene . The disease is inherited in an autosomal dominant manner and exhibits complete penetrance with the average age of manifestation from 35 to 50 years. This disorder belongs to the group of triplets repeat expansions. In the case of Huntington's disease, the number of repeats in the coding part of the huntingtin gene (exon 1) increases [1, 2] . Despite the fact that the mutation responsible for the development of the disease was identified 30 years ago [3], the molecular cellular mechanisms leading to the development of HD have not yet been determined, and the exact functional role of huntingtin has not been established. Huntingtin (HTT) is a large cytoplasmic protein of 347 kDa involved in brain development and maturation of the neurons [4]. To date, a huge amount of data has been accumulated concerning the role of normal and mutant huntingtin in the regulation of such intracellular processes such as regulation of transcription, embryonic development, morphology maintenance, vesicular transport, etc. [5].

According to data in the available literature, huntingtin is involved in biochemical pathways associated with secretory and neurotransmitter activity. Recent reports revealed a full length HTT to be co-isolated with extracellular vesicles from both a porcine model and blood plasma from HD patients [5, 6]. Wild-type and mutant huntingtins were shown to be associated with membranes in the secretory and endocytic pathways [7].

Though there is some understanding of the involvement of huntingtin in a variety of intracellular processes,

a comprehensive approach is required to recognize its role in cell biology. Complex disorders such as Huntington's disease are studied using omics technologies [8, 9]: transcriptomics [10], proteomics [11, 12] and metabolomics [13–16], however the HD secretome remains unstudied.

Fibroblasts are connective tissue cells derived from mesenchymal stem cells. These cells have high proliferative and secretory activity. Fibroblasts have emerged as a valuable model for studying HD due to their accessibility and ability to reflect disease-related changes. Numerous studies have investigated various aspects of fibroblast biology in HD, providing insights into disease pathology and identifying potential biomarkers. Studies utilizing dermal fibroblasts in HD research have revealed alterations in cellular processes such as mitochondrial function, oxidative stress, protein clearance mechanisms, and calcium signaling.

Through the investigation of fibroblasts, valuable insights have been gained into cellular and molecular alterations associated with HD. These findings not only enhance our understanding of disease pathogenesis but also provide potential avenues for developing novel therapeutic strategies. It was discovered (unpublished data by Kraskovskaya et al.) that dermal fibroblasts from a patient with Huntington's disease proliferate more slowly than cells from healthy donors, and their ability to regenerate is also reduced. The study of the mechanisms underlying the regulation of these processes should start from a search for differences in the secretome of these cells.

Here, we describe the results of a proteomic analysis of two dermal fibroblast lines secretomes from patients with HD and healthy donors.

## 2. MATERIALS AND METHODS

### 2.1 Cell Lines

The cell line DF262 was obtained from the Collection of Cell Cultures for Biotechnological and Biomedical Research of the Institute Developmental Biology in the Russian Academy of Sciences (RAS), Moscow. HDDF cell lines with 47 CAG repeats were obtained from the Collection of Vertebrate Cell Cultures of the Institute of Cytology RAS, Saint-Petersburg. Cells were cultured in DMEM/F12 media supplemented with 10% FBS at 37°C in 5 % CO2 until confluence of 80–90 %. The cells from passages 4–7 were used in the experiments. Upon reaching confluency, the cells were subcultured with trypsin-versene solution in a ratio of 1 : 3.

### 2.2 Conditioned Medium Collection for Secretome Extraction

For proteome analysis, cell cultures were seeded at moderate density in flasks (175 cm$^2$). Upon reaching a monolayer density of 70–80%, the cells were sequentially washed to remove the serum and incubated in DMEM/F12 medium (Biolot, Russia) without adding serum for 48 h. Then the conditioned medium was collected in test tubes and sequentially purified from residues in the 750g 10′ at 4°C and 1500g 15′ at 4°C modes. Next, the supernatant was sequentially transferred into special tubes for ultracentrifugation and spin in modes of 20000g 60′ at 4°C and 120000g 90′ at 4°C. Thus, two fractions were obtained, soluble and vesicular phases. The soluble fraction (suspension) was diluted with acetone in a ratio of 1 volume sample to 4 volume acetone and incubated at –20°C for 24 h and used for analysis. Then the samples were centrifuged at 3000 g 50′ at 4°C. The precipitate, after drying, was diluted with MilliQ water. In this form, the sample was filtered and precipitated with a solution of 90% acetonitrile and 0.1% trifluoroacetic acid through a column.

### 2.3 Sample Preparation and LC-MALDI Mass Spectrometry

The protein samples were digested overnight by Trypsin Gold (Promega, USA). Prior to reversed-phase fractionation, digested samples were resuspended in 50 μL of 1% (v/v) formic acid in water and filtered through 0.2 μm PVDF filter. Peptides were separated with a Chromolith CapRod RP-18e HR reversed-phase column (0.1 mm × 150 mm, Merck, Darmstadt, Germany) on a nano LC system (Eksigent NanoLC Ultra 2D+ system, SCIEX, Germany). A total peptide amount of 600 ng was loaded and separated using a linear gradient of 0–50% B over 115 min followed by 50–100% B for 1 min and 100–100% B for 4 min at a flow rate of 400 nL/min. The mobile phases used were A, 5% acetonitrile with 0.2% (v/v) TFA in water and B, 60% (v/v) acetonitrile in water. The column was operated at a room temperature of 22–24°C. The effluent from the column was mixed with matrix solution (CHCA 5 mg/mL, 0.2% (v/v) TFA in 95% methanol) containing
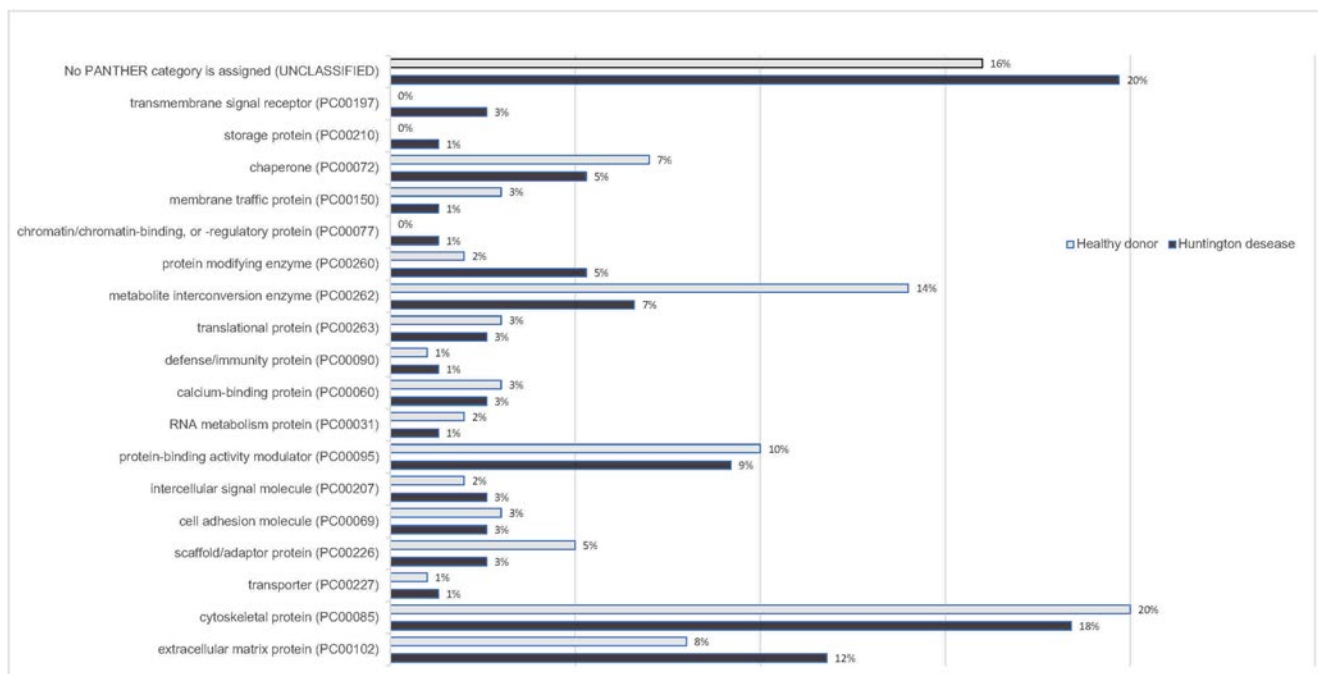
**Fig. 1.** Bar charts representing functional annotation of identified proteins by Gene Ontology Biological Process [17].

two calibration standards bradykinin 2–9 (30 pM/mL) and ACTH 18-39 (60 pM/mL), at a flow rate of 1.4 μL/min. A micro-fraction collector was used to deposit 1 mm spots every 5 s, and a total of 704 fractions were collected in a 44×16 array for each nano LC run. The column was washed with a gradient (0–100–100% B for 5 min and 2 min respectively, at a flow rate of 800 nL/min) and equilibrated to 0% B for 3.5 min before subsequent injections.

We analyzed the fractionated samples with a TOF/TOF 5800 System (SCIEX, Germany) instrument operated in the positive ion mode. We set the MALDI stage to continuous motion mode. MS data were acquired at 3200 laser intensity with 1000 laser shots/spectrum (200 laser shots/sub-spectrum) and MS/MS data were acquired at 3900 laser intensity with a DynamicExit algorithm and a high spectral quality threshold or a maximum of 1000 laser shots/spectrum (250 laser shots/sub-spectrum). Up to 25 top precursors with S/N >40 in the mass range 750–4000 Da were selected from each spot for MS/MS analysis.

### 2.4 Protein Identification

We used the Protein Pilot 5.0 software package (SCIEX, Germany) with the Paragon algorithm 5.0 in thorough mode, for the MS/MS spectra search against the Uniprot human database. Carbamidomethyl cysteine was set as a fixed modification. False discovery rate (FDR) analysis was done by analysis of reversed sequences using the embedded PSEP tool. The MS/MS data was converted to mzidentml format for further analysis using Scaffold 4.0 software. The mass spectrometry proteomics data have been deposited at the Mendeley Data (10.17632/b2k4gmwhmm.1).

Bioinformatics analysis was performed by functional annotation in Scaffold Viewer 5.3.2 (Proteome Software Inc, USA) and DAVID functional annotation tool (6.8) [17].

### 3. RESULTS

We identified 101 proteins in the secretome of cells obtained from a healthy donor and 81 proteins from the secretome of cells from an HD patient (Table 1), proteins which were presented at least in two of four biological replicates analyzed by LC-MALDI shotgun proteomics. The order of proteins in the table is determined by the number of identified peptides for each of them. Of these, 70 proteins were common to both cell types. Thus, 42 proteins specific to the secretome of cells of HD patient differ from healthy patient.

**Table 1.** Proteins, identified in secretomes of dermal fibroblasts of heathy and Huntington disease donors

| Identified proteins | Accession number | Molecular weight | Presence | |
|---|---|---|---|---|
| | | | healthy donor | huntington desease |
| Vimentin | P08670 | 54 kDa | + | + |
| Collagen alpha-2(I) chain | P08123 | 129 kDa | + | + |
| Fibronectin | P02751 | 263 kDa | + | + |
| Neuroblast differentiation-associated protein AHNAK | Q09666 | 629 kDa | + | + |
| Collagen alpha-1(I) chain | P02452 | 139 kDa | + | + |
| Filamin-A | P21333 | 281 kDa | + | |
| Prelamin-A/C | P02545 | 74 kDa | + | + |
| Periostin | Q15063 | 93 kDa | + | |
| Actin, cytoplasmic 2 | P63261 | 42 kDa | + | |
| Collagen alpha-3(VI) chain | P12111 | 344 kDa | + | + |
| Thrombospondin-1 | P07996 | 129 kDa | + | + |
| Tropomyosin alpha-4 chain | P67936 | 29 kDa | + | + |
| Galectin-1 | P09382 | 15 kDa | + | + |
| 72 kDa type IV collagenase | P08253 | 74 kDa | + | + |
| Endoplasmic reticulum chaperone BiP | P11021 | 72 kDa | + | + |
| Triosephosphate isomerase | P60174 | 31 kDa | + | + |
| Interstitial collagenase | P03956 | 54 kDa | + | + |
| Transgelin | Q01995 | 23 kDa | + | + |
| Alpha-enolase | P06733 | 47 kDa | + | + |
| Thymosin beta-4 | P62328 | 5 kDa | + | + |
| Myosin-9 | P35579 | 227 kDa | + | |
| Follistatin-related protein 1 | Q12841 | 35 kDa | + | + |
| Serpin H1 | P50454 | 46 kDa | + | |
| Alpha-actinin-4 | O43707 | 105 kDa | + | |
| Plectin | Q15149 | 532 kDa | + | + |
| Profilin-1 | P07737 | 15 kDa | + | + |
| Thymosin beta-10 | P63313 | 5 kDa | | + |
| Nucleobindin-1 | Q02818 | 54 kDa | + | + |
| SPARC | P09486 | 35 kDa | + | + |
| Moesin | P26038 | 68 kDa | + | + |
| Insulin-like growth factor-binding protein 7 | Q16270 | 29 kDa | + | |
| Caldesmon | Q05682 | 93 kDa | + | + |
| Collagen alpha-1(VI) chain | P12109 | 109 kDa | + | + |
| Metalloproteinase inhibitor 2 | P16035 | 24 kDa | + | + |
| Peptidyl-prolyl cis-trans isomerase A | P62937 | 18 kDa | + | + |
| Transforming growth factor-beta-induced protein ig-h3 | Q15582 | 75 kDa | + | + |
| Fructose-bisphosphate aldolase A | P04075 | 39 kDa | + | + |
| Plasminogen activator inhibitor 1 | P05121 | 45 kDa | + | |
| Heat shock cognate 71 kDa protein | P11142 | 71 kDa | + | |
| Pyruvate kinase PKM | P14618 | 58 kDa | + | |
| Protein disulfide-isomerase A3 | P30101 | 57 kDa | + | + |
| Stromelysin-1 | P08254 | 54 kDa | + | + |
| L-lactate dehydrogenase A chain | P00338 | 37 kDa | + | + |
| Glia-derived nexin | P07093 | 44 kDa | + | + |

**Table 1.** (Contd.)

| Identified proteins | Accession number | Molecular weight | Presence | |
|---|---|---|---|---|
| | | | healthy donor | huntington desease |
| Vinculin | P18206 | 124 kDa | + | + |
| Cofilin-1 | P23528 | 19 kDa | + | + |
| Calumenin | O43852 | 37 kDa | + | + |
| Transgelin-2 | P37802 | 22 kDa | + | + |
| Glutathione S-transferase P | P09211 | 23 kDa | + | |
| Sulfhydryl oxidase 1 | O00391 | 83 kDa | + | + |
| Protein disulfide-isomerase | P07237 | 57 kDa | + | + |
| Metalloproteinase inhibitor 1 | P01033 | 23 kDa | + | + |
| Peptidyl-prolyl cis-trans isomerase B | P23284 | 24 kDa | + | + |
| Cysteine and glycine-rich protein 1 | P21291 | 21 kDa | + | + |
| Filamin-C | Q14315 | 291 kDa | + | + |
| Talin-1 | Q9Y490 | 270 kDa | + | |
| LIM and SH3 domain protein 1 | Q14847 | 30 kDa | + | + |
| Myosin light polypeptide 6 | P60660 | 17 kDa | + | + |
| Peroxiredoxin-1 | Q06830 | 22 kDa | + | + |
| Ribonuclease inhibitor | P13489 | 50 kDa | + | |
| Elongation factor 1-alpha 1 | P68104 (+1) | 50 kDa | + | |
| Beta-2-microglobulin | P61769 | 14 kDa | + | + |
| Prosaposin | P07602 | 58 kDa | + | + |
| Peroxiredoxin-6 | P30041 | 25 kDa | + | |
| Heat shock 70 kDa protein 1A | P0DMV8 (+1) | 70 kDa | + | |
| Lumican | P51884 | 38 kDa | + | + |
| Protein-lysine 6-oxidase | P28300 | 47 kDa | + | + |
| Transitional endoplasmic reticulum ATPase | P55072 | 89 kDa | + | |
| 14-3-3 protein zeta/delta | P63104 | 28 kDa | + | |
| EGF-containing fibulin-like extracellular matrix protein 1 | Q12805 | 55 kDa | + | |
| PDZ and LIM domain protein 1 | O00151 | 36 kDa | | + |
| Myeloid-derived growth factor | Q969H8 | 19 kDa | + | + |
| Gelsolin | P06396 | 86 kDa | + | + |
| Filamin-B | O75369 | 278 kDa | + | |
| Elongation factor 2 | P13639 | 95 kDa | + | |
| Insulin-like growth factor-binding protein 2 | P18065 | 35 kDa | + | |
| Vasorin | Q6EMK4 | 72 kDa | + | + |
| Polyubiquitin-B | P0CG47 (+3) | 26 kDa | | + |
| Fibulin-1 | P23142 | 77 kDa | + | |
| Peptidyl-prolyl cis-trans isomerase FKBP10 | Q96AY3 | 64 kDa | + | |
| Dihydropyrimidinase-related protein 2 | Q16555 | 62 kDa | + | |
| Endoplasmic reticulum resident protein 29 | P30040 | 29 kDa | + | + |
| Galectin-3 | P17931 | 26 kDa | + | + |
| Thioredoxin domain-containing protein 5 | Q8NBS9 | 48 kDa | + | + |
| Programmed cell death protein 5 | O14737 | 14 kDa | + | + |
| Collagen alpha-2(V) chain | P05997 | 145 kDa | + | |
| Collagen alpha-2(IV) chain | P08572 | 168 kDa | + | |
| Keratin, type I cytoskeletal 9 | P35527 | 62 kDa | | + |
| Zyxin | Q15942 | 61 kDa | + | |
| 14-3-3 protein epsilon | P62258 | 29 kDa | + | |
| Calponin-3 | Q15417 | 36 kDa | + | |
| Alpha-2-HS-glycoprotein | P02765 | 39 kDa | + | |

**Table 1.** (Contd.)

| Identified proteins | Accession number | Molecular weight | Presence | |
|---|---|---|---|---|
| | | | healthy donor | huntington desease |
| A-kinase anchor protein 12 | Q02952 | 191 kDa | + | |
| SH3 domain-binding glutamic acid-rich-like protein 3 | Q9H299 | 10 kDa | + | |
| Dickkopf-related protein 3 | Q9UBP4 | 38 kDa | + | |
| Peptidyl-prolyl cis-trans isomerase FKBP2 | P26885 | 16 kDa | + | + |
| Coiled-coil domain-containing protein 80 | Q76M96 | 108 kDa | + | + |
| 14-3-3 protein gamma | P61981 | 28 kDa | + | + |
| Thioredoxin domain-containing protein 17 | Q9BRA2 | 14 kDa | + | + |
| Microtubule-associated protein 1B | P46821 | 271 kDa | + | + |
| Tetranectin | P05452 | 23 kDa | | + |
| Myosin regulatory light polypeptide 9 | P24844 | 20 kDa | + | |
| 40S ribosomal protein S12 | P25398 | 15 kDa | + | |
| 14-3-3 protein beta/alpha | P31946 | 28 kDa | + | |
| UMP-CMP kinase | P30085 | 22 kDa | + | |
| Destrin OS=Homo sapiens | P60981 | 19 kDa | + | |
| Actin-related protein 3 | P61158 | 47 kDa | + | |
| Brain acid soluble protein 1 | P80723 | 23 kDa | | + |
| Gremlin-1 | O60565 | 21 kDa | + | + |
| Macrophage colony-stimulating factor 1 | P09603 | 60 kDa | | + |
| Coactosin-like protein | Q14019 | 16 kDa | + | |
| Neutral alpha-glucosidase AB | Q14697 | 107 kDa | + | |
| Creatine kinase B-type | P12277 | 43 kDa | + | |
| Tripeptidyl-peptidase 1 | O14773 | 61 kDa | + | + |
| Protein S100-A4 | P26447 | 12 kDa | + | + |
| Neudesin | Q9UMX5 | 19 kDa | + | + |
| Disintegrin and metalloproteinase domain-containing protein 10 OS=Homo sapiens | O14672 | 84 kDa | + | |
| Pentraxin-related protein PTX3 OS=Homo sapiens | P26022 | 42 kDa | + | |
| Follistatin OS=Homo sapiens | P19883 | 38 kDa | + | |
| Dickkopf-related protein 1 | O94907 | 29 kDa | + | |
| Tenascin OS=Homo sapiens | P24821 | 241 kDa | | + |
| Cysteine-rich protein 1 | P50238 | 9 kDa | + | |
| Alpha-parvin | Q9NVD7 | 42 kDa | + | |
| Protein CYR61 | O00622 | 42 kDa | + | |
| U6 snRNA-associated Sm-like protein LSm8 | O95777 | 10 kDa | + | |
| Insulin-like growth factor-binding protein 6 | P24592 | 25 kDa | + | |
| Cold-inducible RNA-binding protein | Q14011 | 19 kDa | + | |
| UTP--glucose-1-phosphate uridylyltransferase | Q16851 | 57 kDa | + | |
| Keratin, type II cytoskeletal 6B | P04259 | 60 kDa | | + |
| N-acetylglucosamine-6-sulfatase | P15586 | 62 kDa | | + |
| Target of Nesh-SH3 | Q7Z7G0 | 119 kDa | + | |
| Extracellular sulfatase Sulf-1 | Q8IWU6 | 101 kDa | + | |
| Protein phosphatase 1 regulatory subunit 14A | Q96A00 | 17 kDa | + | |
| T-complex protein 1 subunit eta | Q99832 | 59 kDa | + | + |
| Non-histone chromosomal protein HMG-17 | P05204 | 9 kDa | | + |
| 60S ribosomal protein L7 | P18124 | 29 kDa | | + |
| Tenascin-X | P22105 | 458 kDa | | + |
| Far upstream element-binding protein 2 | Q92945 | 73 kDa | | + |
| 182 kDa tankyrase-1-binding protein | Q9C0C2 | 182 kDa | | + |
| NSFL1 cofactor p47 | Q9UNZ2 | 41 kDa | | + |

We grouped these proteins in clusters in connection with their type and cell function (Fig. 1). Here we show that the most functional groups in secretomes of healthy and HD cells are similar, and it is impossible to distinguish a whole group or groups of cells—based only on cell type—that might indicate the presence or absence of HD. But there are many individual differences with specific proteins. Next, we will look at the different proteins that are most interesting from the point of view of possible functions.

Filamin is a large actin-binding protein with both structural and scaffold functions. It is involved in signal transduction, cell proliferation and differentiation, pseudopodia formation, vesicle transport, tumor resistance and genetic diseases by binding with interacting proteins [18, 19].

Periostin functions by interacting with extracellular matrix components to drive collagen fibrillogenesis and remodeling or by signaling through cell-surface integrin receptors to promote cell adhesion, migration, and proliferation [20] Silencing of the periostin gene (POSTN) can inhibit the biological process of several different cancers, and this inhibition may be related to down-regulation of PI3K/AKT signaling. Transfection of cultured OS cells (Saos-2) by POSTN-shRNA plasmid effectively inhibits the proliferation, invasion, and migration of these cells [21] So, filamin A (FLNA) and periostin—found only in secretome of health donor—are involved in cell proliferation, adhesion and migration. Therefore, these proteins may be a cause for the difference in these processes for dermal fibroblasts with HD genotype.

Alpha-actinin-4 is a non-muscle actin binding protein, also participating in both regulation of NF-kappaB transcription factor activity, RNA metabolism [22] and cell adhesion as a component of integrin-cytoskeleton complex. Serum ACTN4 levels may be valuable diagnostic and prognostic biomarkers for cancer [23] and metastasis as it is a protein involved migration and invasion. It is likely that its presence in the secretome is also associated with differences in cell migration activity.

There are a few actin cytoskeleton proteins, like alpha-actinin-4, talin-1, calponin 3, ARP3, alpha-parvin and myosin 9 that were differentially identified in secretomes of both those with and without HD. Mechanisms of the release of actin into the extracellular environment have not yet been sufficiently characterized. However, the available data demonstrate that different actin isoforms localized on the outer cell surface and in the extracellular matrix are involved in various physiological and pathological processes. At the same time, the actin/angiogenin complex, similarly to actin, can promote plasmin generation due to tissue plasminogen activator (tPA). This complex, in contrast to actin, does not inhibit plasmin activity. In view of this fact, angiogenin promotes degradation of the extracellular matrix, allowing the penetration of endotheliocytes through the basement membrane and migration during angiogenesis [24].

Adrenomedullin (AM) was found only in secretome of fibroblasts from donor with Huntington disease. It is a multifunctional peptide involved in vasodilation, hormone secretion, antimicrobial defense, cellular growth, and angiogenesis. In neurons, AM and related peptides are associated with some structural and functional cytoskeletal proteins that interfere with microtubule dynamics. Furthermore, AM may intervene in neuronal dysfunction through other mechanisms such as immune and inflammatory response, apoptosis, or calcium dyshomeostasis [25]. This peptide has been also detected in the cultured media of cardiovascular, glial tumor, and neurons, which implies that AM is synthesized and secreted by them [26]. Alterations in AM expression have been described in neurodegenerative processes such as Alzheimer's disease or vascular dementia [25].

HSP70, a chaperone with various functions, was not found in secretome of cells from donor with HD. HSP70 is involved in the regulation of growth, development, transfer of genetic material, signaling, and cell death. The neuroprotective properties of HSP70 have been confirmed in various models of ischemic injury in vitro and in vivo. Numerous studies in recent years have noted the important role of HSP70 in the implementation of the mechanisms of metabolic adaptation, neuroplasticity and neuroprotection of brain cells [27]. [28]. Induction of this protein provides an important cellular mechanism to protect vulnerable neurons from excitotoxic overactivation of glutamate receptors by endogenous glutamate, and may be relevant to pathological conditions in which extracellular endogenous glutamate is augmented, such as ischemia [29].

14-3-3 proteins are abundantly expressed adaptor proteins that interact with a vast number of binding partners to regulate their cellular localization and function. These proteins play important roles in pathogenesis through regulating the subcellular localization of target proteins [30]. It was shown, that 14-3-3 ζ might scavenge

misfolded Huntingtin proteins by facilitating the formation of aggregates possibly for neuroprotection [31]. 14-3-3 regulate aspects of neurodegenerative disease with a focus on their protective roles against neurodegeneration. In neurons, 14-3-3 proteins function in diverse processes including differentiation, migration, survival, neurite outgrowth, and ion channel regulation [32].

Brain acid-soluble protein 1 (BASP1) was examined in connection with many neurodegenerative diseases such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease and Amyotrophic Lateral Sclerosis [33]. BASP1 together with growth-associated protein-43 (GAP-43) regulate actin dynamics [34] and presynaptic vesicle cycling at axon terminals, thereby facilitating axonal growth, regeneration, and plasticity. By modulating actin dynamics, GAP-43, and BASP1 take part in neuro-development, synaptic function, and nerve regeneration [33]. Moreover, BASP1 expression inhibits growth and migration of thyroid cancer cells [35], so this protein may play a role in the regulation of these cellular functions.

HSP70 and 14-3-3 have neuroprotective properties, and interestingly, their expression was not detected in the secretome of cells with HD. It may be one of markers of this neurodegenerative disease. Apparently, a change in the secretion of these proteins occurs as a result of the launch of processes associated with the pathological genotype of HD and occurs not only in neurons, but also in other cells. However, these data should be further investigated.

## 4. DISCUSSION

Today we know that cells can contact each other, exchange factors or signaling molecules, secreting them into the extracellular space. This contact could be a distant signal, such as hormones carried through the bloodstream beyond the tissue. It might also be a compound that is endocytosed within the tissue or even by the producing cell itself. The functionality of a certain tissue and a certain type of cell determines its microenvironment, including the secretion [36]. Secreted proteins are known to play a key role in coordinating essential biological functions such as growth, division, differentiation, apoptosis and signaling [37]. Growing evidence from stem cell research makes it the target of many potential regenerative medicine approaches to treat a range of diseases. For example, stem cell transplantation is a possible therapy for many disorders associated with the cardiovascular system, neurodegenerative diseases, and cancers

[38]. However, in this case, the study of intercellular interactions that occur due to the secretion of signaling molecules by autocrine or paracrine mechanisms deserves special attention. In addition , the secretome of healthy cells, which creates a favorable microenvironment for the affected tissue, is also of growing interest.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies involving patients or animals as test objects.

Informed consent was not required for this article.

## CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

## AUTHOR CONTRIBUTION

Author MK—analysis of results and manuscript preparation; author NK—manuscript preparation, cell culturing; author PP—manuscript preparation, isolation of secretomes; author NY—cell culturing; authors YK, EO–isolation of secretomes, cell cuturing; authors ER, SS, AM—mass-spectrometry; author NM—manuscript editing, conceptualization.

## DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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