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Research Article

Protein Profiling of Human Nonpigmented Ciliary Epithelium Cell Secretome: The Differentiation Factors Characterization for Retinal Ganglion Cell line

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The purpose of this paper was to characterize proteins secreted from the human nonpigmented ciliary epithelial (HNPE) cells, which have differentiated a rat retinal ganglion cell line, RGC-5. Undifferentiated RGC-5 cells have been shown to express several marker proteins characteristic of retinal ganglion cells. However, RGC-5 cells do not respond to N-methyl-D aspartate (NMDA), or glutamate. HNPE cells have been shown to secrete numbers of neuropeptides or neuroproteins also found in the aqueous humor, many of which have the ability to influence the activity of neuronal cells. This paper details the profile of HNPE cell-secreted proteins by proteomic approaches. The experimental results revealed the identification of 132 unique proteins from the HNPE cell-conditioned SF-medium. The biological functions of a portion of these identified proteins are involved in cell differentiation. We hypothesized that a differentiation system of HNPE cell-conditioned SF-medium with RGC-5 cells can induce a differentiated phenotype in RGC-5 cells, with functional characteristics that more closely resemble primary cultures of rat retinal ganglion cells. These proteins may replace harsh chemicals, which are currently used to induce cell differentiation.

1. Introduction

Primary open angle glaucoma (POAG), a leading cause of irreversible blindness worldwide, is an optic neuropathy characterized by the gradual and progressive loss of retinal ganglion cells (RGCs), optic nerve degeneration, and excavation of the optic disks [1–4]. The hypothesis has been that larger RGCs were selectively lost in the early stage of glaucoma [5]. Although the mechanisms of optic nerve damage in glaucoma have not been completely determined, it appears that the optic nerve head is a major site of damage [6].

RGCs can generate action potentials that travel along the optic fibers [7]. In general, RGCs are a mixture of more than 20 cell subtypes. They have energy-dependent axonal

transport functions—orthograde and retrograde transports [8]. These terminal projection areas are in the lateral geniculate body. RGCs can be subdivided by their morphology and physiology, but they are usually discussed without classifications.

The *in vitro* study of the physiology and pathophysiology of RGCs has been limited to primary cultures. Previous studies have characterized a transformed rat retinal ganglion cell-line (RGC-5), which expresses many neuronal cell markers, including Thy-1, a cell surface glycoprotein found predominantly in the retinal ganglion cells [6, 9, 10], and Brn-3C, a POU domain transcription factor expressed exclusively in the retinal ganglion cells [11]. RGC-5 cells also express receptors of N-methyl-D aspartate (NMDA), GABA-B, and neurotrophin [6]. However, unlike primary RGCs, these cells were not sensitive to glutamate excitotoxicity in their undifferentiated state. RGC-5 cells pretreated with succinyl concanavalin-A (sCon A) were sensitive to 500 μM glutamate [12]. Lacking glutamate sensitivity causes the difficulties of using the RGC-5 cells in experiments involving glutamate.

Ocular ciliary epithelium cells have been shown to be involved in the synthesis and secretion of various proteins found in aqueous humor [13]. Several proteins, including neuropeptides and their processing enzymes, synthesized and secreted by a human nonpigmented ciliary epithelial (HNPE) cell-line, have been evaluated [14], and it is suggested that these secreted proteins can act in an autocrine or paracrine manner to affect ciliary epithelial functions and other target ocular cells, such as the trabecular meshwork [13]. Because of the neuroendocrine properties of the ciliary epithelium cells, the ability to confer differentiated neuroendocrine phenotypes and the physical locations of these ciliary epithelium cells and RGCs [15], we hypothesized that factors secreted by these HNPE cells may induce the RGC-5 cells to differentiate, and possibly induce glutamate and NMDA sensitivities.

Proteomic analysis, including identification and characterization, is a powerful tool for determination of biological roles and functions of individual proteins. In the present report, we have utilized a system involving HNPE and RGC-5 cells, and this system may result in the morphological and functional differentiation of RGC-5 cells. Although the origin of RGC-5 has been still in question, the expression of neuronal markers was validated [16]. Proteomic approaches have been applied to establish a map of expressed proteins for the characteristics of HNPE cells.

2. Materials and Methods

2.1. Cell Culture. The human non-pigmented ciliary epithelium cells (HNPE) were SV-40 transformed and were a gift from Dr. Miguel Coca-Prados (Yale University). HNPE were maintained at 37°C and 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS, Hyclone Laboratories, Logan, UT), 1% penicillin/streptomycin (Gibco, Grand Island, NY, USA) and 44 mM $NaHCO_3$. After three days, the cells were washed with phosphate buffered saline (PBS) and the medium was replaced by serum-free (SF) DMEM for 12 h.

The HNPE cell conditioned SF-medium was filtered by 0.22 μm filter and diluted 25 times with autoclaved Milli-Q grade water (Millipore Co., Inc.). For each 5 kD cutoff centrifugal tube (Amicon Ultra-15, Millipore Co., Inc.), a 15 mL diluted sample was loaded. Following centrifugation at 5000 $\times$g for 20 min, the sample in the filter unit was collected. The protein concentration of the HNPE cell conditioned SF-medium was measured by the Bio-Rad Bradford total protein assay kit (Bio-Rad Laboratories, Inc.).

RGC-5 cells, a secondary cell culture, were transformed rat retinal ganglion cells developed and obtained from Dr. Agarwal (University of North Texas Health Science Center). RGC-5 cells were maintained in low glucose DMEM in T-150 culture flasks supplemented with 44 mM $NaHCO_3$, 10% FBS, and 1% penicillin/streptomycin (Gibco). Differentiated RGC-5 cells were obtained by using 50% HNPE cell conditioned SF-medium and 50% fresh DMEM (containing 10% FBS). HNPE conditioned medium, which consisted of low glucose DMEM, was incubated with human non-pigmented ciliary epithelial cells (HNPE).

2.2. Immunocytochemistry. RGCs were grown on glass coverslips for 1-2 days prior to experimentation. Coverslips were rinsed with PBS three times and then were fixed in 4% paraformaldehyde for 30 min. These cells were washed with PBS before being permeabilized in 0.1% Triton X-100 for 15 min, washed with PBS, and blocked with 5% bovine serum albumin for 60 min. After rinsing with PBS, the cells were incubated with a mixture of Thy-1 (monoclonal antibodies, Chemicon, Temecula, CA, 1 : 200) and Brn-3C (polyclonal antibodies, Convance Inc, Princeton, NJ, 1 : 1000) for 1.5 h at room temperature and subsequently incubated with a combination of secondary antibodies. After PBS rinses, these cells were incubated for 10 min in the dark with 300 nM DAPI to stain nuclear regions. Cover-slides were mounted on glass slides in antifade medium (FluorSave; Calbiochem, La Jolla, CA) and allowed to dry for 20 min in the dark. Cells were visualized and images were taken using a Zeiss LSM-410 Confocal Scanning Laser Microscope System. Controls were performed by omitting primary antibodies.

2.3. 1D SDS-PAGE. HNPE cell-secreted proteins were separated under denaturing conditions in a 4–12% polyacrylamide gel. The HNPE cell conditioned SF-medium was resuspended in the sample buffer (Invitrogen NuPAGE SDS sample buffer), heated at 80°C for 10 min and then stored on ice. Each well was loaded with 5 μg of sample solution. The SDS-PAGE gel was run in a Bio-Rad protean II xi cell (Richmond CA, USA) at 200 V for 1 h. After completion of electrophoresis, the protein bands in the gel were visualized by silver staining and image acquired using an image scanner (Amersham Biosciences, Uppsala, Sweden), which is operated by the software LabScan 5.00 (Amersham Biosciences).

2.4. Silver Staining. The gels were fixed in an aqueous solution having 40% ethanol and 10% acetic acid overnight,

and then incubated in a buffer solution containing 30% ethanol, 6.8% w/v sodium acetate, and 0.312% w/v sodium thiosulfate for 30 min. After rinsing three times for 5 min each, the gels were stained in a 0.25% w/v silver nitrate solution containing 0.02% formaldehyde for 30 min. The development was performed for 10 min in a solution consisting of 2.5% sodium carbonate and 0.01% formaldehyde. An acetic acid solution (5% v/v) was used to stop the development, and the stained gels were then rinsed three times for 5 min each.

2.5. Protein Identification by Nano-HPLC-ESI-MS/MS. The protein bands were excised manually and digested using sequence grade trypsin (V511A, Promega, USA). The protein samples were reduced, alkylated, and then digested with trypsin using standard protocols [17, 18].

Reverse phase nano-high performance liquid chromatography electrospray ionization tandem mass spectrometry (RP-nano-HPLC-ESI-MS/MS) was used to identify the selected protein bands separated on the SDS-PAGE. The peptides obtained from the tryptic in-gel digestion were analyzed using a nano-HPLC system (LC Packings, Netherlands) coupled to an ion trap mass spectrometer (LCQ Deca XP Plus, ThermoFinnigan, San Jose, CA, USA) equipped with an electrospray ionization source. A linear acetonitrile gradient from 100% buffer A (5% acetonitrile/0.1% formic acid) to 60% buffer B (80% acetonitrile/0.1% formic acid) was used at a flow rate of approximately 200 nL/min for 70 min. The separation was performed on a C18 microcapillary column (Zorbax 300SB-C18, 3.5 μm, 75 μm I.D. $\times$150 mm, Agilent, Germany). Peptides eluted from the microcapillary column were electrosprayed into the nano-HPLC-ESI-MS/MS with the application of a distal 1.3 kV with heated capillary at the temperature of 200°C. Each cycle of one full scan mass spectrum (m/z 450–2000) was followed by three data-dependent tandem mass spectra with the collision energy was set at 35%.

2.6. Database Search. For protein identification, Mascot software (Version 2.2.1, Matrix Science, London, UK) was used to search the human protein sequence database (Swiss-Prot, Release 52.0 of 22-Feb-08). For proteolytic cleavages, only tryptic cleavage was allowed, and the number of maximal internal (missed) cleavage sites was set to 2. Variable modifications of cysteine with carboxyamidomethylation, methionine with oxidation, and asparagine/glutamine with deamidation were allowed. The mass tolerances of the precursor peptide ion and fragment ion were set to 1 Da. Positive protein identifications were defined if the Mowse scores of greater than 50 were considered significant ($P < 0.05$). Proteins were initially annotated by similar searches using UniProtKB/Swiss-Prot databases (Last modified September 22, 2009) [19–21].

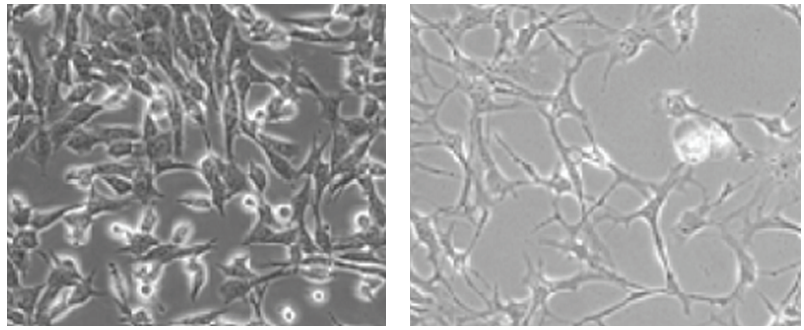
3. Results and Discussion

Cell secretome (cell-conditional medium) studies can make major contributions in understand biomarker discovery and cell pathophysiological mechanisms. It is composed of proteins that are found in the extracellular growth medium. The cell secretome consists of proteins that are secreted, shed from the cell surface and intracellular proteins released into the supernatant due to cell lysis, apoptosis, and necrosis [22, 23]. The secretome which consists of proteins or peptides secreted from cells into the extracellular medium represents the major class of molecules involved in the intercellular communication in multicellular organisms. It constitutes an important class of proteins that control and regulate a multitude of biological and physiological processes and indicates a clinically relevant source for biomarker and therapeutic target discoveries [24].

Thus, secreted proteins constitute an important category of active molecules that play crucial roles in a number of physiological and pathological processes and may reflect a broad variety of pathological conditions and thus represent a rich source of biomarkers. Proteomic characterization of proteins for identification of specific biomarkers provides a powerful tool to gain deep insights into disease mechanisms in which proteins play major roles. In this study, we have used gel electrophoresis associated with mass spectrometry for identification of the proteome and secretome of HNPE cell conditioned SF-medium samples.

3.1. RGC-5 Cell Differentiation. The differentiation system consisted of RGC-5 cells on coverslips inside 6-well plates, which were exposed to the conditioned medium from HNPE cells. RGC-5 cells proliferated rapidly with a doubling time of less than a day. Decreasing the percentage of serum in the medium may slow down proliferation. The control RGC-5 cells were heterogeneous in shape. Morphological changes of RGC-5 cells were induced by HNPE cell conditioned SF-medium (Figure 1) and caused the shrinkage of the cell body with elongated neurite outgrowth (Figure 1(b)), which allows comparison with undifferentiated RGC-5 cells (Figure 1(a)). The overall morphology of RGC-5 cells after the treatment was similar to those seen in primary cultures of rat retinal ganglion cells [25]. Moreover, the morphology of RGC-5 cells differentiated by our method was similar to the ones induced by a broad-spectrum protein kinase inhibitor staurosporine [26]. Nevertheless, Frassetto and coworkers did not conclude this to be the possible differentiation mechanism. This secretome map is a preliminary study to unveil the mechanism since the differentiation is probably the consequence of the action of several proteins and/or enzymes. It was also noted that the differentiation treatment led to decreased culture density compared with the control cells. This finding is consistent with the study from Wood et al. [27]. For subsequent studies, the conditioned medium from confluent flasks containing HNPE cells was used and found to be equally effective in promoting differentiation of RGC-5 cells.

Thy-1 expression in undifferentiated RGC-5 cells was used as a marker to identify retinal ganglion cells [28]. After treatment with HNPE cell conditioned SF-medium, RGC-5 cells have an enhanced Thy-1 expression, compared to the undifferentiated cells (Figure 2). In the retina, the



(a) (b)

Figure 1: Morphological changes in RGC-5 cells after treatment with HNPE conditioned SF-medium (40x) (a) before, and (b) after. The RGC-5 cells treated with HNPE conditioned SF-medium induced morphological changes, including longer axons and more neurite outgrowth (Figure 1(b)), compared to RGC-5 cells without treatment (Figure 1(a)).

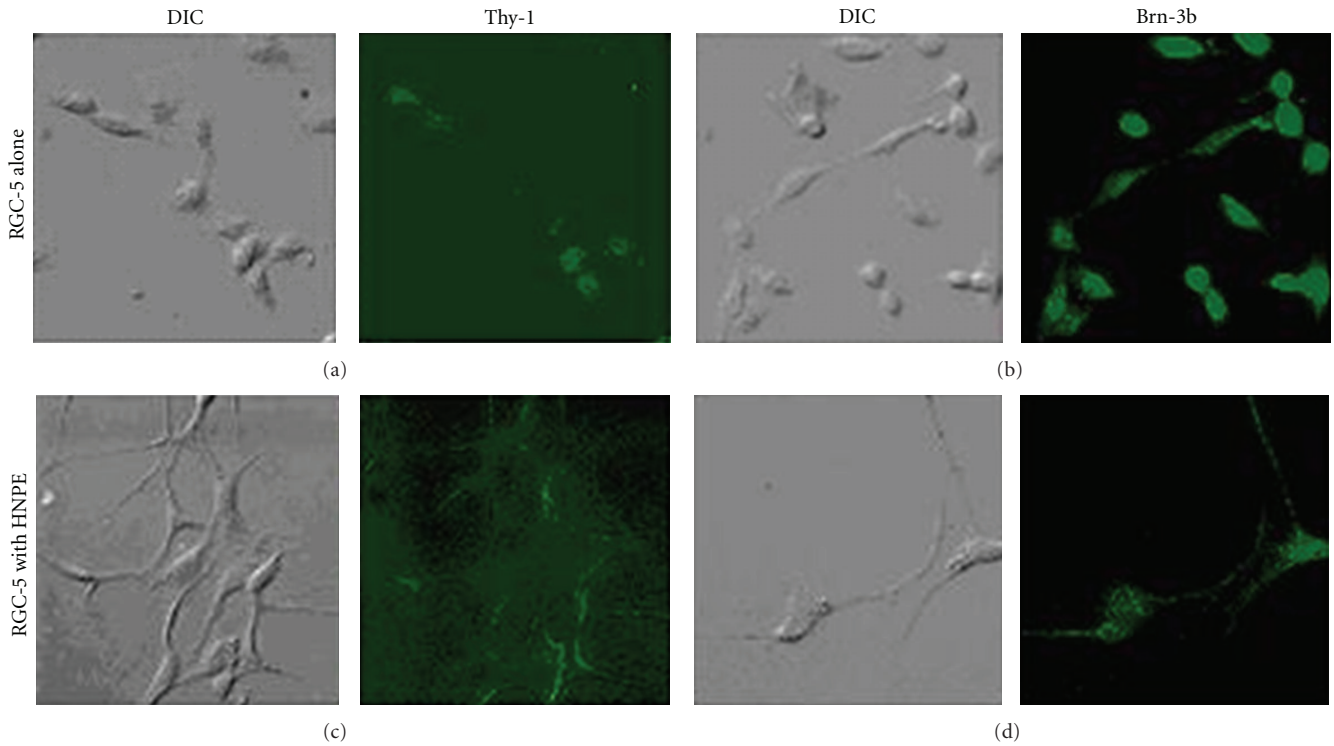


Figure 2: Immunocytochemical analysis of Thy-1 and Brn-3b expression in RGC-5 cells differentiated by treatment with HNPE cell conditioned SF-medium. Staining with antibodies to the cell surface glycoprotein, Thy-1, have been commonly used as a marker to identify retinal ganglion cells. After cultivation with HNPE conditioned medium, RGC-5 cells have an enhanced Thy-1 expression, compared to the undifferentiated cells. RGC-5 cells without cultivation with HNPE conditioned medium express Brn-3b in a different pattern compared with treated RGC-5 cells. Specifically, Brn-3b has a nuclear localization in RGC-5 cells without cultivation with HNPE conditioned medium; however, upon treatment, RGC-5 cells express Brn-3b in a more punctate cytosolic manner.

class IV POU domain transcription factor, Brn-3b, was expressed almost exclusively in subpopulations of ganglion cells and used to identify RGCs [29]. Brn-3b was regarded as a marker for differentiation of RGCs, since Brn-3 factors were not necessary for the initial specification of sensory neurons, but were essential for their normal differentiation and survival [30]. Specifically, Brn-3b was localized in the nuclear in RGC-5 cells; however, upon treatment with HNPE cell conditioned SF-medium, RGC-5 cells express Brn-3b in a more punctate cytosolic manner (Figure 2).

3.2. Proteome Analysis. The SDS-PAGE followed by silver staining resolved the protein bands from HNPE cell conditioned SF-medium. Figure 3 shows the silver-stained 1D SDS-PAGE of secreted proteins from HNPE cells. Five micrograms of secreted protein was loaded on a gel for

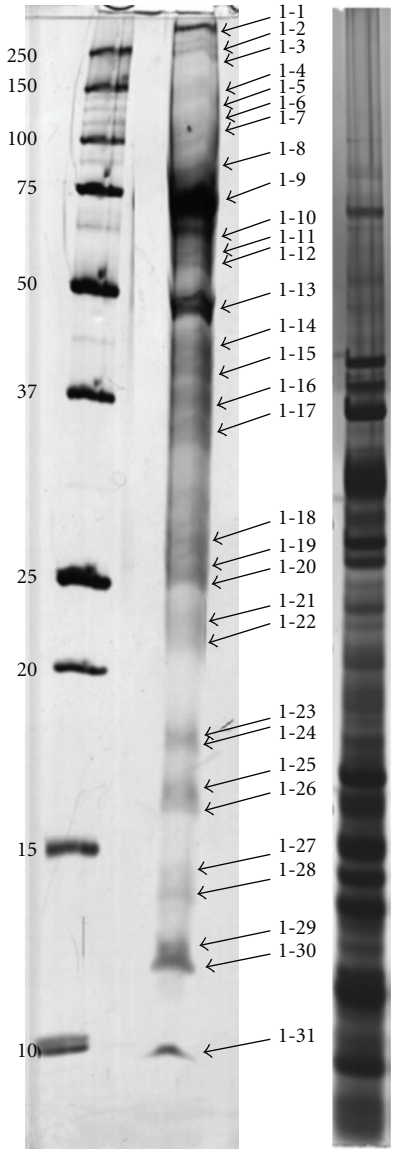


Figure 3: 1D SDS-PAGE image of HNPE conditioned SF-medium (5 μg/well, silver stained, left-hand side: molecular weight marker, kDa). The gel bands on the middle lane with serial numbers were analyzed by nano-HPLC-ESI-MS/MS. In the 30 bands, 132 proteins were identified. The gel bands on the right-hand side were the cell lysised proteins.

visualization, and more than 30 protein bands were detected in the HNPE conditioned SF-medium using the image analysis software. To identify the proteins, the position of the 1D SDS-PAGE lane was excised from the gel, washed to remove the stain, and subjected to tryptic digestion. The resulting peptides were characterized by nano-HPLC-MS/MS for protein identification. When a protein was identified by three or more unique peptides possessing MASCOT scores, no visual assessment of spectra was conducted and the protein was considered present in the sample.

In this study, all MS/MS spectra were manually confirmed (even if the above criteria were passed) by the visual assessment for their overall quality. In addition, the criteria for manual validation reported by Jaffe et al., which requires a readily observable series of at least four y-ions, was used [31]. Thus, the criteria should be enough for the validation of the identified proteins. By using this strategy, 132 unique proteins with at least three unique peptide sequences matched were identified, and a summary of the protein identifications achieved is listed in Table 1.

In this study, 47 proteins (35.6%) were known to be present in cytoplasm. Twenty-two proteins (16.7%) were known to be secreted into the extracellular space. Twenty-five proteins (18.9%) were known to be nuclear proteins. Eleven proteins (8.3%) were known to be membrane proteins. Ten proteins (7.6%) were known to be cytosol proteins. A few mitochondrial, endoplasmic reticulum, intracellular, cytoskeleton, and golgi apparatus proteins were also identified. A considerable portion of the identified proteins (6%, 8 proteins) has not been reported for their synthesized locations. Some proteins were described as found in different subcellular locations, which explains the total sum being substantially larger than 100%.

Some identified proteins in the distribution of cellular location were not secreted proteins, but they were still present in the secreted medium. To clarify the puzzle, a cell viability test was applied. The survival rate of HNPE cells was determined by the dimethylthiazol-diphenyltetrazolium bromide (MTT) assay, which was about 97%. Thus, those identified proteins were not corresponding to released proteins from dead cells. Also, according the protein profiles in Figure 3, the protein patterns obtained from secreted medium and cell lysate were very different. As a result, these proteins identified in this study can be considered as secreted proteins, which may have been synthesized inside the cells and transferred out.

Based on the functional categories in the Swiss-Prot and TrEMBL protein database, the identified proteins were classified into several groups. The Swiss-Prot identifiers could be employed for linkages of proteins to defined vocabulary of terms describing the cellular components, biological processes, and molecular functions of known gene ontology (GO). Gene Ontology Consortium provides annotations of each protein and its structure, which allowed us to organize selected proteins into biologically relevant groups. These groupings can be utilized as the basis for identifying biological information showing correlated protein changes [20, 32]. Such protein functions were listed in Table 2.

In this study, some of the proteins secreted by HNPE cells, which were confirmed by the Western blotting method, may be candidate factors responsible for promoting differentiation of RGC-5 cells including thrombospondin-1, 2, 3 precursor (1-2, 1-3, 1-13), galectin-3-binding protein (1-5∼1-7), neurogenic locus notch homolog protein 3 (Notch-3, 1-11), follistatin-related protein 1 precursor (1-11), sPARC precursor (1-14), peroxiredoxin-1 (1-21, 1-22), cofilin 1 (1-24, 1-27), profilin 1 (1-27, 1-28), galectin-1 (1-28), and myotrophin (1-30). Cell differentiation is directed by a variety of intra- and extracellular events including signals generated by extracellular matrix (ECM) components, which mediate adhesive cell-to-cell interactions and trigger a cascade of post-receptor intracellular signaling pathways. The

Table 1: Proteins identified in HNPE conditioned SF-medium by proteomic analyses.

Serial No.	SwissProt No.	Protein name	MW	Score	Subcellular location	Sequence coverage	Molecular function	Biological process
1-1	P02751	Fibronectin precursor	262442	1728	Secreted	25%	Collagen/heparin binding	cell adhesion/migration
1-2	Q08378	Golgin subfamily A member 3	167252	37	Cytoplasm	4%	Transporter activity	Intra-Golgi vesicle-mediated transport
	P02751	Fibronectin precursor	262442	810	Secreted	11%	Collagen/heparin binding	Cell adhesion/migration
	P02452	Collagen α-1(I) chain precursor	138799	163	Secreted	3%	Protein binding	Skeletal/epidermis development
	P11047	Laminin γ-1 chain precursor	177492	74	Secreted	2%	Extracellular matrix structural constituent	Cell adhesion/migration
	P07996	Thrombospondin-1 precursor	129330	50	Secreted	1%	Signal transducer activity	Multicellular organismal development
	O95239	Chromosome-associated kinesin KIF4A	139794	46	Nucleus	2%	Protein binding	Anterograde axon cargo transport
	Q5VTR2	Ubiquitin-protein ligase BRE1A	113592	39	Nucleus	1%	Transcription coactivator activity, ubiquitin-protein ligase activity and binding, zinc ion binding	Regulation of gene-specific transcription, protein polyubiquitination, negative regulation of cell migration
1-3	Q8TF76	Serine/threonine-protein kinase Haspin	88405	36	Nucleus	6%	ATP binding/protein kinase activity	Protein amino acid phosphorylation
	P07996	Thrombospondin-1 precursor	129330	427	Secreted	11%	Signal transducer activity	Multicellular organismal development
	P02452	Collagen α-1(I) chain precursor	138799	231	Secreted	8%	Protein binding	Epidermis/ skeletal development
	P01024	Complement C3 precursor	187046	145	Secreted	2%	Receptor binding	Complement activation
	P02751	Fibronectin precursor	262442	86	Secreted	3%	Collagen/heparin binding	Cell adhesion/migration
	Q14980	Nuclear mitotic apparatus protein 1	238130	50	Nucleus	4%	Protein binding/structural molecule activity	Mitotic anaphase
	P35442	Thrombospondin-2 precursor	129872	48	Secreted	1%	Heparin binding	
	P07814	Bifunctional aminoacyl-tRNA synthetase	162923	39	Cytoplasm	1%	Protein binding	Protein complex assembly
	P81274	G-protein signaling modulator 2	75798	56	Cytoplasm	5%	Identical protein binding	G-protein coupled receptor protein signaling pathway
	O14686	Myeloid/lymphoid or mixed-lineage leukemia protein 2	563831	48	Nucleus	1%	Protein/DNA binding	Regulation of transcription, DNA-dependent
	Q9UQ26	Regulating synaptic membrane exocytosis protein 2	160303	43	Cell membrane	2%	Zinc ion binding, Rab GTPase binding	Intracellular protein transport
	Q9UM54	Myosin-6	148620	35	Golgi apparatus	4%	ADP/calmodulin binding	DNA damage response, intracellular protein transport
	O15020	Spectrin β chain, brain 2	271127	38	Cytoplasm, cytoskeleton	2%	Actin binding	Vesicle-mediated transport
1-4	O00339	Matrilin-2 precursor	106768	109	Secreted	3%	Calcium ion binding	
1-5	Q08380	Galectin-3-binding protein precursor	65289	404	Secreted	23%	Protein binding/scavenger receptor activity	Cellular defense response/signal transduction
	O94985	Calsyntenin-1 precursor	109724	55	Endoplasmic reticulum membrane, nucleus, Golgi membrane	1%	Calcium ion binding, protein binding	Homophilic cell adhesion

Table 1: Continued.

Serial No.	SwissProt No.	Protein name	MW	Score	Subcellular location	Sequence coverage	Molecular function	Biological process
	P13569	Cystic fibrosis transmembrane conductance regulator	168066	37	Membrane	1%	ATP-binding and phosphorylation-dependent chloride channel activity	Respiratory gaseous exchange, transport
	P12814	α-actinin-1	102993	168	Cytoplasm	7%	Integrin binding	Regulation of apoptosis
1-6	O43707	α-actinin-4	104788	544	Nucleus	11%	Integrin binding	Regulation of apoptosis
	P12814	α-actinin-1	102993	521	Cytoplasm	14%	Integrin binding	Regulation of apoptosis
	Q08380	Galectin-3-binding protein precursor	65289	467	Secreted	18%	Protein binding/scavenger receptor activity	Cellular defense response/signal transduction
	P35609	α-actinin-2	103788	165	Cytoplasm	6%	Integrin binding	Regulation of apoptosis
	P34932	Heat shock 70 kDa protein 4	94240	82	Cytoplasm	4%	ATP binding	Response to unfolded protein
	P35711	Transcription factor SOX-5	83973	40	Nucleus	8%	Transcription factor activity	Transcription from RNA polymerase II promoter
1-7	Q08380	Galectin-3-binding protein precursor	65289	277	Secreted	15%	Protein binding/scavenger receptor activity	cellular defense response/signal transduction
	O43707	α-actinin-4	104788	163	Nucleus	10%	Integrin binding	Regulation of apoptosis
	P08238	Heat shock protein HSP 90-β	83081	159	Cytoplasm	5%	Nitric-oxide synthase regulator activity	Response to unfolded protein
	P29400	Collagen α-5(IV) chain precursor	160943	47	Secreted	4%	Binding, extracellular matrix structural constituent	
	Q13740	CD166 antigen precursor	65091	40	Membrane	4%	Receptor binding	Cell adhesion/signal transduction
	P07900	Heat shock protein HSP 90- α	84476	225	Cytoplasm	5%	ATP binding/nitric-oxide synthase regulator activity	Response to unfolded protein/signal transduction
	P12814	α-actinin-1	102993	194	Cytoplasm	6%	Integrin binding	Regulation of apoptosis
	P35609	α-actinin-2	103788	74	Cytoplasm	3%	Integrin binding	Regulation of apoptosis
	O00469	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2 precursor	84632	48	Rough endoplasmic reticulum membrane	4%	Protein binding	Protein modification process/response to hypoxia
	P34932	Heat shock 70 kDa protein 4	94240	44	Cytoplasm	3%	ATP binding	Response to unfolded protein
	Q13045	Protein flightless-1 homolog	144659	43	Nucleus	3%	Actin binding	Muscle contraction
1-8	P02768	Serum albumin precursor	69321	87	Secreted	3%	Protein binding/pyridoxal phosphate binding	Negative regulation of apoptosis /transport
	Q8TEU7	Rap guanine nucleotide exchange factor 6	179294	44	Cytoplasm	3%	GTP-dependent protein binding	Ras protein signal transduction
1-9	O95248	SET-binding factor 1	208125	41	Nucleus	1%	Protein tyrosine/serine/threonine phosphatase activity	Protein amino acid dephosphorylation
	Q9UPQ9	Trinucleotide repeat-containing 6B protein	182703	36	Cytoplasmic mRNA processing body	2%	RNA binding, nucleotide binding	Gene silencing by RNA, regulation of translation
	P29401	Transketolase	67835	134	Cytosol	7%	Protein binding	Transketolase activity
	P02768	Serum albumin precursor	69321	82	Secreted	5%	Protein binding/pyridoxal phosphate binding	Negative regulation of apoptosis/transport

Table 1: Continued.

Serial No.	SwissProt No.	Protein name	MW	Score	Subcellular location	Sequence coverage	Molecular function	Biological process
	O60333	Kinesin-like protein KIF1B	204305	58	Cytoplasmic vesicle	1%	ATPase activity/microtubule motor activity	Neuromuscular synaptic transmission/nerve-nerve synaptic transmission
	O75095	Multiple epidermal growth factor-like domains 6 precursor	128524	46		6%	Calcium ion binding	
1-10	P06744	Glucose-6-phosphate isomerase	62976	334	Cytoplasm	8%		Humoral immune response/carbohydrate metabolic process
	P27797	Calreticulin precursor	48112	67	Endoplasmic reticulum lumen, Cytoplasm, Secreted	5%	DNA/protein binding	Regulation of apoptosis/transcription, DNA-dependent, protein export from nucleus
	P09493	Tropomyosin 1 α chain	32689	44	Cytoplasm	8%	Structural constituent of muscle	Cell motility, regulation of heart/muscle contraction
	Q06495	Sodium-dependent phosphate transport protein 2A	68893	49	Membrane	1%	Sodium-dependent phosphate transmembrane transporter activity	Body fluid secretion, phosphate transport
1-11	P37268	Squalene synthetase	48084	40	Endoplasmic reticulum membrane	5%	Farnesyl-diphosphate farnesyltransferase activity	Steroid biosynthetic process
	Q12799	T-complex protein 10A homolog	45440	39	Cytosol	7%		
	O75095	Multiple epidermal growth factor-like domains 6 precursor	128524	46		1%	Calcium ion binding	
	Q9UM47	Neurogenic locus notch homolog protein 3 precursor	243496	37	Cell membrane	5%	Protein binding	
	P39191	Alu subfamily SB2 sequence contamination warning entry	65263	59		5%		
	Q12841	Follistatin-related protein 1 precursor	34963	52	Secreted	2%	Heparin binding	BMP signaling pathway
	Q14980	Nuclear mitotic apparatus protein 1	238130	41	Nucleus	2%	Protein binding/structural molecule activity	Mitotic anaphase/nuclear organization and biogenesis
	P14136	Glial fibrillary acidic protein, astrocyte	49850	80	Cytoplasm	7%	Structural constituent of cytoskeleton	
	P78527	DNA-dependent protein kinase catalytic subunit	468788	39	Nucleus	1%	DNA-dependent protein kinase activity	Peptidyl-serine phosphorylation
1-12	Q9UPQ9	Trinucleotide repeat-containing 6B protein	182703	49		4%		
	P06744	Glucose-6-phosphate isomerase	62976	401	Cytoplasm	16%		Humoral immune response/carbohydrate metabolic process
	P14618	Pyruvate kinase isozymes M1/M2	57769	145	Cytosol	7%	Pyruvate kinase activity	Glycolysis
	P00390	Glutathione reductase, mitochondrial precursor	56221	69	Mitochondrion	2%	Glutathione-disulfide reductase activity/electron carrier activity	

Table 1: Continued.

Serial No.	SwissProt No.	Protein name	MW	Score	Subcellular location	Sequence coverage	Molecular function	Biological process
	P09622	Dihydrolipoyl dehydrogenase, mitochondrial precursor	54116	47	Mitochondrion matrix	4%	Dihydrolipoyl dehydrogenase activity	
	P30154	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A β isoform	66159	42		3%	Protein heterodimerization activity	
	O95271	Tankyrase-1	141922	37	Cytoplasm	2%	NAD+ ADP-ribosyltransferase activity	Peptidyl-serine/threonine phosphorylation
1-13	P06733	α-enolase	47008	697	Cytoplasm	27%	Transcription factor activity, phosphopyruvate hydratase activity	Negative regulation of cell growth
	Q15113	Procollagen C-endopeptidase enhancer 1 precursor	47942	114	Secreted	10%		Multicellular organismal development
	P13929	β-enolase	46826	242	Cytoplasm	10%	Phosphopyruvate hydratase activity	
	P09104	γ-enolase	47108	135	Cytoplasm	4%	Phosphopyruvate hydratase activity	
	Q7Z3E2	Protein C10orf118	103623	42		2%		
	P11117	Lysosomal acid phosphatase precursor	48285	42	Lysosome	2%	Acid phosphatase activity	
	P02686	Myelin basic protein	33097	40	Myelin membrane	7%		Axon ensheathment, immune response, central nervous system development
	P50395	Rab GDP dissociation inhibitor β	50631	40	Cytoplasm	2%	Rab GDP-dissociation inhibitor activity	Signal transduction
	Q13371	Phosducin-like protein	34260	40	Cytoplasm	8%	Regulator of G-protein signaling activity	Signal transduction
	P35573	Glycogen debranching enzyme	174523	39	Cytosol	1%	Amylo-α-1,6-glucosidase activity	
	Q6ZU80	Protein C14orf145	73526	38		4%		
	P49746	Thrombospondin-3 precursor	104135	36	Secreted	1%	Calcium ion binding	Cell-matrix adhesion
	Q7L1I2	Synaptic vesicle glycoprotein 2B	77393	35	Cytoplasmic vesicle	3%		
1-14	P09486	SPARC precursor	34610	36	Secreted	14%	Calcium/calcium ion binding	Ossification, transmembrane receptor protein tyrosine kinase signaling pathway
1-15	P04075	Fructose-bisphosphate aldolase A	39264	341	Cytoskeleton	37%	Fructose-bisphosphate aldolase activity	Fructose metabolic process, glycolysis
	P09972	Fructose-bisphosphate aldolase C	39300	134	Cytoskeleton	13%	Fructose-bisphosphate aldolase activity	Fructose metabolic process
	P00505	Aspartate aminotransferase, mitochondrial precursor	47445	45	Mitochondrion matrix	3%	Aspartate transaminase activity	Fatty acid transport, response to ethanol
1-16	P40925	Malate dehydrogenase, cytoplasmic	36272	40	Cytoplasm	11%	L-malate dehydrogenase activity, malic enzyme activity	
	O00623	Peroxisome assembly protein 12	40771	37	Peroxisome membrane	5%	Zinc ion/protein C-terminus binding	Protein import into peroxisome matrix
	Q9NVP4	Protein C20orf12	62868	33	Intracellular	2%	zinc ion binding	
	P07195	L-lactate dehydrogenase B chain	36484	52	Cytoplasm	5%	L-lactate dehydrogenase activity	
	P10909	Clusterin precursor	52461	42	Secreted	5%	Protein binding	Complement activation, lipid metabolic process

Table 1: Continued.

Serial No.	SwissProt No.	Protein name	MW	Score	Subcellular location	Sequence coverage	Molecular function	Biological process
1-17	P11117	Lysosomal acid phosphatase precursor	48285	44	Lysosome	2%	Acid phosphatase activity	
	P09493	Tropomyosin 1 α chain	32689	59	Cytoplasm	3%	Structural constituent of muscle	Cell motility, regulation of heart/muscle contraction
1-18	P18669	Phosphoglycerate mutase 1	28655	151	Cytosol	16%	Bisphosphoglycerate 2-phosphatase activity	Glycolysis
	P63104	14-3-3 protein zeta/delta	27728	110	Cytoplasm	12%	Transcription factor binding	Antiapoptosis, signal transduction
	O60242	Brain-specific angiogenesis inhibitor 3 precursor	171379	38	Cell membrane	1%		
	O00459	Phosphatidylinositol 3-kinase regulatory subunit β	81574	34	Cytosol	2%	Protein binding	Negative regulation of antiapoptosis
	P46940	Ras GTPase-activating-like protein IQGAP1	189134	34	Cell membrane	2%	GTPase activator/inhibitor activity, calmodulin binding	Signal transduction
1-19	P60174	Triosephosphate isomerase	26522	156	Cytosol	18%	Triose-phosphate isomerase activity	
	P26232	α-2 catenin	105085	34	Cytoplasm	1%	Structural constituent of cytoskeleton	Cell adhesion
1-20	P60174	Triosephosphate isomerase	26522	373	Cytosol	37%	Triose-phosphate isomerase activity	
	Q06495	Sodium-dependent phosphate transport protein 2A	68893	49	Membrane	1%	Sodium-dependent phosphate transmembrane transporter activity, protein binding	Body fluid secretion, phosphate metabolic process and transport
	Q14980	Nuclear mitotic apparatus protein 1	238115	44	Nucleus	4%	Protein binding, structural molecule activity	Mitotic anaphase, nuclear organization and biogenesis
	P78527	DNA-dependent protein kinase catalytic subunit	468788	36	Nucleus	1%	DNA-dependent protein kinase activity/protein binding	Peptidyl-serine phosphorylation
1-21	Q06830	Peroxiredoxin-1	22096	231	Cytoplasm, melanosome	19%	Peroxidase activity/protein binding	Cell proliferation, hydrogen peroxide catabolic process, skeletal development
	P30086	Phosphatidylethanolamine-binding protein 1	20913	122	Cytoplasm	10%	Phosphatidylethanolamine binding/protein binding	
	P16035	Metalloproteinase inhibitor 2 precursor	24383	104	Secreted	13%	Metalloendopeptidase inhibitor activity/protein binding	
	P04179	Superoxide dismutase	24707	66	Mitochondrion matrix	6%	Superoxide dismutase activity	Age-dependent response to reactive oxygen species, regulation of transcription from RNA polymerase II promoter, response to superoxide, superoxide metabolic process
	P53618	Coatomer subunit β	107071	51	Cytoplasm	2%	Protein binding	COPI coating of Golgi vesicle, intra-Golgi vesicle-mediated transport, retrograde vesicle-mediated transport, Golgi to ER
	O43395	U4/U6 small nuclear ribonucleoprotein Prp3	77481	45	Nucleus speckle	3%	Protein binding/RNA splicing factor activity, transesterification mechanism	Nuclear mRNA splicing, via spliceosome

Table 1: Continued.

Serial No.	SwissProt No.	Protein name	MW	Score	Subcellular location	Sequence coverage	Molecular function	Biological process
	O15240	Neurosecretory protein VGF precursor	67247	44	Secreted	7%		Response to cAMP
	Q06495	Sodium-dependent phosphate transport protein 2A	68893	42	Membrane	1%	Protein binding/sodium-dependent phosphate transmembrane transporter activity	Body fluid secretion, phosphate metabolic process, phosphate transport
	Q9Y587	AP-4 complex subunit sigma-1	16994	41	Golgi apparatus	9%	Transporter activity	
	Q04760	Lactoylglutathione lyase	20575	40	Cytoplasm	4%	Lactoylglutathione lyase activity	Antiapoptosis, carbohydrate metabolic process
	P11117	Lysosomal acid phosphatase precursor	48285	40	Lysosome	2%	Acid phosphatase activity	
	Q9P0K1	ADAM 22 precursor	100368	39	Membrane	5%	Integrin binding	Central nervous system development, negative regulation of cell adhesion
	Q96Q42	Alsin	183550	38	Cytosol	1%	Protein homodimerization activity/protein serine/threonine kinase activator activity/Rab GTPase binding/Rac guanyl-nucleotide exchange factor activity	Cell death, endosome organization, neuron projection morphogenesis, positive regulation of Rac GTPase activity, positive regulation of Rac protein signal transduction, positive regulation of protein kinase activity, regulation of endosome size
1-22	Q06830	Peroxiredoxin-1	22096	110	Cytoplasm, Melanosome	21%	Peroxidase activity/protein binding	Cell proliferation, hydrogen peroxide catabolic process, skeletal development
	Q8N1I0	Dedicator of cytokinesis protein 4	225005	41	Intracytoplasmic membrane	1%	PDZ domain binding/Rac GTPase activator activity/Rac GTPase binding	
	P11117	Lysosomal acid phosphatase precursor	48285	39	Lysosome	2%	Acid phosphatase activity	
	O95274	Ly6/PLAUR domain-containing protein 3 precursor	35948	38	Cell membrane	8%		
1-23	P00441	Superoxide dismutase [Cu-Zn]	15795	73	Cytoplasm, cytosol, nucleus	4%	Chaperone/phosphatase 2B/copper ion/zinc ionbinding, protein homodimerization activity, superoxide dismutase activity/antioxidant activity	Cell aging, oxidation reduction, regulation of organ growth, positive regulation of apoptosis
	O15516	Circadian locomoter output cycles protein kaput	95244	55	Cytoplasm, nucleus	1%	Transcription factor activity	Circadian rhythm, photoperiodism, positive regulation of transcription from RNA polymerase II promoter, signal transduction
	O00160	Myosin If	124725	39		2%	Actin binding/ATP binding/calmodulin binding	
	Q13075	Baculoviral IAP repeat-containing protein 1	159479	39	Intracellular	1%	Nucleoside-triphosphatase activity, nucleotide binding, zinc ion binding	Antiapoptosis, nervous system development
	Q6ZUB1	Protein C9orf79	157037	35	Membrane	1%		
1-24	Q9BXM0	Periaxin	154906	35	cytoplasm, nucleus	1%	Protein binding	Axon ensheathment

Table 1: Continued.

Serial No.	SwissProt No.	Protein name	MW	Score	Subcellular location	Sequence coverage	Molecular function	Biological process
	Q15084	Protein disulfide-isomerase A6 precursor	48091	38	Endoplasmic reticulum lumen, melanosome	2%	Protein binding/protein disulfide isomerase activity	Protein folding
	Q13136	Liprin-α-1	135695	42	Cytoplasm	2%	Protein binding/signal transducer activity	Cell-matrix adhesion, signal transduction
	Q32MQ0	Protein ZNF750	77312	44	Intracellular	5%	Zinc ion binding	
	Q15113	Procollagen C-endopeptidase enhancer 1 precursor	47942	48	Secreted	3%		Multicellular organismal development
	P15531	Nucleoside diphosphate kinase A	17138	67	Cytoplasm, nucleus	17%	Deoxyribonuclease activity, DNA/GTP/magnesium ion binding, nucleoside diphosphate kinase activity	Negative regulation of cell proliferation, positive regulation of DNA binding, positive regulation of epithelial cell proliferation, regulation of apoptosis
	P00441	Superoxide dismutase [Cu-Zn]	15795	70	Cytoplasm, cytosol, nucleus	19%	Chaperone/phosphatase 2B/copper ion/zinc ionbinding, protein homodimerization activity, superoxide dismutase activity/antioxidant activity	Cell aging, oxidation reduction, regulation of organ growth, positive regulation of apoptosis
	P23528	Cofilin-1	18360	139	Nucleus matrix, cytoplasm	17%	Protein binding	Actin cytoskeleton organization and biogenesis, antiapoptosis, Rho protein signal transduction
1-25	Q96EZ8	Microspherule protein 1	51771	35	Nucleus	2%	Protein binding	Protein modification process
	O95271	Tankyrase 1	141922	35	Cytoplasm, Golgi apparatus membrane, nucleus	1%	NAD+ ADP-ribosyltransferase activity	Peptidyl-serine phosphorylation, peptidyl-threonine phosphorylation, telomere maintenance via telomerase
	Q14980	Nuclear mitotic apparatus protein 1	238115	35	Nucleus	1%	Protein binding, structural molecule activity	Mitotic anaphase, nuclear organization and biogenesis
	P17612	cAMP-dependent protein kinase, α-catalytic subunit	40433	37	Cytoplasm, nucleus	1%	ATP binding, cAMP-dependent protein kinase activity	Hormone-mediated signaling, protein amino acid phosphorylation, protein kinase cascade
	P11117	Lysosomal acid phosphatase precursor	48285	39	Lysosome	2%	acid phosphatase activity	
	P10071	Zinc finger protein GLI3	169743	39	Nucleus	1%	Protein binding, transcription factor activity	Anatomical structure morphogenesis, multicellular organismal development, protein import into nucleus, translocation, signal transduction
	P78527	DNA-dependent protein kinase catalytic subunit	468788	40	Nucleus	1%	DNA-dependent protein kinase activity	Peptidyl-serine phosphorylation
	Q06495	Sodium-dependent phosphate transport protein 2A	68893	40	Membrane	1%	Protein binding, sodium-dependent phosphate transmembrane transporter activity	Body fluid secretion, phosphate metabolic process, phosphate transport

Table 1: Continued.

Serial No.	SwissProt No.	Protein name	MW	Score	Subcellular location	Sequence coverage	Molecular function	Biological process
	P15531	Nucleoside diphosphate kinase A	17138	86	Cytoplasm, nucleus	17%	Deoxyribonuclease activity, DNA/GTP/magnesium ion binding, nucleoside diphosphate kinase activity	Negative regulation of cell proliferation, positive regulation of DNA binding, positive regulation of epithelial cell proliferation, regulation of apoptosis
	P62937	Peptidyl-prolyl cis-trans isomerase A	17870	422	Cytoplasm	37%	Unfolded protein binding, virion binding	Initiation of viral infection, protein folding, provirus integration, regulation of viral genome replication
1-26	P62937	Peptidyl-prolyl cis-trans isomerase A	17870	171	Cytoplasm	32%	Unfolded protein binding, virion binding	Initiation of viral infection, protein folding, provirus integration, regulation of viral genome replication
	O95352	Autophagy-related protein 7	77909	34	Cytoplasm	2%	Protein homodimerization activity, ubiquitin activating enzyme activity	Membrane fusion, positive regulation of protein modification process, protein amino acid lipidation
	Q9UPT6	C-jun-amino-terminal kinase-interacting protein 3	146962	54	Cytoplasm	2%	kinesin/protein kinase binding, MAP-kinase scaffold activity	Regulation of JNK cascade, vesicle-mediated transport
1-27	Q8IWJ2	GRIP and coiled-coil domain-containing protein 2	184545	38	Cytoplasm, Golgi apparatus membrane	1%	Identical protein binding	
	Q9NX63	Coiled-coil-helix-coiled-coil-helix domain-containing protein 3	26136	39	Mitochondrion	13%	Protein binding	
	P02452	Collagen α-1(I) chain precursor	138799	44	Secreted	3%	Protein binding	Epidermis development, skeletal development
	P59998	Actin-related protein 2/3 complex subunit 4	19523	45	Cytoplasm	2%	Protein binding, structural constituent of cytoskeleton	Actin nucleation
	P01034	Cystatin C precursor	15789	56	Secreted	7%	Cysteine protease inhibitor activity, protein homodimerization activity	
	P23528	Cofilin-1	18360	178	Nucleus matrix, cytoplasm	19%	Protein binding	Actin cytoskeleton organization and biogenesis, antiapoptosis, Rho protein signal transduction
	P07737	Profilin-1	14914	478	Cytoplasm	45%	Actin binding, proline-rich region binding	Actin cytoskeleton organization
1-28	P09382	Galectin-1	14575	41	Cytoplasm	7%	Protein binding, signal transducer activity	Positive regulation of I-κB kinase, NF-κB cascade, regulation of apoptosis
	P10599	Thioredoxin	11599	59	Cytoplasm	13%	Protein binding	Cell motility, cell proliferation, cell-cell signaling, signal transduction

Table 1: Continued.

Serial No.	SwissProt No.	Protein name	MW	Score	Subcellular location	Sequence coverage	Molecular function	Biological process
	P04080	Cystatin B	11133	61	Cytoplasm, nucleus	12%	Endopeptidase inhibitor activity, protein binding	
	P07737	Profilin-1	14914	320	Cytoplasm	42%	Actin binding, proline-rich region binding	Actin cytoskeleton organization
1-29	Q9UPQ9	Trinucleotide repeat-containing 6B protein	182703	34	Cytoplasmic mRNA processing body	4%	RNA binding, nucleotide binding	Gene silencing by RNA, regulation of translation
	P78527	DNA-dependent protein kinase catalytic subunit	468788	35	Nucleus	1%	DNA-dependent protein kinase activity, protein binding	Peptidyl-serine phosphorylation
	P81605	Dermcidin precursor	11277	45	Secreted	12%	Protein binding	
	P10599	Thioredoxin	11599	76	Cytoplasm	19%	Protein binding	Cell motility, cell proliferation, cell-cell signaling, signal transduction
	Q6NUM9	All-trans-retinol 13,14-reductase precursor	66777	39	Endoplasmic reticulum membrane	3%	All-trans-retinol 13,14-reductase activity	Oxidation reduction, retinol metabolic process
1-30	P14174	Macrophage migration inhibitory factor	12337	39	Cytoplasm, secreted	11%	Cytokine activity	Cell proliferation, cell surface receptor linked signal transduction, negative regulation of apoptosis, prostaglandin biosynthetic process, regulation of macrophage activation
	Q6KC79	Nipped-B-like protein	315854	36	Nucleus	1%	Protein C-terminus binding	Maintenance of mitotic sister chromatid cohesion
	P82279	Crumbs homolog 1 precursor	154081	36	Secreted	1%	Calcium ion binding	Cell-cell signaling, establishment and/or maintenance of cell polarity, response to stimulus
	Q9NX63	Coiled-coil-helix-coiled-coil-helix domain-containing protein 3	26136	39	Mitochondrion	13%	Protein binding	
	Q8N0X7	Spartin	72788	41		2%		Cell death
	P14174	Macrophage migration inhibitory factor	12337	60	Cytoplasm, secreted	5%	Cytokine activity	Cell proliferation, cell surface receptor linked signal transduction, negative regulation of apoptosis, prostaglandin biosynthetic process, regulation of macrophage activation
	P58546	Myotrophin	12756	71	Cytoplasm	7%	Protein binding	Cell growth, neuron differentiation
	Q8WUT4	Uncharacterized protein C20orf75 precursor	78794	37	Membrane	5%	Protein binding	
1-31	O94851	Protein MICAL-2	126609	40	Cytoplasm	1%	Monooxygenase activity, zinc ion binding	Metabolic process
	Q9Y333	U6 snRNA-associated Sm-like protein LSm2	10828	69	Nucleus	20%	Protein/U6 snRNA binding	Nuclear mRNA splicing

These serial numbers are designated as in Figure 3.
Swiss-Prot/TrEMBL accession number was given from http://us.expasy.org/.

Table 2: The functions of 132 proteins identified in this study were presented into functional categories based on their annotations in the GO database.

SwissProt No.	Protein name	Protein function
O00160	Myosin If	Myosins are actin-based motor molecules with ATPase activity. Unconventional myosins serve in intracellular movements
O00339	Matrilin-2 precursor	Involved in matrix assembly
O00459	Phosphatidylinositol 3-kinase regulatory subunit β	Binds to activated (phosphorylated) protein-tyrosine kinases, through its SH2 domain, and acts as an adapter, mediating the association of the p110 catalytic unit to the plasma membrane.
O00469	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2 precursor	Forms hydroxylysine residues in -Xaa-Lys-Gly- sequences in collagens. These hydroxylysines serve as sites of attachment for carbohydrate units and are essential for the stability of the intermolecular collagen cross-links
O00623	Peroxisome assembly protein 12	Required for protein import into peroxisomes
O14686	Myeloid/lymphoid or mixed-lineage leukemia protein 2	Histone methyltransferase. Methylates "Lys-4" of histone H3. H3 "Lys-4" methylation represents a specific tag for epigenetic transcriptional activation. Plays a central role in β-globin locus transcription regulation by being recruited by NFE2. Acts as a coactivator for estrogen receptor by being recruited by ESR1, thereby activating transcription
O15020	Spectrin β chain, brain 2	Probably plays an important role in neuronal membrane skeleton
O15240	Neurosecretory protein VGF precursor	May be involved in the regulation of cell-cell interactions or in synaptogenesis during the maturation of the nervous system
O15516	Circadian locomotors output cycles protein kaput	ARNTL/2-CLOCK heterodimers activate E-box element (3′-CACGTG-5′) transcription of a number of proteins of the circadian clock. Activates transcription of PER1 and PER2. This transcription is inhibited in a feedback loop by PER and CRY proteins. Has intrinsic histone acetyltransferase activity and this enzymatic function contributes to chromatin-remodeling events implicated in circadian control of gene expression
O43395	U4/U6 small nuclear ribonucleoprotein Prp3	Participates in pre-mRNA splicing. May play a role in the assembly of the U4/U5/U6 tri-snRNP complex
O43707	α-actinin-4	F-actin cross-linking protein which is thought to anchor actin to a variety of intracellular structures. This is a bundling protein
O60242	Brain-specific angiogenesis inhibitor 3 precursor	Might be involved in angiogenesis inhibition and suppression of glioblastoma
O60333	Kinesin-like protein KIF1B	Motor for anterograde transport of mitochondria. Has a microtubule plus end-directed motility
O75095	Multiple epidermal growth factor-like domains 6 precursor	
O94851	Protein MICAL-2	
O94985	Calsyntenin-1 precursor	Induces KLC1 association with vesicles and functions as a cargo in axonal anterograde transport. Complex formation with APBA2 and APP, stabilizes APP metabolism and enhances APBA2-mediated suppression of β-APP40 secretion, due to the retardation of intracellular APP maturation. In complex with APBA2 and C99, a C-terminal APP fragment, abolishes C99 interaction with PSEN1 and thus APP C99 cleavage by γ-secretase, most probably through stabilization of the direct interaction between APBA2 and APP. The intracellular fragment AlcICD suppresses APBB1-dependent transactivation stimulated by APP C-terminal intracellular fragment (AICD), most probably by competing with AICD for APBB1-binding. May modulate calcium-mediated postsynaptic signals

Table 2: Continued.

SwissProt No.	Protein name	Protein function
O95239	Chromosome-associated kinesin KIF4A	Motor protein that translocates PRC1 to the plus ends of interdigitating spindle microtubules during the metaphase to anaphase transition, an essential step for the formation of an organized central spindle midzone and midbody and for successful cytokinesis. May play a role in mitotic chromosomal positioning and bipolar spindle stabilization
O95248	SET-binding factor 1	Probable pseudophosphatase. Lacks several amino acids in the catalytic pocket which renders it catalytically inactive as a phosphatase. The pocket is, however, sufficiently preserved to bind phosphorylated substrates, and may be protect them from phosphatases. Inhibits myoblast differentiation *in vitro* and induces oncogenic transformation in fibroblasts
O95271	Tankyrase 1	Regulate vesicle trafficking and modulate the subcellular distribution of SLC2A4/GLUT4-vesicles. Has PARP activity and can modify TERF1, and thereby contribute to the regulation of telomere length
O95274	Ly6/PLAUR domain-containing protein 3 precursor	Supports cell migration. May be involved in urothelial cell-matrix interactions. May be involved in tumor progression
O95352	Autophagy-related protein 7	E1 enzyme essential for multisubstrates such as GABARAPL1 and ATG12
P00390	Glutathione reductase, mitochondrial precursor	Maintains high levels of reduced glutathione in the cytosol
P00441	Superoxide dismutase (Cu-Zn)	Destroys radicals which are normally produced within the cells and which are toxic to biological systems
P00505	Aspartate aminotransferase, mitochondrial precursor	Facilitates cellular uptake of long-chain free fatty acids
P01024	Complement C3 precursor	C3 plays a central role in the activation of the complement system. Its processing by C3 convertase is the central reaction in both classical and alternative complement pathways. After activation C3b can bind covalently, via its reactive thioester, to cell surface carbohydrates or immune aggregates. Derived from proteolytic degradation of complement C3, C3a anaphylatoxin is a mediator of local inflammatory process. It induces the contraction of smooth muscle, increases vascular permeability, and causes histamine release from mast cells and basophilic leukocytes
P01034	Cystatin C precursor	As an inhibitor of cysteine proteinases, this protein is thought to serve an important physiological role as a local regulator of this enzyme activity
P02452	Collagen α-1(I) chain precursor	Type I collagen is a member of group I collagen (fibrillar forming collagen)
P02686	Myelin basic protein	The classic group of MBP isoforms (isoform 4-isoform 14) are with PLP the most abundant protein components of the myelin membrane in the CNS. They have a role in both its formation and stabilization. The smaller isoforms might have an important role in remyelination of denuded axons in multiple sclerosis. The nonclassic group of MBP isoforms (isoform 1-isoform 3/Golli-MBPs) may preferentially have a role in the early developing brain long before myelination, maybe as components of transcriptional complexes, and may also be involved in signaling pathways in T-cells and neural cells. Differential splicing events combined with optional posttranslational modifications give a wide spectrum of isomers, with each of them potentially having a specialized function. Induces T-cell proliferation
P02751	Fibronectin precursor	Fibronectins bind cell surfaces and various compounds including collagen, fibrin, heparin, DNA, and actin. Fibronectins are involved in cell adhesion, cell motility, opsonization, wound healing, and maintenance of cell shape. Interaction with TNR mediates inhibition of cell adhesion and neurite outgrowth
P02768	Serum albumin precursor	Serum albumin, the main protein of plasma, has a good binding capacity for water, Ca(2+), Na(+), K(+), fatty acids, hormones, bilirubin, and drugs. Its main function is the regulation of the colloidal osmotic pressure of blood

Table 2: Continued.

SwissProt No.	Protein name	Protein function
P04075	Fructose-bisphosphate aldolase A	
P04080	Cystatin B	Intracellular thiol proteinase inhibitor
P04179	Superoxide dismutase	Destroys radicals which are normally produced within the cells and which are toxic to biological systems
P06733	α-enolase	Multifunctional enzyme that, as well as its role in glycolysis, plays a part in various processes such as growth control, hypoxia tolerance, and allergic responses. May also function in the intravascular and pericellular fibrinolytic system due to its ability to serve as a receptor and activator of plasminogen on the cell surface of several cell-types such as leukocytes and neurons. May be a tumor suppressor
P06744	Glucose-6-phosphate isomerase	Neuroleukin is a neurotrophic factor for spinal and sensory neurons
P07195	L-lactate dehydrogenase B chain	
P07737	Profilin-1	Binds to actin and affects the structure of the cytoskeleton. At high concentrations, profilin prevents the polymerization of actin, whereas it enhances it at low concentrations. By binding to PIP2, it inhibits the formation of IP3 and DG
P07814	Bifunctional aminoacyl-tRNA synthetase	Catalyzes the attachment of the cognate amino acid to the corresponding tRNA in a two-step reaction: the amino acid is first activated by ATP to form a covalent intermediate with AMP and is then transferred to the acceptor end of the cognate tRNA
P07900	Heat shock protein HSP 90-α	Molecular chaperone. Has ATPase activity
P07996	Thrombospondin-1 precursor	Adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions. Can bind to fibrinogen, fibronectin, laminin, type V collagen, and integrins α-V/ β-1, α-V/ β-3 and α-IIb/ β-3
P08238	Heat shock protein HSP 90- β	Molecular chaperone. Has ATPase activity.
P09104	γ-enolase	Has neurotrophic and neuroprotective properties on a broad spectrum of central nervous system (CNS) neurons. Binds, in a calcium-dependent manner, to cultured neocortical neurons and promotes cell survival
P09382	Galectin-1	Cell apoptosis and cell differentiation
P09486	SPARC precursor	Appears to regulate cell growth through interactions with the extracellular matrix and cytokines. Binds calcium and copper, several types of collagen, albumin, thrombospondin, PDGF and cell membranes. There are two calcium binding sites; an acidic domain that binds 5 to 8 Ca(2+) with a low affinity and an EF-hand loop that binds a Ca(2+) ion with a high affinity
P09493	Tropomyosin 1 α chain	Binds to actin filaments in muscle and nonmuscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments
P09622	Dihydrolipoyl dehydrogenase, mitochondrial precursor	Lipoamide dehydrogenase is a component of the glycine cleavage system as well as of the α-ketoacid dehydrogenase complexes
P09972	Fructose-bisphosphate aldolase C	
P10071	Zinc finger protein GLI3	A role in limb and brain development
P10599	Thioredoxin	Participates in various redox reactions through the reversible oxidation of its active center dithiol to a disulfide and catalyzes dithiol-disulfide exchange reactions

Table 2: Continued.

SwissProt No.	Protein name	Protein function
P10909	Clusterin precursor	Not yet clear. It is known to be expressed in a variety of tissues, and it seems to be able to bind to cells, membranes, and hydrophobic proteins. It has been associated with programmed cell death (apoptosis)
P11047	Laminin γ-1 chain precursor	Binding to cells via a high-affinity receptor, laminin is thought to mediate the attachment, migration, and organization of cells into tissues during embryonic development by interacting with other extracellular matrix components
P11117	Lysosomal acid phosphatase precursor	
P12814	α-actinin-1	F-actin cross-linking protein which is thought to anchor actin to a variety of intracellular structures. This is a bundling protein
P13569	Cystic fibrosis transmembrane conductance regulator	Involved in the transport of chloride ions. May regulate bicarbonate secretion and salvage in epithelial cells by regulating the SLC4A7 transporter
P13929	β-enolase	Appears to have a function in striated muscle development and regeneration
P14136	Glial fibrillary acidic protein, astrocyte	GFAP, a class-III intermediate filament, is a cell-specific marker that, during the development of the central nervous system, distinguishes astrocytes from other glial cells
P14174	Macrophage migration inhibitory factor	Mediator in regulating the function of macrophage in host defense
P14618	Pyruvate kinase isozymes M1/M2	Glycolytic enzyme that catalyzes the transfer of a phosphoryl group from phosphoenolpyruvate (PEP) to ADP, generating ATP
P15531	Nucleoside diphosphate kinase A	Synthesis of nucleoside triphosphates other than ATP
P16035	Metalloproteinase inhibitor 2 precursor	Complexes with metalloproteinases (such as collagenases) and irreversibly inactivates them. Known to act on MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-13, MMP-14, MMP-15, MMP-16, and MMP-19
P17612	cAMP-dependent protein kinase, α-catalytic subunit	Phosphorylates a large number of substrates in the cytoplasm and the nucleus
P18669	Phosphoglycerate mutase 1	Interconversion of 3- and 2-phosphoglycerate with 2,3-bisphosphoglycerate as the primer of the reaction. Can also catalyze the reaction of synthase and phosphatase, but with a reduced activity
P23528	Cofilin-1	Controls reversibly actin polymerization and depolymerization
P26232	α-2 catenin	
P27797	Calreticulin precursor	Molecular calcium binding chaperone promoting folding, oligomeric assembly and quality control in the ER via the calreticulin/calnexin cycle. This lectin interacts transiently with almost all of the monoglucosylated glycoproteins that are synthesized in the ER. Interacts with the DNA-binding domain of NR3C1 and mediates its nuclear export
P29400	Collagen α-5(IV) chain precursor	Type IV collagen is the major structural component of glomerular basement membranes (GBM), forming a "chicken-wire" meshwork together with laminins, proteoglycans and entactin/nidogen
P29401	Transketolase	
P30086	Phosphatidylethanolamine-binding protein 1	Binds ATP, opioids, and phosphatidylethanolamine. Has lower affinity for phosphatidylinositol and phosphatidylcholine. Serine protease inhibitor which inhibits thrombin, neuropsin, and chymotrypsin but not trypsin, tissue type plasminogen activator, and elastase/HCNP may be involved in the function of the presynaptic cholinergic neurons of the central nervous system. HCNP increases the production of choline acetyltransferase but not acetylcholinesterase. Seems to be mediated by a specific receptor
P30154	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A β isoform	The PR65 subunit of protein phosphatase 2A serves as a scaffolding molecule to coordinate the assembly of the catalytic subunit and a variable regulatory B subunit
P34932	Heat shock 70 kDa protein 4	

Table 2: Continued.

SwissProt No.	Protein name	Protein function
P35442	Thrombospondin-2 precursor	Adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions. Can bind to fibrinogen, fibronectin, laminin, and type V collagen
P35573	Glycogen debranching enzyme	Multifunctional enzyme acting as 1,4-α-D-glucan:1,4-α-D-glucan 4-α-D-glycosyltransferase and amylo-1,6-glucosidase in glycogen degradation
P35609	α-actinin-2	F-actin cross-linking protein which is thought to anchor actin to a variety of intracellular structures. This is a bundling protein
P35711	Transcription factor SOX-5	Binds specifically to the DNA sequence 5′-AACAAT-3′. Activates transcription of COL2A1 and AGC1 *in vitro*
P37268	Squalene synthetase	
P39191	Alu subfamily SB2 sequence contamination warning entry	
P40925	Malate dehydrogenase, cytoplasmic	
P46940	Ras GTPase-activating-like protein IQGAP1	Binds to activated CDC42 but does not stimulate its GTPase activity. It associates with calmodulin. Could serve as an assembly scaffold for the organization of a multimolecular complex that would interface incoming signals to the reorganization of the actin cytoskeleton at the plasma membrane. May promote neurite outgrowth
P49746	Thrombospondin-3 precursor	Adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions. Can bind to fibrinogen, fibronectin, laminin, and type V collagen
P50395	Rab GDP dissociation inhibitor β	Regulates the GDP/GTP exchange reaction of most Rab proteins by inhibiting the dissociation of GDP from them, and the subsequent binding of GTP to them
P53618	Coatomer subunit β	The coatomer is a cytosolic protein complex that binds to dilysine motifs and reversibly associates with Golgi nonclathrin-coated vesicles, which further mediate biosynthetic protein transport from the ER, via the Golgi up to the trans Golgi network. Coatomer complex is required for budding from Golgi membranes, and is essential for the retrograde Golgi-to-ER transport of dilysine-tagged proteins. In mammals, the coatomer can only be recruited by membranes associated to ADP-ribosylation factors (ARFs), which are small GTP-binding proteins; the complex also influences the Golgi structural integrity, as well as the processing, activity, and endocytic recycling of LDL receptors
P58546	Myotrophin	Cerebellar morphogenesis
P59998	Actin-related protein 2/3 complex subunit 4	Actin-binding component of the Arp2/3 complex which is involved in regulation of actin polymerization and together with an activating nucleation-promoting factor (NPF) mediates the formation of branched actin networks
P60174	Triosephosphate isomerase	
P62937	Peptidyl-prolyl cis-trans isomerase A	PPIases accelerate the folding of proteins
P63104	14-3-3 protein zeta/delta	Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathway. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner

Table 2: Continued.

SwissProt No.	Protein name	Protein function
P78527	DNA-dependent protein kinase catalytic subunit	Serine/threonine-protein kinase that acts as a molecular sensor for DNA damage. Involved in DNA nonhomologous end joining (NHEJ) required for double-strand break (DSB) repair and V(D)J recombination. Must be bound to DNA to express its catalytic properties. Promotes processing of hairpin DNA structures in V(D)J recombination by activation of the hairpin endonuclease artemis (DCLRE1C). The assembly of the DNA-PK complex at DNA ends is also required for the NHEJ ligation step. Required to protect and align broken ends of DNA. May also act as a scaffold protein to aid the localization of DNA repair proteins to the site of damage. Found at the ends of chromosomes, suggesting a further role in the maintenance of telomeric stability and the prevention of chromosomal end fusion. Also involved in modulation of transcription. Recognizes the substrate consensus sequence (ST)-Q. Phosphorylates "Ser-139" of histone variant H2AX/H2AFX, thereby regulating DNA damage response mechanism. Phosphorylates DCLRE1C, c-Abl/ABL1, histone H1, HSPCA, c-jun/JUN, p53/TP53, PARP1, POU2F1, DHX9, SRF, XRCC1, XRCC1, XRCC4, XRCC5, XRCC6, WRN, c-myc/MYC, and RFA2. Can phosphorylate C1D not only in the presence of linear DNA but also in the presence of supercoiled DNA. Ability to phosphorylate TP53/p53 in the presence of supercoiled DNA is dependent on C1D
P81274	G-protein signaling modulator 2	Plays an important role in spindle pole orientation. Interacts and contributes to the functional activity of G(i) α proteins. Acts to stabilize the apical complex during neuroblast divisions
P81605	Dermcidin precursor	DCD-1 displays antimicrobial activity thereby limiting skin infection by potential pathogens in the first few hours after bacterial colonization. Highly effective against *E. coli*, *E. faecalis*, *S. aureus* and *C. albicans*. Optimal pH and salt concentration resemble the conditions in sweat.
P82279	Crumbs homolog 1 precursor	Survival-promoting peptide promotes survival of neurons and displays phosphatase activity. It may bind IgG Photoreceptor morphogenesis in the retina/May maintain cell polarization and adhesion
Q04760	Lactoylglutathione lyase	Catalyzes the conversion of hemimercaptal, formed from methylglyoxal and glutathione, to S-lactoylglutathione
Q06495	Sodium-dependent phosphate transport protein 2A	May be involved in actively transporting phosphate into cells via Na(+) cotransport in the renal brush border membrane. Probably mediates 70–80% of the apical influx
Q06830	Peroxiredoxin-1	Involved in redox regulation of the cell. Reduces peroxides with reducing equivalents provided through the thioredoxin system but not from glutaredoxin. May play an important role in eliminating peroxides generated during metabolism. Might participate in the signaling cascades of growth factors and tumor necrosis factor-α by regulating the intracellular concentrations of H_2O_2
Q08378	Golgin subfamily A member 3	Golgi autoantigen; probably involved in maintaining Golgi structure
Q08380	Galectin-3-binding protein precursor	Promotes integrin-mediated cell adhesion. May stimulate host defense against viruses and tumor cells
Q12799	T-complex protein 10A homolog	
Q12841	Follistatin-related protein 1 precursor	May modulate the action of some growth factors on cell proliferation and differentiation. Binds heparin

Table 2: Continued.

SwissProt No.	Protein name	Protein function
Q13045	Protein flightless-1 homolog	May play a role as coactivator in transcriptional activation by hormone-activated nuclear receptors (NR) and acts in cooperation with NCOA2 and CARM1. Involved in estrogen hormone signaling. Involved in early embryonic development (By similarity). May play a role in regulation of cytoskeletal rearrangements involved in cytokinesis and cell migration
Q13075	Baculoviral IAP repeat-containing protein 1	Prevents motor-neuron apoptosis induced by a variety of signals
Q13136	Liprin-α-1	Regulate the disassembly of focal adhesions/may localize receptor-like tyrosine phosphatases type 2A at specific sites on the plasma membrane, possibly regulating their interaction with the extracellular environment and their association with substrates
Q13371	Phosducin-like protein	
Q13740	CD166 antigen precursor	Cell adhesion molecule that binds to CD6. Involved in neurite extension by neurons via heterophilic and homophilic interactions. May play a role in the binding of T- and B-cells to activated leukocytes, as well as in interactions between cells of the nervous system
Q14980	Nuclear mitotic apparatus protein 1	May be a structural component of the nucleus
Q15084	Protein disulfide-isomerase A6 precursor	Catalyzes the rearrangement of -S-S- bonds in proteins
Q15113	Procollagen C-endopeptidase enhancer 1 precursor	Binds to the C-terminal propeptide of type I procollagen and enhances procollagen C-proteinase activity
Q32MQ0	Protein ZNF750	
Q5VTR2	Ubiquitin-protein ligase BRE1A	E3 ubiquitin-protein ligase that mediates monoubiquitination of "Lys-120" of histone H2B. H2B "Lys-120" ubiquitination gives a specific tag for epigenetic transcriptional activation and is also prerequisite for histone H3 "Lys-4" and "Lys-79" methylation. Forms a ubiquitin ligase complex in cooperation with the E2 enzyme UBE2E1/UBCH6. It thereby plays a central role in histone code and gene regulation. Required for transcriptional activation of Hox genes. Recruited to the MDM2 promoter, probably by being recruited by p53/TP53, and thereby acts as a transcriptional coactivator
Q6KC79	Nipped-B-like protein	Probably plays a structural role in chromatin. Involved in sister chromatid cohesion, possibly by interacting with the cohesin complex
Q6NUM9	All-trans-retinol 13,14-reductase precursor	Play a role in the metabolism of vitamin A
Q6ZU80	Protein C14orf145	
Q6ZUB1	Protein C9orf79	
Q7L1I2	Synaptic vesicle glycoprotein 2B	Probably plays a role in the control of regulated secretion in neural and endocrine cells
Q7Z3E2	Protein C10orf118	
Q8IWJ2	GRIP and coiled-coil domain-containing protein 2	Function probably involved in maintaining Golgi structure
Q8N0X7	Spartin	May be implicated in endosomal trafficking, or microtubule dynamics, or both.
Q8N1I0	Dedicator of cytokinesis protein 4	Involved in regulation of adherens junction between cells. Functions as a guanine nucleotide exchange factor (GEF), which activates Rap1 small GTPase by exchanging bound GDP for free GTP
Q8TEU7	Rap guanine nucleotide exchange factor 6	Guanine nucleotide exchange factor (GEF) for Rap1A, Rap2A, and M-Ras GTPases. Does not interact with cAMP

Table 2: Continued.

SwissProt No.	Protein name	Protein function
Q8TF76	Serine/threonine-protein kinase Haspin	Required for normal alignment of chromosomes at metaphase. Phosphorylates histone H3 "Thr-3" during mitosis
Q8WUT4	Uncharacterized protein C20orf75 precursor	May play an important role in hippocampus-dependent long-lasting memory
Q96EZ8	Microspherule protein 1	Modulates the transcription repressor activity of DAXX by recruiting it to the nucleolus. May be an inhibitor of TERT telomerase activity
Q96Q42	Alsin	May act as a GTPase regulator. Controls survival and growth of spinal motoneurons
Q9BXM0	Periaxin	Is required for maintenance of peripheral nerve myelin sheath/may have a role in axon-glial interactions
Q9NVP4	Protein C20orf12	
Q9NX63	Coiled-coil-helix-coiled-coil-helix domain-containing protein 3	
Q9P0K1	ADAM 22 precursor	Probable ligand for integrin in the brain. This is a noncatalytic metalloprotease-like protein. Involved in regulation of cell adhesion and spreading and in inhibition of cell proliferation
Q9UM47	Neurogenic locus notch homolog protein 3 precursor	Functions as a receptor for membrane-bound ligands Jagged1, Jagged2, and Delta1 to regulate cell-fate determination. Upon ligand activation through the released notch intracellular domain (NICD) it forms a transcriptional activator complex with RBP-Jκ and activates genes of the enhancer of split locus. Affects the implementation of differentiation, proliferation, and apoptotic programs
Q9UM54	Myosin-6	Myosins are actin-based motor molecules with ATPase activity. Unconventional myosins serve in intracellular movements. Myosin 6 is a reverse-direction motor protein that moves towards the minus-end of actin filaments. Has slow rate of actin-activated ADP release due to weak ATP binding. Functions in a variety of intracellular processes such as vesicular membrane trafficking and cell migration. Required for the structural integrity of the Golgi apparatus via the p53-dependent prosurvival pathway. Appears to be involved in a very early step of clathrin-mediated endocytosis in polarized epithelial cells. May act as a regulator of F-actin dynamics. May play a role in transporting DAB2 from the plasma membrane to specific cellular targets. Required for structural integrity of inner ear hair cells
Q9UPQ9	Trinucleotide repeat-containing 6B protein	Plays a role in RNA-mediated gene silencing by both micro-RNAs (miRNAs) and short interfering RNAs (siRNAs). Required for miRNA-dependent translational repression and siRNA-dependent endonucleolytic cleavage of complementary mRNAs by argonaute family proteins
Q9UPT6	C-jun-amino-terminal kinase-interacting protein 3	Mediates JNK signaling by aggregating specific components of the MAPK cascade to form a functional JNK signaling module. May function as a regulator of vesicle transport, through interactions with the JNK-signaling components and motor proteins
Q9UQ26	Regulating synaptic membrane exocytosis protein 2	Rab effector involved in exocytosis. May act as scaffold protein.
Q9Y333	U6 snRNA-associated Sm-like protein LSm2	Binds specifically to the 3′-terminal U-tract of U6 snRNA. May be involved in pre-mRNA splicing
Q9Y587	AP-4 complex subunit sigma-1	Subunit of novel type of clathrin- or nonclathrin-associated protein coat involved in targeting proteins from the trans-Golgi network (TGN) to the endosomal-lysosomal system

* sort by SwissProt No.

roles for ECM proteins in cell growth and differentiation can be indicated by their abilities to modulate a variety of growth factors [33].

Thrombospondin (TSP, MW~420 kDa), which belongs to a multigene family of modular modular glycoproteins, is composed of three identical subunits within a disulfide linkage. TSP is synthesized by several matrix-forming cells and is incorporated into their extracellular matrix. In several cell types, this protein supports cell growth and proliferation. As a component of ECM, TSP is involved in the regulation of mediate platelet aggregation, inflammation, and angiogenesis as well as adhesion, migration, growth, and differentiation of a number of normal and transformed cells [34, 35]. The expression of the TSP has been also investigated during the process of differentiation of embryonal carcinoma cells, granulose cells and HL-60 cells *in vitro* [36–39]. Although the TSP is prevalent in differentiated cells, the induced TSP syntheses during the differentiation may function differently during neurogenesis.

In the eye, TSP-1 is localized in the epiretinal membrane and between the retinal pigment epithelial layer and Bruch's membrane, which is a cell-attachment factor with cell-specific affinity. TSP-1 production by retinal pigment epithelial cells is affected by the state of proliferation and cell density. With its anti-angiogenic activity, TSP-1 may play several biologic roles on Bruch's membrane [35]. In another report, the authors evaluated the bone marrow stromal cells (BMSCs) secretion of TSP-1, which is a putative mechanistic agent acting on RGCs for survival and growth [40]. The BMSC-derived TSP-1 is identified as a specific mediator of reparative processes in neurons, which functions included enhanced RGC neurite formation, cell survival, and expression of synaptophysin. It suggested that the TSP-1 signaling pathway might be an important role in neural-like differentiation in BMSCs and outgrowth in RGCs [40]. These observations suggest that the synthesis of TSP contributes to the differentiation options/alternatives of RGC-5 cells toward a neural fate, reminiscent of their neural crest origin.

TSP-2 and SPARC (secreted protein, acidic and rich in cysteine) are classified as matricellular proteins. TSP-2 appears to play a role in reducing proliferation, while SPARC may have a positive role in progenitor cell expansion. TSP-2 and SPARC have been shown to positively influence osteoblast differentiation, with the ability to limit adipogenesis [41, 42].

TSP-3 is structurally similar to cartilage oligomeric matrix protein (COMP/TSP-5), and was a recently described member with the calcium binding Type 3 repeats. Like Type 1 and 2 repeats, TSP-3 is absence of the complement and contains four epidermal growth factor receptors with a distinct N terminus that has no significant homology to other TSPs. TSP-3 is also an oligomeric heparin binding protein present in both the cell layer and medium [43].

Galectin-3-binding protein (G3BP), also known as Mac-2 binding protein, is a secreted glycoprotein with a molecular mass of ~90 kDa present in the extracellular matrix of cells. Gelectins and their binding proteins have primarily been described in cell-cell and cell-matrix interactions and play roles in autoimmunity, inflammation and tumor progression or metastasis [44]. G3BP promotes integrin-mediated cell adhesion and functions in cancer progression of human tumor cells. It also binds to multiple proteins in the extracellular matrix including collagen, fibronectin, and nidogen, and to molecules mediating cell-cell and cell-matrix adhesions that are critical during tumor cell invasion and migration [45–48].

Notch-3 was the third discovered human homologue of the Drosophila melanogaster type I membrane protein notch. In Drosophila, the interaction of notch with its cell-bound ligands (delta and serrate) establishes an intercellular signaling pathway that plays a key role in neural development. Members of the Notch gene family were thought to be involved as receptors for membrane-bound ligands Jagged1, Jagged2, and Delta1 in the regulation of cell fate in a variety of neurogenesis of embryos, particularly in the developing central nervous system (CNS) from the homogenous cell population of the neural tube [49, 50]. The Notch-3 activation induces the increase of the progenitor cell number in the CNS and affected CNS development. The Notch-3 mutation may lead to cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). CADASIL leads to stroke and dementia and is the main feature of recurrent subcortical ischemic events and vascular dementia. Such mutations affect highly-conserved cysteine residues in epidermal growth factor- (EGF-) like repeat domain in the extracellular part of the receptor [51, 52].

Follistatin-related protein (FSRP) is a recently discovered glycoprotein that is highly homologous in both primary sequence and exon/intron domain structure to the activin-binding protein, follistatin (FS). FS is a secreted monomeric glycoprotein and a member of a large group of proteins containing a highly conserved module of cysteine-rich sequence termed the follistatin domain. It was first isolated from ovarian follicular fluid on the basis of its ability to suppress FSH secretion by pituitary cells *in vitro* [53]. This follistatin gene family includes follistatin, follistatin-related gene (FLRG) protein, follistatin-related protein (FSRP), agrin, secreted protein acidic, and it is rich in cysteine (SPARC), and Mac25 [54]. A follistatin-like sequence containing 10 conserved cysteine residues may modulate the action of some growth factors on cell proliferation and differentiation. It was also thought to be an autoantigen associated with rheumatoid arthritis [55].

SPARC, also known as osteonectin, 43 K protein, or BM-40, is a 32.7 kDa calcium- and copper-binding glycoprotein, which is a product of natural synthesis from osteoblasts, endothelial cells, and megakaryocytes. It functions as a counteradhesive protein, as a modulator of growth factor activity, and as a cell-cycle inhibitor [56]. SPARC belongs to matrix-associated factors that mediate cell-matrix interactions. Other members of this group include TSP-1 and -2, osteopontin (OPN), tenascins, and the SPARC-related proteins. Expressed during many stages of development in a variety of organisms, the expression of this matricellular protein, SPARC, is restricted in adult vertebrates primarily to tissues that undergo consistent turnover or to sites of injuries and diseases [56]. Vertebrate SPARC binds to a number of different ECM components

including albumin, thrombospondin 1, PDGF, vitronectin, entactin/nidogen, fibrillar collagens (types I, II, III, and V), and collagen type IV, the prevalent collagen in basement membranes [57]. The ability of SPARC to bind to several resident ECM proteins affects the expression of matrix metalloproteinases and adjusts effects of growth factors; as a counteradhesive factor of cell shape change, this supports SPARC to regulate cell interactions during their development [57]. SPARC appears to regulate cell growth through interactions with the extracellular matrix and cytokines. It is also a matricellular protein that modulates cell adhesion and proliferation and is thought to function in tissue remodeling and angiogenesis [58, 59].

Peroxiredoxin (PRDX) is a recently identified family of antioxidative proteins that includes six isoforms in mammals. They share a common reactive Cys residue in the N-terminal region and are capable of serving as a peroxidase, involving thioredoxin and/or glutathione as the electron donor. PRDX 1–4 have an additional reactive Cys residue in the conserved C-terminal region and show >70% amino acid sequence homology. In this capacity, they may be involved in the protection of cells from oxidative stress. Peroxiredoxin1 (PRDX1) is ubiquitously expressed and functions as an antioxidant enzyme, which reduces hydrogen peroxide and alkyl hydroperoxide and is involved in cellular proliferation, differentiation, apoptosis, and innate immunity [60]. PRDX1 may participate in the signal cascades of growth factors and tumor necrosis factor-α by regulating the intracellular concentrations of hydrogen peroxide [61–63]. A previous study also applied a proteomic approach to study PRDX1, -2, and -3 expressions in Alzheimer's diseases and Down's syndrome, and found a significant increase in PRDX1 expression associated with the neurodegenerative diseases [64].

The human cofilin protein has a molecular weight of approximately 21 kDa. It is a member of the actin depolymerization factor (ADF)/cofilin family. Cofilin is an essential cellular protein that can bind the barbed end of actin and is required for cell viability [65]. In cells, cofilin acts in harmony with other regulatory proteins to mediate the response of the actin cytoskeleton to extracellular signals. In vertebrates, cofilin is regulated by pH, phosphorylation and phosphoinositides. It is involved in the translocation of the actin-cofilin complex from cytoplasm to nucleus. Cofilin plays an essential role in actin filament dynamics by enhancing depolymerization and severance of actin filaments [66]. These activities of cofilin can be abolished by phosphorylation at Ser-3; therefore, phosphorylation/dephosphorylation of cofilin at Ser-3 is regarded as one of the important mechanisms for regulating cofilin activities and actin filament dynamics [67]. Sinha et al. reported that the suppression of cofilin might lead to cancer regression [68].

Profilin-1 (PFN1) is a widely and highly expressed 14- to 17-kDa cytoplasmic and nuclear ligand protein of the microfilament system. It is a ubiquitous actin monomer-binding protein involved in actin polymerization in response to extracellular signaling pathways. PFN1 plays a central role in the regulation of *de novo* actin assembly by preventing spontaneous actin polymerisation through the binding of actin monomers and addition of monomeric actin to the barbed actin-filament ends [69]. The importance of profilins for normal cell proliferation, differentiation, cellular survival, motility, adhesion, migration, and cytoskeleton remodelling has been verified [69–72]. PFN1 may be a tumor suppressor because its expression was reduced in several types of invasive cancers and it was able to suppress tumorigenicity when overexpressed [73]. In addition, the immunohistochemistry analysis also showed low levels of PFN1 in several human breast cancers. Other than being a tumor suppressor, PFN1 was reported as a necessary element for differentiation of human epithelial cells [74].

Galectins are a family of structurally related carbohydrate-binding proteins and widely distributed in nematodes, insects, and porifer, as well as vertebrates and fungi [75]. They are defined by their affinity for poly-*N*-acetyllactosamine-enriched glycoconjugates and sequence similarities in the carbohydrate recognition domain. The galectins are a family of β-galactoside-binding proteins implicated in modulating cell-cell and cell-matrix interactions, which would be required for protein secretion through the classical secretory pathways found in the extracellular space [76].

Galectin-1 is expressed during human embryogenesis, and many adult cell types express and secrete galectin-1 into the extracellular matrix [76]. Galectin-1 contributes to different events associated with cancer biology, including tumour transformation, proliferation, differentiation, cell cycle regulation, growth arrest, apoptosis, cell adhesion, migration, inflammation, and inhibition of full cell activation [77]. A previous study has shown that galectin-1 induced sustained exposure of phosphatidylserine on the cell surface in a carbohydrate-dependent fashion, but phosphatidylserine exposure is not associated with cell death by apoptosis and does not affect cell viability. There is evidence that galectin-1 contributes to tumour evasion of immune responses [78].

A positive correlation has recently been shown between galectin-3 expression and the degree of malignant transformation in certain types of cell lines, and the amount of galectin-3 expression is expected to possibly serve as an index of degree for neoplastic transformation, tumor cell survival, angiogenesis, tumor metastasis, and tumor malignancy [79, 80]. Recent studies have revealed that intracellular galectin-3 exhibits the activity to suppress drug-induced apoptosis and anoikis that contribute to cell survival. Resistance to apoptosis is essential for cancer cell survival and plays a role in tumor progression [81].

Moreover, both galectin-1 and galectin-3 expressions are necessary for the initiation of the transformed phenotype of tumors. Inhibition of galectin-1 expression can suppress the transformed phenotype of human glioma cells [82]. In addition, following the inhibition of galectin-3 expression, breast carcinoma cells and thyroid papillary carcinoma cells lose their transformed characteristic phenotypes in cell culture [83, 84].

Myotrophin, a 12 kDa protein consists of 117 amino acids, has a potential role in cerebellar morphogenesis and

may be involved in differentiation of cerebellar neurons, particularly of granule cells, and associated with cardiac hypertrophy. It appears to be a primary modulator for myocardial cell growth and differentiation [85]. Myotrophin accelerates myocyte growth by stimulating protein synthesis and may be correlated with cardiac hypertrophy in the pathogenesis, where it is involved in the conversion of NF-κB p50-p65 heterodimers to p50-p50 and p65-p65 homodimers as well as in the normal development of cardiac myocytes [86]. A previous study also indicated that myotrophin may be involved in the upregulation of myofibrillar protein and the activation of cardiac gene transcription during the growth and hypertrophy of myocardium; thus, the induction of early response of gene expression may be linked to this response [87].

The 132 proteins identified in this study may be involved in some biologic processes that are associated with cell differentiation, proliferation, and adhesion. We have tested some proteins incorporated into the medium; however, none of those proteins can solely induce cell differentiation. The results form a database with a diversity and relative abundance of various proteins found in the HNPE cell-secreted proteins. The database provides not only information on the nature of protein contents in HNPE cells but also potential proteins to be examined in further investigations.

4. Conclusions

In this study, we established the first secretome database for HNPE cells. The experimental results obtained by SDS-PAGE and nano-high performance liquid chromatography electrospray ionization tandem mass spectrometry (nano-HPLC-ESI-MS/MS) system revealed the identification of 132 unique proteins from HNPE cell secretome. Among these 132 proteins identified with higher confidence levels, some proteins have been reported involving in cell differentiation, such as thrombospondin-1, 2, 3 precursor, galectin-3-binding protein, neurogenic locus notch homolog protein 3, follistatin-related protein 1 precursor, sPARC precursor, peroxiredoxin-1, cofilin 1, profilin 1, galectin-1, and myotrophin. However, none of those proteins can induce cell differentiation solely. This list serves as a starting point for buildingup a comprehensive database of the proteome of this cell-line. The database can include diverse repertoires of proteins expressed by HNPE cells. All of this data will enhance our understanding of the molecular mechanisms involved in maintaining the differentiated states of HNPE cells and directing their differentiation and, in turn, will bring us closer to fulfill the vast clinical potentials of the cells.

In conclusion, we have demonstrated that RGC-5 cells upon coculturing with HNPE cell conditioned SF-medium developed a differentiated morphology and continued to express the necessary RGC markers. The differentiated RGC-5 cells would therefore be useful to study apoptotic pathways of retinal ganglion cell death. The findings from this study may have significant impacts on HNPE cell biology and cell engineering.

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