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

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24

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

50 **1. INTRODUCTION**

51 The extracellular environment, also referred as extracellular matrix (ECM), is principally formed by
52 glycosaminoglycans involved in several biological functions mainly related to their molecular
53 structure [1]. Among these, hyaluronic acid (HA), an unbranched glycosaminoglycan formed by
54 repetitive disaccharide units of N-acetyl-D-glucosamine and D-glucuronic acid linked through
55 alternating ß-1,3- and ß-1,4-glycosidic bonds, is synthesized in human by different haluronan
56 synthase isoforms (HAS1, HAS2 and HAS3). Hyaluronidases (HYALs) and reactive oxygen species
57 (ROS) are instead responsible for around 30% of its degradation. The remaining is systemically
58 metabolized by endothelial cells of the lymphatic vessel and liver [2]. Because of the diverse degrees
59 of physiological enzymatically and non-enzymatically polymerization, HA is usually classified as
60 low-molecular-weight (LMW-HA) when $\leq 10^6$ Da, or as high-molecular-weight (HMW-HA) when
61 $> 10^6$ Da. Nevertheless, a precise cutoff is not defined.

62 About biological functions, endogenous HMW-HA demonstrated a positive role in the control of
63 tissue hydration, inflammatory and immune processes, tissue repair, and endothelial cellular growth
64 [3, 4, 5]. On the other hand, endogenous LWM-HA may induce pro-inflammatory activity stimulating
65 cytokines, chemokines and growth factors as well as the ECM remodeling, uncontrolled cellular
66 growth, and angiogenesis during wound healing [3, 6]. Since last century, HA has been attracting the
67 attention of many industrial fields, from pharmaceutical to cosmetic ones due to its widespread
68 distribution in humans and its diversified physiochemical proprieties including biocompatibility,
69 biodegradability, mucoadhesivity, viscoelasticity and hygroscopicity [7]. In cosmetics HA is widely
70 used as anti-ageing especially for its ability to induce tissue boost, skin hydration and collagen
71 stimulation [8]. Pavicic et al. [9] for example demonstrated that HMW-HA improves only hydration
72 in aged skin probably because of its low skin penetration, while LMW-HA (50-800 kDa) shows better
73 results on skin elasticity. Moreover, exogenous LWM-HA has showed to cross the *corneum stratum*
74 [10, 11] and the epidermis [12] more easily than the HMW-HA, supporting its currently increasing

75 use in the topical formulations, although the detailed intra- and extra-cellular changes induced by
76 exogenous LMW-HA are poorly described. The aim of this work is to quantitatively describe the
77 proteome alterations induced by 20-50 kDa LMW-HA in normal human dermal fibroblasts by
78 advanced mass spectrometric technique and network analysis. Indeed, the current development of
79 'omics sciences (genomics, proteomics, metabolomics etc.) supported by performing analytical tools
80 is showing a new molecular panorama due to a huge amount of data available. To own our knowledge,
81 this is the first proteomics study applied to exogenous 20-50 kDa LWM-HA to gain a deeper insight
82 into its molecular effects at fibroblast level. Therefore, the results here obtained will allow reaching
83 a more complete comprehension about biological processes influenced by 20-50 kDa LWM-HA,
84 useful information for the improvement also of existing cosmetics formulations and for the
85 optimization of personalized treatments.
86
87 ## 2. MATERIAL AND METHODS
88 ### 2.1 Cell culture
89 The adult normal human dermal fibroblasts (NHDF-Ad 28887; Lonza) were cultured as a monolayer
90 in Dulbecco's Modified Eagle's Medium (DMEM; Gibco) containing 10% FBS (Euroclone), 1%
91 glutamine and 1% penicillin-streptomycin antibiotic (Lonza), at 37 °C in a humidified atmosphere of
92 5% CO_2.
93
94 ### 2.2 Cell viability assays
95 The viability of cells was evaluated using MTT reduction assay (Sigma-Aldrich) and Real Time Glo-
96 MT kit assay (Promega). Briefly, $9x10^3$ NHDF-Ad cells were seeded on a 96-well plate overnight.
97 LMW-HA powder (RENOVHYAL 20-50 kDa; SOLIANCE – Pomacle, France) was dissolved in
98 complete DMEM to obtain different concentrations (w/v): 0.125 %, 0.25 %, 0.50 %, 1.00 % and 2.50
99 %. Each LMW-HA cell media solution was added to the cells. Treated and untreated cells, as control,
100 were incubated in biological duplicate for 24 hours. For the viability cells' assessment, the MTT and

101 RealTime Glo-MT assays were performed following the standard protocols. All statistical analyses
102 were done by the GraphPad software (v 6.0).
103
104 **2.3 Cell treatment**
105 Three experimental conditions were planned considering the results of cell viability assays and the
106 usual concentrations in the cosmetic products. NHDF-Ad (7th passage, 90% of confluence) seeded in
107 T75 flasks were treated in biological duplicate with 0.125 %, 0.25 % and 0.5 % LMW-HA, w/v in
108 cell media, respectively for 24 hours considering the physiological turn-over. Two untreated flasks
109 were used as control. Whole experiment was replicate three times to increase the reliability of results.
110
111 **2.4 Sample preparation**
112 Once treated, all cells were trypsinized (Gibco) and pelleted by two cycle of centrifuge at 400 g, room
113 temperature for 5 min. The whole protein was extracted by using a buffer composed by 8 M urea in
114 50 mM Tris-HCl, 30 mM NaCl (Bio-Rad) at 8.5 pH and 1 % of protease inhibitor cocktail (Sigma-
115 Aldrich) followed by centrifugation at 14000 x g, 4° C for 30 min. The amount of proteins was
116 quantified by the Bradford Reagent (Sigma-Aldrich) following the standard procedure. 20 µg of
117 proteins in 50 mM NH_4HCO_3 were reduced with 5 mM DL-dithiothreitol (DTT, Sigma-Aldrich) for
118 30 min at 52°C, then centrifuged at 500 rpm and alkylated with 15 mM iodoacetamide (Sigma-
119 Aldrich) for 20 min in the dark at room temperature. The trypsin digestion was performed in 1:20
120 enzyme:protein ratio (w/w) (Trypsin Sequencing Grade; Roche, Monza, Italy) overnight at 37°C [13].
121
122 **2.5 High-resolution mass spectrometry analysis (nLC-HRMS)**
123 To increase the quality of instrumental analysis, the digested samples were further purified and
124 concentrated by 0.2 µL C-18 resin ZipTip (Millipore, Milan, Italy). Tryptic peptides were analyzed
125 using a Dionex Ultimate 3000 nano-LC system (Sunnyvale CA, USA) connected to Orbitrap
126 Fusion™ Tribrid™ Mass Spectrometer (Thermo Scientific, Bremen, Germany) equipped with a

127 nano-electrospray ion source (nESI). Peptide mixtures were pre-concentrated onto an Acclaim
128 PepMap 100 - 100 μm × 2 cm C18 and separated on EASY-Spray column, 25 cm × 75 μm ID packed
129 with Thermo Scientific Acclaim PepMap RSLC C18, 3 μm, 100 Å. The temperature was set to 35 °C
130 and the flow rate was 300 nL min^{-1}. Mobile phases were the following: 0.1% formic acid (FA) in
131 water (solvent A); 0.1% FA in water/acetonitrile with 2/8 ratio (solvent B). The elution gradient was
132 from 96% buffer A to 40% buffer B for 110 min. MS spectra were collected over an *m/z* range of
133 375-1500 Da at 120,000 resolutions, operating in data dependent scan mode, cycle time 3 sec between
134 master scans. Higher-energy collision dissociation (HCD) was performed with collision energy set at
135 35 eV in positive polarity. Each sample was analyzed in three technical replicates.
136
137 **2.6 Data analysis**
138 The instrumental raw files were analyzed by MaxQuant software v1.6.6.0 [14] set on
139 *Uniprot_Homosapiens* database against the Andromeda search engine. The quantification of peptides
140 and related proteins for each control and treated sample in biological duplicate and technical
141 triplicates was based on the LFQ intensities. Trypsin as the digestive enzyme, variable modification
142 of carbamidomethylation of cysteine (+57.021 Da), fixed modification of methionine oxidation
143 (+15.995 Da), N-terminal acetylation (+42.011 Da) and LFQ minimum ratio count to 2 were set as
144 further parameters. The interpretation and visualization of results from MaxQuant software were
145 performed by a two-sample t-test using Perseus (v1.6.1.3, Max Planck Institute of Biochemistry,
146 Germany). The protein variations (log_2 fold changes) were evaluated by using (0.125 % LMW-HA
147 vs control: 0.25 % LMW-HA vs control; 0.50 % LMW-HA vs control). Statistical parameters (p
148 <0.05; $q<0.05$, q= FDR adjusted p-value) were set to identify the differentially expressed proteins
149 between samples. The proteins were selected with a minimum of two peptides. Variability of
150 biological replicates were measured using the scatter plot with Pearson correlation coefficient values
151 of the LFQ intensities. The network protein analyses related to significantly altered proteins were

152 carried out by Reactome, STRING (v 11.0) and Ingenuity Pathways Analysis (last release; Qiagen)
153 based on Gene Ontology database.
154

155 **3. RESULTS**

156 Considering the widespread use of HA in topical formulations and lack of deep knowledge about
157 intra- and extra-cellular biological effects of LMW-HA, the goal of this study was to quantify the
158 proteome's changes induced by different concentrations of LMW-HA in normal human dermal
159 fibroblasts.
160

161 **3.1 Treatments with LMW-HA: identification and differential proteomic analysis**

162 Based on the MTT and RealTime-Glo results **(Figure 1**) and the plausible exposition in the real
163 setting, 0.125, 0.25 and 0.50 % LMW-HA were selected for the treatments. Automatic count of cells
164 supported the cell viability results. After the treatment in biological duplicate with 0.125, 0.25 and
165 0.50 % LWM-HA respectively, applying MS-based label-free quantitative (LFQ) proteomic analysis,
166 a total of 2328 proteins were identified and quantified from treated and control samples. The quality
167 and reproducibility of biological and technical replicates were confirmed by multi-scatter plot
168 (Pearson coefficient values ≥ 0.98; **Figure S1**). As consequence of 0.125 % LWM-HA, 39 proteins
169 resulted significantly altered (25 up-regulated, 14 down-regulated) **(Figure 2a and Supplementary**
170 **Table 1)**, 149 by 0.25 % LWM-HA (72 up-regulated, 77 down-regulated) **(Figure 2b and**
171 **Supplementary Table 1)** and 496 by 0.50 % LWM-HA (334 up-regulated, 172 down-regulated)
172 **(Figure 2c and Supplementary Table 1)**. Moreover, some of these were affected by all LWM-HA
173 concentrations tested **(Figure S2 and Table 1)** or by at least two out of three concentrations **(Table**
174 **1)**.
175

176 **3.2 Protein Network Analyses**

177 After the identification and quantification of differentially regulated proteins, we applied protein
178 network analyses using String, Reactome and Ingenuity Pathways Analysis (IPA) based on Gene
179 Ontology (GO) terms to describe functional protein modules and pathways. See the **Supplementary**
180 **Table 2** for the complete list.
181
182 ***3.2.1 Proteome's changes induced by 0.125 % LWM-HA***
183 This study demonstrated limited proteome's changes related to 0.125 % LWM-HA. Nevertheless,
184 despite the low number of significantly altered proteins compared to controls (n=39; **Supplementary**
185 **Table 1**), we mainly showed a noticeable increase of intracellular reorganization and mitochondrial
186 activity (**Supplementary Table 2)**. In details, we found a pronounced overexpression of organelle
187 (FDR= 0.023) and mitochondrial matrix (FDR = 3.2 e-04) reorganization. The low number of down-
188 regulated proteins (n=14) did not allow us the identification of relevant nodes.
189
190 ***3.2.2 Proteome's changes induced by 0.25 % LWM-HA***
191 By increasing the concentration of LWM-HA from 0.125 to 0.25 % a more robust biological effect
192 was observed with the significant alteration of 149 proteins **(Table 1 and Supplementary Table 1)**
193 involved both in intra- and extra-cellular environment.
194 Regarding the intracellular activity, we observed the up-regulation of several pathways such as
195 chromosome organization (FDR = 0.0046) and oxidation-reduction process (FDR = 0.0437) **(Figure**
196 **3)** but also cellular component organization or biogenesis involving 40 genes (FDR = 1.8 e-04), and
197 protein processing (FDR = 0.040). More interesting were the LWM-HA effects outside the cell by
198 raising the expression of proteins involved in collagen binding (FDR = 0.0266) and extracellular
199 matrix organization (FDR = 0.0046) (Figure 3), elastic fibers formation (FDR = 0.03) or syndecans
200 interactions (FDR = 0.014). ECM proteoglycans biosynthesis was also enriched (FDR = 0.015)
201 especially for dermatan sulfate (HSPG2; fold change = 9.74) and chondroitin sulfate (CSPG4; fold
202 change = 1.73). Conversely, a reduced expression of several immune pathways such as IL-12

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

206

207 ***3.2.3 Proteome's changes induced by 0.50 % LWM-HA***

208 The main intra- and extracellular impact was shown in cells treated with 0.50 % LWM-HA: we found
209 in fact the highest number of significantly altered proteins, 496 vs 149 and 36 with 0.125 and 0.25 %
210 LWM-HA, respectively, and related pathways. The intracellular effects were mainly supported by an
211 increasing of cells' proliferation by translation process (FDR = 6.02 e-11), of oxidation-reduction
212 process (FDR = 7.75 e-06) and of immune process (FDR = 4.4 e-04) **(Figure 4a)**. More in details,
213 cells growth was demonstrated by enhancing of VEGFA-VEGFR2 complex (p value= 3.35 e-03), of
214 RNA expression (p value = 6.72 e-05) and fibroblasts proliferation pathway (p value = 7.50 e-03)
215 **(Figure 4b)**. Furthermore, mitochondrial activity by acid citric cycle II (p value = 1.89 e-04), EIF2
216 pathway (p value= 9.34 e-05) and Wnt signaling (FDR = 0.076) were also increased. In addition, 0.50
217 % LWM-HA seemed to provide an increasing immune response by an up-regulation of IL-12 family
218 signaling (FDR= 1.04 e-05) **(Figure 4a)** as well as IL2 (p value = 3.21 e-03; z score = 1.342), IL4 (p
219 value = 6.91 e-03; z score = 1.633) and TNF signaling (p value = 2.73 e-01; z score = 1.788) **(Figure**
220 **4c)**. In addition, also IL-1 family signaling (FDR = 3.72 e-04), NF-kB pathway (FDR = 2.15 e-05),
221 and neutrophil degranulation (FDR = 0.007) showed an increasing vs control. Finally, in line with the
222 previous concentration we observed a pronounced extracellular activity by an over-expression of
223 HSPG2 (fold change = 8.15), FN1 (fold change = 3.18) and CPSG4 (fold change = 1.77) suggesting
224 a high ECM reorganization mainly based on proteoglycans biosynthesis **(Table 1 and**
225 **Supplementary Table 2)**.

226

227 ## 4. DISCUSSION

228 A deepening knowledge of molecular effects induced by any active principle is fundamental to
229 support its use, due to demonstration of safety and efficacy. Although the general attention was
230 mainly focused on the intracellular environment, an increasing number of evidences has pointed out
231 the biological importance also of the extracellular one. Indeed, the extracellular matrix (ECM) has
232 showed to be involved in several physiological and pathological pathways [1]. High-molecular-
233 weight hyaluronic acid (HWM-HA) is one of the major ECM's constituents, but also a widespread
234 commercial ingredient for its superficial hydration proprieties. More recently, LMW-HA became
235 commercially available especially for anti-aging use [8]. Despite its diffusion, the detailed intra- and
236 extra- cellular impact of commercially available low-molecular-weight HA (LMW-HA) is not yet
237 defined. At this regard, the objective of this study was to describe and quantify the proteins profile's
238 change induced by different concentrations of 20-50 kDa LWM-HA (0.125 %, 0.25 % and 0.50 %
239 respectively) on normal human dermal fibroblasts. To do this, a quantitative proteomics approach
240 was applied considering the large number of molecular information available by omics science,
241 including proteomics, and the currently improvement of instrumental technique. The high-resolution
242 mass spectrometric technique coupled with nano-LC was used and the results were explained by
243 network and pathways analyses. In line with previous evidences [3, 6] but in a deeper way, here we
244 demonstrated both an intra- and extra-cellular impact of 20-50 kDa LWM-HA. Indeed, the 24 hours
245 treatment with LWM-HA induced, for example, an increasing of cell proliferation and growth as well
246 as of extracellular matrix reorganization or proteoglycans biosynthesis. Moreover, at the highest
247 concentration (0.50 %) the inflammatory and immune responses were activated, among all, by the
248 stimulation of lymphocytes, interleukins (IL-12, IL-1, IL-2, IL-4 etc.) or necrosis tumor factor
249 signaling. However, the global cells' wellness was still sustained as demonstrated, for example, by
250 the significant up-regulation of EIF2 pathway implicated in the protein synthesis, of citrate acid cycle
251 that is a pivotal factor of mitochondrial functionality and of fibroblasts proliferation. In addition, 0.50
252 % LWM-HA enhanced the VEGFA-VEGFR2 signaling that is actively involved in angiogenesis by
253 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

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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.

374

375 **Supplementary figure legends**

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

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

382

383 **Table Legend**

384 **Table 1.** Some of differentially regulated proteins by LWM-HA concentrations (0.125 %, 0.25 % and
385 0.50 %, respectively). In bold those more relevant for the networking explanation.

386

387 **Supplementary table legends**

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

390 **Table S2.** Complete list of significantly altered pathways for each concentration of LWM-HA
391 (0.125%, 0.25% and 0.50%) provided by String and Reactome software, respectively.

392

393 **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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