ADVANCED QUANTITATIVE PROTEOMICS TO EVALUATE MOLECULAR EFFECTS OF LOW-MOLECULAR-WEIGHT HYALURONIC ACID IN HUMAN DERMAL FIBROBLASTS

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ABSTRACT

Hyaluronic acid (HA) is physiologically synthesized by several human cells types but it is also a widespread ingredient of commercial products, from pharmaceuticals to cosmetics. Despite its extended use, the precise intra- and extra-cellular effects of HA at low-molecular-weight (LWM-HA) are currently unclear. At this regard, the aim of this study is to in-depth identify and quantify proteome's changes in normal human dermal fibroblasts after 24 hours treatment with 0.125, 0.25 and 0.50 % LMW-HA (20-50 kDa) respectively, vs controls. To do this, a label-free quantitative proteomic approach based on high-resolution mass spectrometry was used. Overall, 2328 proteins were identified of which 39 significantly altered by 0.125 %, 149 by 0.25 % and 496 by 0.50 % LMW-HA. Protein networking studies indicated that the biological effects involve the enhancement of intracellular activity at all concentrations, as well as the extracellular matrix reorganization, proteoglycans and collagen biosynthesis. Moreover, the cell's wellness was confirmed, although mild inflammatory and immune responses were induced at the highest concentration. The more complete comprehension of intra- and extra-cellular effects of LMW-HA here provided by an advanced analytical approach and protein networking will be useful to further exploit its features and improve current formulations.

Abbreviations: HA, hyaluronic acid; LMW-HA, low-molecular-weight hyaluronic acid; HMW-HA, high-molecular-weight hyaluronic acid; nLC-HRMS, nano liquid chromatography- high resolution mass spectrometry

Keywords: Low-molecular-weight hyaluronic acid; dermal fibroblasts; mass spectrometry; quantitative proteomics; networking

1. INTRODUCTION

The extracellular environment, also referred as extracellular matrix (ECM), is principally formed by glycosaminoglycans involved in several biological functions mainly related to their molecular structure [1]. Among these, hyaluronic acid (HA), an unbranched glycosaminoglycan formed by repetitive disaccharide units of N-acetyl-D-glucosamine and D-glucuronic acid linked through alternating ß-1,3- and ß-1,4-glycosidic bonds, is synthesized in human by different haluronan synthase isoforms (HAS1, HAS2 and HAS3). Hyaluronidases (HYALs) and reactive oxygen species (ROS) are instead responsible for around 30% of its degradation. The remaining is systemically metabolized by endothelial cells of the lymphatic vessel and liver [2]. Because of the diverse degrees of physiological enzymatically and non-enzymatically polymerization, HA is usually classified as low-molecular-weight (LMW-HA) when $\leq 10^6$ Da, or as high-molecular-weight (HMW-HA) when $> 10^6$ Da. Nevertheless, a precise cutoff is not defined.

About biological functions, endogenous HMW-HA demonstrated a positive role in the control of tissue hydration, inflammatory and immune processes, tissue repair, and endothelial cellular growth [3, 4, 5]. On the other hand, endogenous LWM-HA may induce pro-inflammatory activity stimulating cytokines, chemokines and growth factors as well as the ECM remodeling, uncontrolled cellular growth, and angiogenesis during wound healing [3, 6]. Since last century, HA has been attracting the attention of many industrial fields, from pharmaceutical to cosmetic ones due to its widespread distribution in humans and its diversified physiochemical proprieties including biocompatibility, biodegradability, mucoadhesivity, viscoelasticity and hygroscopicity [7]. In cosmetics HA is widely used as anti-ageing especially for its ability to induce tissue boost, skin hydration and collagen stimulation [8]. Pavicic et al. [9] for example demonstrated that HMW-HA improves only hydration in aged skin probably because of its low skin penetration, while LMW-HA (50-800 kDa) shows better results on skin elasticity. Moreover, exogenous LWM-HA has showed to cross the *corneum stratum* [10, 11] and the epidermis [12] more easily than the HMW-HA, supporting its currently increasing

use in the topical formulations, although the detailed intra- and extra-cellular changes induced by exogenous LMW-HA are poorly described. The aim of this work is to quantitatively describe the proteome alterations induced by 20-50 kDa LMW-HA in normal human dermal fibroblasts by advanced mass spectrometric technique and network analysis. Indeed, the current development of 'omics sciences (genomics, proteomics, metabolomics etc.) supported by performing analytical tools is showing a new molecular panorama due to a huge amount of data available. To own our knowledge, this is the first proteomics study applied to exogenous 20-50 kDa LWM-HA to gain a deeper insight into its molecular effects at fibroblast level. Therefore, the results here obtained will allow reaching a more complete comprehension about biological processes influenced by 20-50 kDa LWM-HA, useful information for the improvement also of existing cosmetics formulations and for the optimization of personalized treatments.

2. MATERIAL AND METHODS

2.1 Cell culture

The adult normal human dermal fibroblasts (NHDF-Ad 28887; Lonza) were cultured as a monolayer in Dulbecco's Modified Eagle's Medium (DMEM; Gibco) containing 10% FBS (Euroclone), 1% glutamine and 1% penicillin-streptomycin antibiotic (Lonza), at 37 °C in a humidified atmosphere of 5% CO_2**.**

2.2 Cell viability assays

The viability of cells was evaluated using MTT reduction assay (Sigma-Aldrich) and Real Time Glo-MT kit assay (Promega). Briefly, $9x10^3$ NHDF-Ad cells were seeded on a 96-well plate overnight. LMW-HA powder (RENOVHYAL 20-50 kDa; SOLIANCE – Pomacle, France) was dissolved in complete DMEM to obtain different concentrations (w/v): 0.125 %, 0.25 %, 0.50 %, 1.00 % and 2.50 %. Each LMW-HA cell media solution was added to the cells. Treated and untreated cells, as control, were incubated in biological duplicate for 24 hours. For the viability cells' assessment, the MTT and

RealTime Glo-MT assays were performed following the standard protocols. All statistical analyses were done by the GraphPad software (v 6.0).

2.3 Cell treatment

Three experimental conditions were planned considering the results of cell viability assays and the usual concentrations in the cosmetic products. NHDF-Ad (7^{th} passage, 90% of confluence) seeded in T75 flasks were treated in biological duplicate with 0.125 %, 0.25 % and 0.5 % LMW-HA, w/v in cell media, respectively for 24 hours considering the physiological turn-over. Two untreated flasks were used as control. Whole experiment was replicate three times to increase the reliability of results.

2.4 Sample preparation

Once treated, all cells were trypsinized (Gibco) and pelleted by two cycle of centrifuge at 400 g, room temperature for 5 min. The whole protein was extracted by using a buffer composed by 8 M urea in 50 mM Tris-HCl, 30 mM NaCl (Bio-Rad) at 8.5 pH and 1 % of protease inhibitor cocktail (Sigma-Aldrich) followed by centrifugation at 14000 x g, 4° C for 30 min. The amount of proteins was quantified by the Bradford Reagent (Sigma-Aldrich) following the standard procedure. 20 µg of proteins in 50 mM NH_4HCO_3 were reduced with 5 mM DL-dithiothreitol (DTT, Sigma-Aldrich) for 30 min at 52°C, then centrifuged at 500 rpm and alkylated with 15 mM iodoacetamide (Sigma-Aldrich) for 20 min in the dark at room temperature. The trypsin digestion was performed in 1:20 enzyme:protein ratio (w/w) (Trypsin Sequencing Grade; Roche, Monza, Italy) overnight at 37°C [13].

2.5 High-resolution mass spectrometry analysis (nLC-HRMS)

To increase the quality of instrumental analysis, the digested samples were further purified and concentrated by 0.2 µL C-18 resin ZipTip (Millipore, Milan, Italy). Tryptic peptides were analyzed using a Dionex Ultimate 3000 nano-LC system (Sunnyvale CA, USA) connected to Orbitrap Fusion™ Tribrid™ Mass Spectrometer (Thermo Scientific, Bremen, Germany) equipped with a

nano-electrospray ion source (nESI). Peptide mixtures were pre-concentrated onto an Acclaim PepMap 100 - 100 µm × 2 cm C18 and separated on EASY-Spray column, 25 cm × 75 µm ID packed with Thermo Scientific Acclaim PepMap RSLC C18, 3 µm, 100 Å. The temperature was set to 35 °C and the flow rate was 300 nL min^{-1}. Mobile phases were the following: 0.1% formic acid (FA) in water (solvent A); 0.1% FA in water/acetonitrile with 2/8 ratio (solvent B). The elution gradient was from 96% buffer A to 40% buffer B for 110 min. MS spectra were collected over an *m/z* range of 375-1500 Da at 120,000 resolutions, operating in data dependent scan mode, cycle time 3 sec between master scans. Higher-energy collision dissociation (HCD) was performed with collision energy set at 35 eV in positive polarity. Each sample was analyzed in three technical replicates.

2.6 Data analysis

The instrumental raw files were analyzed by MaxQuant software v1.6.6.0 [14] set on *Uniprot_Homosapiens* database against the Andromeda search engine. The quantification of peptides and related proteins for each control and treated sample in biological duplicate and technical triplicates was based on the LFQ intensities. Trypsin as the digestive enzyme, variable modification of carbamidomethylation of cysteine (+57.021 Da), fixed modification of methionine oxidation (+15.995 Da), N-terminal acetylation (+42.011 Da) and LFQ minimum ratio count to 2 were set as further parameters. The interpretation and visualization of results from MaxQuant software were performed by a two-sample t-test using Perseus (v1.6.1.3, Max Planck Institute of Biochemistry, Germany). The protein variations (log_2 fold changes) were evaluated by using (0.125 % LMW-HA vs control: 0.25 % LMW-HA vs control; 0.50 % LMW-HA vs control). Statistical parameters ($p < 0.05$; $q < 0.05$, q= FDR adjusted p-value) were set to identify the differentially expressed proteins between samples. The proteins were selected with a minimum of two peptides. Variability of biological replicates were measured using the scatter plot with Pearson correlation coefficient values of the LFQ intensities. The network protein analyses related to significantly altered proteins were

carried out by Reactome, STRING (v 11.0) and Ingenuity Pathways Analysis (last release; Qiagen) based on Gene Ontology database.

3. RESULTS

Considering the widespread use of HA in topical formulations and lack of deep knowledge about intra- and extra-cellular biological effects of LMW-HA, the goal of this study was to quantify the proteome's changes induced by different concentrations of LMW-HA in normal human dermal fibroblasts.

3.1 Treatments with LMW-HA: identification and differential proteomic analysis

Based on the MTT and RealTime-Glo results **(Figure 1**) and the plausible exposition in the real setting, 0.125, 0.25 and 0.50 % LMW-HA were selected for the treatments. Automatic count of cells supported the cell viability results. After the treatment in biological duplicate with 0.125, 0.25 and 0.50 % LWM-HA respectively, applying MS-based label-free quantitative (LFQ) proteomic analysis, a total of 2328 proteins were identified and quantified from treated and control samples. The quality and reproducibility of biological and technical replicates were confirmed by multi-scatter plot (Pearson coefficient values ≥ 0.98; **Figure S1**). As consequence of 0.125 % LWM-HA, 39 proteins resulted significantly altered (25 up-regulated, 14 down-regulated) **(Figure 2a and Supplementary Table 1)**, 149 by 0.25 % LWM-HA (72 up-regulated, 77 down-regulated) **(Figure 2b and Supplementary Table 1)** and 496 by 0.50 % LWM-HA (334 up-regulated, 172 down-regulated) **(Figure 2c and Supplementary Table 1)**. Moreover, some of these were affected by all LWM-HA concentrations tested **(Figure S2 and Table 1**) or by at least two out of three concentrations **(Table 1)**.

3.2 Protein Network Analyses

After the identification and quantification of differentially regulated proteins, we applied protein network analyses using String, Reactome and Ingenuity Pathways Analysis (IPA) based on Gene Ontology (GO) terms to describe functional protein modules and pathways. See the **Supplementary Table 2** for the complete list.

3.2.1 Proteome's changes induced by 0.125 % LWM-HA

This study demonstrated limited proteome's changes related to 0.125 % LWM-HA. Nevertheless, despite the low number of significantly altered proteins compared to controls (n=39; **Supplementary Table 1**), we mainly showed a noticeable increase of intracellular reorganization and mitochondrial activity **(Supplementary Table 2)**. In details, we found a pronounced overexpression of organelle (FDR= 0.023) and mitochondrial matrix (FDR = 3.2 e-04) reorganization. The low number of down-regulated proteins (n=14) did not allow us the identification of relevant nodes.

3.2.2 Proteome's changes induced by 0.25 % LWM-HA

By increasing the concentration of LWM-HA from 0.125 to 0.25 % a more robust biological effect was observed with the significant alteration of 149 proteins **(Table 1 and Supplementary Table 1)** involved both in intra- and extra-cellular environment.

Regarding the intracellular activity, we observed the up-regulation of several pathways such as chromosome organization (FDR = 0.0046) and oxidation-reduction process (FDR = 0.0437) **(Figure 3)** but also cellular component organization or biogenesis involving 40 genes (FDR = 1.8 e-04), and protein processing (FDR = 0.040). More interesting were the LWM-HA effects outside the cell by raising the expression of proteins involved in collagen binding (FDR = 0.0266) and extracellular matrix organization (FDR = 0.0046) (Figure 3), elastic fibers formation (FDR = 0.03) or syndecans interactions (FDR = 0.014). ECM proteoglycans biosynthesis was also enriched (FDR = 0.015) especially for dermatan sulfate (HSPG2; fold change = 9.74) and chondroitin sulfate (CSPG4; fold change = 1.73). Conversely, a reduced expression of several immune pathways such as IL-12

signaling (FDR = 9.92 e-07) and neutrophil degranulation (FDR= 0.123) was observed, suggesting a great cells' wellness when exposed to the medium concentration (0.25 % LWM-HA) **(Table 1 and Supplementary Table 2)**.

3.2.3 Proteome's changes induced by 0.50 % LWM-HA

The main intra- and extracellular impact was shown in cells treated with 0.50 % LWM-HA: we found in fact the highest number of significantly altered proteins, 496 vs 149 and 36 with 0.125 and 0.25 % LWM-HA, respectively, and related pathways. The intracellular effects were mainly supported by an increasing of cells' proliferation by translation process (FDR = 6.02 e-11), of oxidation-reduction process (FDR = 7.75 e-06) and of immune process (FDR = 4.4 e-04) **(Figure 4a)**. More in details, cells growth was demonstrated by enhancing of VEGFA-VEGFR2 complex (p value= 3.35 e-03), of RNA expression (p value = 6.72 e-05) and fibroblasts proliferation pathway (p value = 7.50 e-03) **(Figure 4b)**. Furthermore, mitochondrial activity by acid citric cycle II (p value = 1.89 e-04), EIF2 pathway (p value= 9.34 e-05) and Wnt signaling (FDR = 0.076) were also increased. In addition, 0.50 % LWM-HA seemed to provide an increasing immune response by an up-regulation of IL-12 family signaling (FDR= 1.04 e-05) **(Figure 4a)** as well as IL2 (p value = 3.21 e-03; z score = 1.342), IL4 (p value = 6.91 e-03; z score = 1.633) and TNF signaling (p value = 2.73 e-01; z score = 1.788) **(Figure 4c**). In addition, also IL-1 family signaling (FDR = 3.72 e-04), NF-kB pathway (FDR = 2.15 e-05), and neutrophil degranulation (FDR = 0.007) showed an increasing vs control. Finally, in line with the previous concentration we observed a pronounced extracellular activity by an over-expression of HSPG2 (fold change = 8.15), FN1 (fold change = 3.18) and CPSG4 (fold change = 1.77) suggesting a high ECM reorganization mainly based on proteoglycans biosynthesis **(Table 1 and Supplementary Table 2)**.

4. DISCUSSION

A deepening knowledge of molecular effects induced by any active principle is fundamental to support its use, due to demonstration of safety and efficacy. Although the general attention was mainly focused on the intracellular environment, an increasing number of evidences has pointed out the biological importance also of the extracellular one. Indeed, the extracellular matrix (ECM) has showed to be involved in several physiological and pathological pathways [1]. High-molecular-weight hyaluronic acid (HWM-HA) is one of the major ECM's constituents, but also a widespread commercial ingredient for its superficial hydration proprieties. More recently, LMW-HA became commercially available especially for anti-aging use [8]. Despite its diffusion, the detailed intra- and extra- cellular impact of commercially available low-molecular-weight HA (LMW-HA) is not yet defined. At this regard, the objective of this study was to describe and quantify the proteins profile's change induced by different concentrations of 20-50 kDa LWM-HA (0.125 %, 0.25 % and 0.50 % respectively) on normal human dermal fibroblasts. To do this, a quantitative proteomics approach was applied considering the large number of molecular information available by omics science, including proteomics, and the currently improvement of instrumental technique. The high-resolution mass spectrometric technique coupled with nano-LC was used and the results were explained by network and pathways analyses. In line with previous evidences [3, 6] but in a deeper way, here we demonstrated both an intra- and extra-cellular impact of 20-50 kDa LWM-HA. Indeed, the 24 hours treatment with LWM-HA induced, for example, an increasing of cell proliferation and growth as well as of extracellular matrix reorganization or proteoglycans biosynthesis. Moreover, at the highest concentration (0.50 %) the inflammatory and immune responses were activated, among all, by the stimulation of lymphocytes, interleukins (IL-12, IL-1, IL-2, IL-4 etc.) or necrosis tumor factor signaling. However, the global cells' wellness was still sustained as demonstrated, for example, by the significant up-regulation of EIF2 pathway implicated in the protein synthesis, of citrate acid cycle that is a pivotal factor of mitochondrial functionality and of fibroblasts proliferation. In addition, 0.50 % LWM-HA enhanced the VEGFA-VEGFR2 signaling that is actively involved in angiogenesis by inducing the proliferation, survival and migration of endothelial cells, and by increasing endothelial

permeability [15, 16]. As further confirmation of the cells' viability and ECM reorganization after the 0.25 and 0.50 % LMW-HA treatment, a significant upregulation was demonstrated by FN1, HSPG2 and EMILIN 1 genes. FN1 encodes for fibronectin, a glycoprotein of the extracellular matrix that plays a key role in cell adhesion and migration processes as well as in wound healing binding membrane-spanning receptor proteins as integrins but also collagen, fibrin, and heparan sulfate proteoglycans (i.e. syndecans, gypicans and perlecans) [17, 18]. Then, HSPG2 encodes exactly for heparan sulfate proteoglycans (HSPGs) that show angiogenic and growth-promoting attributes primarily by acting as a coreceptor for basic fibroblast growth factor (FGF2) [19, 20] as well as a cross-linker among many extracellular matrix components and cell-surface molecules (laminin, prolargin, collagen type IV etc.). Additionally, as resulted also in this study, HSPGs demonstrated a pivotal role in regulating developmental signaling pathways including transforming growth factor-β or β-catenin independent Wnt signaling [17], where Wnt are lipid-modified proteins strictly associated with cell surface and ECM. EMILIN 1, a multidomain glycoprotein, is also involved in skin wound reparation and in control of cell proliferation, in the matrix anchoring fibroblast to keratinocytes [20].

This study has strong points and limitations. The first include: *i)* the analytical instruments and the applied methodology, that allowed us a detailed and quantified description of a huge number of proteins; *ii)* the investigation of less known form of HA (i.e. LMW-HA); *iii)* the network analysis conducted by several software, which allowed to depict a complete intra- and extra-cellular overview; *iv)* the primary and healthy selected cell line; *v)* the LMW-HA concentrations tested that reflected those commonly used. With regard to the main limitations, it should be noted that a simple *in vitro* environment like that of cells, less complex that whole skin, could offer a limited view of the biological effects of a compound.

In conclusion, treating normal human dermal fibroblast with hyaluronic acid at low-molecular-weight resulted in positive intra- and extra-cellular effects enhancing the nucleus and mitochondria functionality as well as the ECM reorganization. In addition, the inflammatory and immune activity

induced by the highest concentration seemed to be well tolerated. As next step, considering the close connection between proteins and other metabolites, also the lipidome profile's changes will be analyzed to provide a more complete comprehension about the *in vitro* molecular effects of LWM-HA supporting and improving its commercial use and safety.

CONFLICT OF INTEREST

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors state no conflict of interest.

ACKNOWLEDGEMENTS

We thank UNITECH OMICs, mass spectrometry platform of Università degli Studi di Milano, for running mass spectrometry analyses; Dr. Martina Contilli for her contribution in experimental procedures and data analysis.

CRediT Authors statements

Silvia Radrezza: Methodology, Investigation, Formal analysis, Data Curation, Writing - Original Draft, Visualization**; Giovanna Baron:** Formal analysis, Writing - Review & Editing**; Sarath Babu Nukala:** Investigation**; Gabriele Depta:** Resources; **Giancarlo Aldini**: Supervision; **Marina Carini**: Conceptualization, Supervision, Writing - Review & Editing; **Alfonsina D'Amato:** Methodology, Project administration, Writing - Review & Editing

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Figure Legends

Figure 1. Cell viability assays. a) MTT and b) RealTime Glo with LWM-HA 0.125 %, 0.25 %, 0.50 %, 1.00 % and 2.50 % respectively.

Figure 2. Distribution of differentially regulated proteins with a) LWM-HA 0.125 %, b) LWM-HA 0.25 % and c) LWM-HA 0.50 %. Green color indicates up-regulation (log2 fold change ≥ 0.6), red color represents down-regulation (log2 fold change ≤ -0.6); Scatter plots of log2 fold change on x-axis against -log p-value on y-axis of significantly quantified proteins.

Figure 3. Networking of up-regulated proteins by LMW-HA 0.25 % (String). In label some of the most interesting pathways significantly altered.

Figure 4. a) Networking of up-regulated proteins by LMW-HA 0.50 % (String). In label, some of more interesting pathways significantly altered. b) Proliferation's pathways enhanced by LMW-HA 0.50 % (IPA) c) Pathways involved in immune response significantly altered by LWM-HA 0.50% (IPA). For b) and c) in red the increased genes, in green those decreased. The color intensity is positive related to the up- or down-gene's regulation; orange line leads to activation, yellow lines for findings inconsistent with state of downstream molecule; grey line for effect not predicted.

Supplementary figure legends

Figure S1. Variabilities of biological replicates measured using the scatter plot (Perseus software) with Pearson correlation coefficient values of the LFQ intensities.

Figure S2. Different expression of proteins significantly altered by LWM-HA 0.125 % (1st column, blue), LWM-HA 0.25% (2nd column, orange) and LWM-HA 0.50 % (3th column, grey) respectively. On x-axis, the list of gene names while on y-axis the log2 fold change (difference) of treated sample vs control.

Table Legend

Table 1. Some of differentially regulated proteins by LWM-HA concentrations (0.125 %, 0.25 % and 0.50 %, respectively). In bold those more relevant for the networking explanation.

Supplementary table legends

Table S1. Complete list of identified proteins (Perseus). Among these, those significant for each concentration of LWM-HA (0.125%, 0.25% and 0.50%).

Table S2. Complete list of significantly altered pathways for each concentration of LWM-HA (0.125%, 0.25% and 0.50%) provided by String and Reactome software, respectively.

Table 1.

Accession numbers	Protein name	Gene Name	T0.125% vs C	T0.25% vs C	T0.50% vs C
Q2L6I2	ATP-binding cassette sub-family F member 1	ABCF1	1.36	1.56	1.55
Q9Y6K8	Adenylate kinase isoenzyme 5	AK5	0.73	0.76	0.71

Q53F35	Acidic leucine-rich nuclear phosphoprotein 32 family member B	ANP32B	1.37	1.41	1.56
A0A087WZT3	BolA-like protein 2	BOLA2	0.71	0.74	-
A8K651	Complement component 1 Q subcomponent-binding protein, mitochondrial	C1QBP	1.41	-	1.46
Q6IAW5	Calumenin	CALU	1.24	1.21	1.29
C9JEZ4	Cdc42 effector protein 3	CDC42EP3	-	0.73	0.80
Q6UVK1	**Chondroitin sulfate proteoglycan 4**	**CSPG4**	**-**	**1.73**	**1.77**
Q5VTU3	Dynein light chain Tctex-type 1	DYNLT1	-	1.38	1.50
P47813	Eukaryotic translation initiation factor 1A	EIF1AX	1.57	1.52	1.46
Q9Y6C2	**EMILIN-1**	**EMILIN1**	**-**	**4.75**	**3.31**
A0A0A0MT60	Peptidyl-prolyl cis-trans isomerase;FK506-binding protein 15	FKBP15	1.59	1.31	1.32
A0A024R462	**Fibronectin**	**FN1**	**-**	**3.91**	**3.18**
Q53TX0	Glutaminase kidney isoform, mitochondrial	GLS	1.39	-	1.51
B2R6K4	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	GNB1	-	0.72	0.83
F5GZQ3	Trifunctional enzyme subunit beta, mitochondrial	HADHB	1.41	1.78	2.04
P16401	Histone H1.5	HIST1H1B	1.90	3.01	2.33
P16403	Histone H1.2	HIST1H1C	1.30	2.14	1.83
Q8IUE6	Histone H2A type 2-B	HIST2H2AB	2.11	1.87	-
X6RGJ2	Heterochromatin protein 1-binding protein 3	HP1BP3	1.44	1.63	1.56
A0A0S2Z410	3-hydroxyacyl-CoA dehydrogenase type-2	HSD17B10	1.34	1.40	1.67
A0A024RAB6	**Basement membrane-specific heparan sulfate proteoglycan core protein**	**HSPG2**	**-**	**9.74**	**8.15**
B4DT20	MICOS complex subunit MIC60	IMMT	-	1.23	1.70

B4DWZ7	LanC-like protein 2	LANCL2	-	0.72	0.82
P30533	Alpha-2-macroglobulin receptor-associated protein	LRPAP1	-	0.77	0.84
Q8IV28	Nidogen-2	NID2	-	2.17	1.77
Q15113	Procollagen C-endopeptidase enhancer 1	PCOLCE	-	1.46	1.25
B4DDC8	Protein phosphatase 1G	PPM1G	1.26	1.29	1.48

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