Research Paper

Plasma exosome proteomics reveals the pathogenesis mechanism of post-stroke cognitive impairment

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ABSTRACT

Exploration and utilization of exosome biomarkers and their related functions provide the possibility for the diagnosis and treatment of post-stroke cognitive impairment (PSCI). To identify the new diagnostic and prognostic biomarkers of plasma exosome were used label-free quantitative proteomics and biological information analysis in PSCI patients. Behavioral assessments were performed, including the Mini-Mental Status Examination (MMSE), the Montreal Cognitive Assessment (MoCA), the Barthel index, the Morse Fall Seale (MFS) between control group (*n* = 10) and PSCI group (*n* = 10). The blood samples were collected to analyze the biomarker and differentially expressed proteins of plasma exosome using label-free quantitative proteomics and biological information. The exosomes marker proteins were determined by Western blot. The exosome morphology was observed by transmission electron microscopy. The scores of MMSE and MoCA were significantly decreased in the PSCI group. The PT% and high-density lipoprotein decreased and the INR ratio increased in PSCI group. The mean size of exosome was approximately 71.6 nm and the concentration was approximately 6.8E+7 particles/mL. Exosome proteomics identified 259 differentially expressed proteins. The mechanisms of cognitive impairment are related to regulate the degradation of ubiquitinated proteins, calcium dependent protein binding, cell adhesive protein binding, formation of fibrin clot, lipid metabolism and ATP-dependent degradation of ubiquitinated proteins in plasma exosome of PSCI patients. Plasma levels of YWHAZ and BAIAP2 were significantly increased while that of IGHD, ABCB6 and HSPD1 were significantly decreased in PSCI patients. These proteins might be target-related proteins and provide global insights into pathogenesis mechanisms of PSCI at plasma exosome proteins level.

INTRODUCTION

Ischemic stroke is induced by cerebral artery occlusion, which causes damage to endothelial cells, vascular smooth muscle, glial cells, neurons and related neuro-vascular units, and ultimately leads to brain tissue death and focal neurological damage [1, 2]. Post-stroke cognitive impairment (PSCI) is a type of vascular cognitive impairment that manifests throughout 6 months following a stroke. Patients suffering from

stroke lesions taking place in various regions of the brain that are not traditionally cognition-included may also result in development of PSCI. The plasma exosome biomarkers of PSCI have been emphasized. Plasma exosome biomarkers exploration and utilization and their associated functions allowed PSCI diagnosis and treatment possible.

Exosomes are small membrane vesicles existed in extracellular fluid and contain important genetic materials such as DNA, RNA, protein, lipid and miRNA [3]. Exosomes exist in extracellular fluid and contain important genetic materials such as DNA, RNA, protein, lipid and miRNA [3]. Exosomes can directly or indirectly act on target cells through the release of membrane contents or signal molecules for intercellular information transmission. In different pathological stages of ischemic stroke, exosomes released by different types of nerve cells contain specific signal molecules. Exosome miRNA-122-5p and miR-300-3p were biomarkers for the diagnosis of hyperacute (less than 6 h), subacute (8–14 d) and convalescent (greater than 14 d) ischemic cerebral infarction [4]. Exosomes secreted by circulating EPCs can transfer their inclusion to recipient endothelial cells, which contain miRNA related to PI3K/Akt signaling pathway and miRNA related to angiogenesis, such as miR-126 and miR-296. In the brain, exosomes secreted by cultured glioma cells provide angiogenic proteins, mRNAs and miRNAs to cerebrovascular endothelial cells and induce angiogenesis [5]. Neurons and glial cells interact with exosomes released by them to transfer biomolecules, regulate axonal growth and myelin sheath formation, and participate in brain nerve remodeling. Exosomes derived from multipluripotent mesenchymal stromal cells can effectively promote vascular and nerve regeneration, reduce inflammatory response and improve traumatic brain injury [6, 7]. Proteomics is an indispensable omics science to elucidate the proteome diversity. Faced with the limitations of diagnosis and treatment of PSCI, the task of finding effective methods to diagnose, predict and prevent PSCI has become more important.

In order to find a new prognostic and diagnostic plasma exosome biomarkers utilizing label-free quantitative proteomics and analysis of biological information in individuals with PSCI. In this study, a variety of psychological evaluations, which include the Mini-Mental Status Examination (MMSE), the Montreal Cognitive Assessment (MoCA), the Barthel index and blood biomarker detection were performed. The plasma exosome from control participants and PSCI patients were collected and analyzed by label-free quantitative proteomics. The differentially expressed proteins and its biological information analysis were conducted to establish global prospective on the PSCI pathogenesis mechanisms at proteins level.

METHODS

Study design

The Chinese Clinical Trial Registry (ChiCTR) has registered and recorded this clinical trial (registration number: ChiCTR1900023739, registration date: June 10, 2019), and the research protocol was approved by the Ethics Committee of Dongzhimen Hospital, a department of Beijing University of Chinese Medicine (approval number: DZMEC-KY-2019-04). Patients suffering from acute ischemic stroke were registered from Dongzhimen Hospital (eastern area) associated with Beijing University of Chinese Medicine between June 9, 2019 to December, 2019. The present investigation was conducted based on the ethical principles outlined in the Helsinki Declaration of 1975 (and as modified in 2013). This study contains the control group and PSCI group.

Participants

This investigation included 20 participants who signed an informed consent form. There are 10 participants in the PSCI group and control group, respectively. PSCI patients' inclusion criteria involved the following: (1) Age ≥35 years and ≤70 years; (2) Every patient has either an MRI or CT scan to validate the acute ischemic stroke; (3) Cognitive evaluation was done by MMSE within the first 7 days after the development of acute ischemic stroke. Patients having MMSE score ≤26 were determined as the PSCI group. (4) Cognitive impairment occurred after stroke [8, 9]. The exclusion criteria comprise the subsequent aspects : (1) Symptoms exhibited a range of complex to severe primary disorders of the heart, kidney, liver and hematopoietic system; (2) Consciousness acute disturbance; (3) Dementia and brain, different causes are because of mental or physiological diseases; (4) With severe vision, hearing or even speech impairment conflicts with rehabilitation; and (5) Furthermore the onset of cognitive impairment, there were no additional focal indications of cerebrovascular disease.

Behavioral assessment

The informed consent forms were signed by all participants and a variety of behavioral evaluations were conducted, such as the MMSE, the MoCA, the Barthel index, the Morse Fall Seale (MFS), and The Braden Scale for the prediction of pressure sore risk, as well as the Padua Prediction Score. The patients received

antiplatelet therapy (aspirin, 100 mg, QD) before assessments.

Blood biomarker detection

After a fasting period of 12-h, blood samples were obtained in the morning. Two milliliters of whole blood were drawn from peripheral vein of each participant and kept in a polypropylene tube including EDTA. The Dongzhimen Hospital affiliated with Beijing University of Chinese Medicine was requested to furnish the blood specimens. Following that, the fibrinogen, red blood cell specific volume (HCT), total cholesterol (CHO), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and other different markers were measured using whole blood samples.

The isolation and determination of plasma exosomes characteristics

The isolation procedure of exosomes

The isolation of plasma exosomes using TiO2 with a slight modification [10]. Remove the plasma sample from storage and place it on ice. Centrifuge the plasma sample at 2000 × g at room temperature for 20 min to extract cells and debris. Transfer 100 μL plasma to a new tube and processed using 0.2 mm pore size syringe filters (PALL Life Sciences, USA) for extracting apoptotic bodies and the large microvesicles. Following that, 5 mg of TiO2 microspheres were combined with the plasma sample (GL Science Inc, Japan) and mixed the sample thoroughly by vortexing at 4°C for a period of 5 min. The mixture underwent centrifugation at a force of 20,000 × g for 3 min at a temperature of 4°C, subsequently, the supernatant was extracted. The exosomes on the TiO2 microspheres were hydrated with PBS three times to eliminate unspecific contaminants. After washing with PBS, exosomes were lysed and digested directly with trypsin (Promega, Madison, WI) from the surface of microspheres at 37°C in 50 mM ammonium bicarbonate for 16 h. A new tube was used to transfer the supernatant and the TiO2 microspheres were hydrated twice with 100 μL of 0.1% formic acid after centrifugation at 12,000 g at 25°C for 5 min. The washing fraction was extracted and pooled with the supernatant. NanoDrop (Thermo Fisher, USA) was utilized to measure the concentration of peptide at an absorbance of 280 nm.

Western blot analysis of exosomes marker proteins

Detect the quality of plasma protein in 0.5 mL fraction sample. The fraction of vesicles with the least plasma protein content was selected for subsequent analysis. The concentration of protein samples was measured utilizing the BCA method. 12% SDS-PAGE was used for separating 10 μg of the protein, then the protein transferred to a 0.45 μm PVDF membrane, and inhibited utilizing a blocking solution including 5% bovine serum albumin for a period of 1 h at room temperature. Exosomal marker protein antibodies were add and incubate at a temperature of 4°C overnight, containing anti-CD63 rabbit polyclonal antibody (1:600) (Cat No. 25682-1-AP, Proteintech Group, Chicago, IL, USA), anti-TSG101 rabbit polyclonal antibody (1:2000) (Cat No. 28283-1-AP, Proteintech Group, USA), anti-CD81 mouse polyclonal antibody (1:3500) (Cat No. 66866-1-Ig, Proteintech Group, USA), anti-CD9 mouse polyclonal antibody (1:3000) (Cat No. 20597-1-AP, Proteintech Group, USA) and HRP-conjugated secondary antibodies (1:6000) (Cat No. SA00001-2; Proteintech Group, USA). The western blotting was examined utilizing an eECL Western blot kit (Cat No. CW0049 M, CWBIO, Jiangsu, China). After being eluted with 1 × TBST buffer, secondary antibody was supplemented and incubated at room temperature for a period of 90 min. After 3 hydrations by TBST, the color was generated utilizing SuperSignal West Femto Substrate Trial Kit (34094, Pierce, Rockford, IL, USA).

Transmission electron microscopy (TEM)

The exosome morphology was observed utilizing TEM. Exosome sample drops have the ability to adsorb for 5 min on formvar-coated EM grids, and were stained negatively utilizing 2% (w/v) phosphotungstic acid for 1 min. At 80 kV of acceleration voltage, transmission electron microscope (H-7650; Hitachi, Ltd., Tokyo, Japan) was utilized to perform TEM analysis.

Nanoparticle tracking analyzer (NTA) and Dynamic light scattering (DLS) analysis

The particle size and concentration analysis of model exosomes were performed on ZetaView Nanoparticle Tracker (Particle Metrix, Meerbusch, Germany). Calibrate the instrument with polystyrene particles with approximately 100 nm of a particle size, Dilute the model exosomes to approximately 1×10^8 particles/mL, and put them into the instrument for analysis. Each group of samples is automatically scanned 11 times to remove abnormal data. The data was recorded and analyzed by ZetaView 8.03.04.01 software.

Label-free quantitative proteomics

MALDI-TOF-MS/MS and database searching were utilized for the identification of proteins. An online liquid chromatography-tandem mass spectrometry (LC-MS/MS) setup including an EasynanoLC system and a Q-Exactive mass spectrometer (Thermo Scientific, Germany) installed with a nanoelectrospray ion source was utilized for all LC-MS/MS investigations.

(1) Mobile phase A included 0.1% FA, 2% acetonitrile dissolved within water, and mobile phase B included 0.1% FA, 98% acetonitrile in water. The flow rate has been measured to be 300 nL/min.

(2) At 2 kV, the source was operated. In order to perform a full MS survey scan, AGC target was 3e6, scan range was from m/z 300 to 1400 and the result of resolution was 70,000. The 50 highest intense peaks with charge state 2 and above were chosen in order to sequence and fragmented in the ion trap by HCD with normalized collision energy of 27%. Exclude isotope item was enabled and dynamic exclusion time was adjusted as 18 s.

(3) MaxQuant software was utilized to search the Raw MS files against UniProt database. (Version 1.5.2.8). The fixed modification was C (carbamidomethyl) and the variable modification was M (oxidation) and protein N-term (acetyl). The tolerance for the first search peptide was 20 ppm and the tolerance for the main search peptide was 6 ppm. The MS/MS tolerance result was 0.02Da. The PSM and protein false discovery level result was 1%. The employed among the runs and minimum score required for modified peptides was 40.

Biological information analysis

Protein-protein interaction (PPI) networks analysis

To gain a deeper comprehension from the perspective of the biological context of differentially expressed proteins, the protein interaction analysis was conducted by utilizing the free web-based search tool STRING10.5. The STRING database is a fundamental data resource within the ELIXIR's core, containing both identified and anticipated protein interactions. These data will be collected and integrated by the STRING database, with consolidating identified and anticipated protein-protein correlation data for the organism's large number [11]. STRING was needed to incorporate additional predicted functional partners for the purpose of improving the PPI networks formation. The protein IDs list that was differentially expressed was entered into the STRING database (https://string-db.org) to determine identified and anticipated protein functional correlation networks.

GO analysis

For exhibiting the differentially expressed proteins presence, to categorize the proteins in accordance with their biological process, protein classification, cellular composition, and pathway, GO enrichment analysis was conducted. In order to find out how these experimentally discovered proteins are distributed, GO enrichment annotation tools were used to examine each protein with higher speed and to comprehend the relationship between protein and biological function in its entirety. GO function enrichment analysis of protein describes this distribution comparison with the overall protein distribution, confirming which biological processes or molecular functions were significantly enriched with experimentally identified proteins, many regulatory, metabolic, and signal transduction pathways are resistant within the organism, and these pathways frequently form various pathways. Pathway analysis permits the identification of the highest significant biochemical-metabolic and signal transduction pathways in which the proteins were contained.

ELISA quantification analysis

To detect the levels of IGHD, ABCB6, HSPD1, YWHAZ and BAIAP2, enzyme-linked immunosorbent assay (ELISA) kits were utilized to determine the plasma. According to the manufacturer guidelines, plasma samples were thawed and submitted to ELISA quantitative analysis using human IgD ELISA kit (Abcam, ab157708, Cambridge, UK), human ATP Binding Cassette Subfamily B Member 6, Mitochondrial (ABCB6) ELISA Kit (Abbexa, abx385541, Cambridge, UK), human Heat Shock Protein 60, HSP-60 ELISA Kit (CSB-E08560h, Cusabio, China), human 14-3-3 protein zeta/delta (YWHAZ) ELISA kit (CSB-EL026293HU, Cusabio, China), human Brain-Specific Angiogenesis Inhibitor 1-Associated Protein 2 (BAIAP2) ELISA Kit (Abbexa, abx386001, Cambridge, UK). ELISA microplates were read using MK3 microplate reader (Thermo, Helsinki, Finland).

Statistical analysis

Each value was reported as the mean ± standard deviation (SD). Statistical analysis was conducted by SPSS20.0. The Demographic data and MMSE, MoCA, blood marker were analyzed with independent-samples *t*-test between control group and PSCI group. The Chi-square statistical test was utilized to investigate potential differences in gender variation between two distinct groups. *P*-values < 0.05 were reported as statistically significant.

RESULTS

Demographics, behavioral assessment and detection of blood biomarker results

There were no variations in age ($P = 0.182$), gender ($P = 0.653$) or education ($P = 0.072$) among the PSCI and the control groups. The MMSE and MoCA scores were significantly reduced throughout the PSCI groups ($P < 0.01$) (Table 1). According to the control group,

the PT% and high-density lipoprotein reduced ($P < 0.05$) and the INR ratio reduced ($P < 0.05$) in the PSCI group (Table 2).

Isolation and characterization results of plasma exosomes

The content detection results of plasma protein in 0.5 mL fraction sample were shown in Figure 1A. The content of plasma protein in fractions 6–10 is relatively lower, and the purity of exosomes is relatively high. Therefore, the 5 vesicle fractions 6–10 were selected for subsequent analysis. Western blot analysis was utilized to detect the exosomal protein markers in all the plasma exosome samples. The exosomal markers of protein expression levels (TSG101, CD9, CD63, and CD81) were shown in Figure 1B. The exosome morphology and size were visualized by TEM (Figure 1C). TEM observation revealed highly homogenous exosome combination with a regular round morphology having a diameter range of 30–200 nm. A representative laser scattering microscopy image of isolated exosomes is shown in Figure 1D. NTA was exploited to measure the size distribution of particles and view the isolated vesicle samples (Figure 1E, 1F). The samples fluorescent detection examined in the scatter mode. The size is calculated by the diffusion behavior. The study determined that the mean size of the particles was 71.6 nm, while the concentration was estimated to be around 6.8E+7 particles/mL.

Differentially expressed exosome proteins determination among the control and PSCI groups

In total, 259 differentially expressed exosome proteins have been measured and determined between the control group and PSCI group by Label-free quantitative proteomics, of which 131 proteins demonstrated up-regulated expression and 128 proteins showed down-regulated expression. The differentially expressed proteins with PSCI/control ratios that are high or less than 1.2-fold change having a set *P*-value < 0.05 were shown to be significantly changed. The differentially expressed proteins volcano plot was presented in Figure 2. The heat map of the whole differential expressed proteins were presented in Figure 3. The identification results of main 30 up-regulated proteins were presented in Table 3 and the other 101 up-regulated proteins were shown in Supplementary Table 1. The identification results of main 30 down-regulated proteins were shown in Table 4 and the other 98 down-regulated proteins were shown in Supplementary Table 2. The results of these proteins are summarized in detail. According to Table 5, the biological process category of 30 up-regulated proteins and molecular function were conducted according to biological function. The biological process category of 27 down-regulated proteins and molecular function were shown in Table 6.

GO analysis outcomes of the differentially expressed proteins

A GO analysis was employed to categorize the protein classification, molecular function, cellular composition, biological process, and mechanism.

GO analysis outcomes of the up-regulated proteins

Protein classification results indicated that 7.0%, 5.5%, 4.7%, 4.7%, 5.5%, 3.9% and 4.7% of these 128 up-regulated proteins were cytoskeletal protein, membrane traffic protein, metabolite interconversion enzyme, protein modifying enzyme, protein-binding activity modulator, scaffold/adaptor protein, and transporter, respectively (Figure 4A). Molecular function classification results indicated that 26.6%, 17.2%, 4.7% of these 128 up-regulated proteins were binding, catalytic activity, and transporter activity, respectively (Figure 4B). In biological process classification, the majority of the proteins were identified to be contributed in biological regulation (28.1%), organization of cellular component or biogenesis (18.0%), cellular process (39.8%), localization (18.8%), metabolic process (15.6%), response to stimulus (21.9%), and signaling (16.4%), respectively (Figure 4C). Based on the categorization of pathways, most of these proteins were associated with blood coagulation (4.8%), CCKR signaling map (3.1%), EGF receptor signaling pathway (3.1%), endothelin signaling pathway (3.9%), FGF signaling pathway (3.1%), inflammation induced by chemokine and cytokine signaling pathway (7.0%), integrin signaling pathway (6.3%), PI3 kinase pathway (2.3%) (Figure 5). The blood coagulation pathway included proteinase-activated receptor 4, platelet glycoprotein V, integrin alpha-IIb, platelet glycoprotein Ib alpha chain, platelet glycoprotein Ib beta chain, and platelet glycoprotein IX. The endothelin signaling pathway included endothelin-converting enzyme 1, cAMP-dependent protein kinase catalytic subunit beta, protein kinase C beta type, mitogen-activated protein kinase 1, and guanine nucleotide-binding protein subunit alpha-14.

GO analysis outcomes of the down-regulated proteins

Protein classification results indicated that 5.9%, 5.9%, 8.5%, 3.4%, 10.2% and 4.2% of these 118 down-regulated proteins were cytoskeletal protein, extracellular matrix protein, metabolite interconversion enzyme, nucleic acid binding protein, protein modifying enzyme, and protein-binding activity modulator, respectively (Figure 6A). Molecular function

Table 1. Demographic information and behavioral scale assessment.

	Control group (n = 10)	PSCI group (n = 10)	P-value
Age (y)	55.80 ± 6.92	60.30 ± 9.17	0.231
Gender (M/F)	6/4	5/5	0.653
Education (y)	10.30 ± 2.16	8.30 ± 2.50	0.072
MMSE	29.80 ± 0.42	20.30 ± 3.83	<0.01
MoCA	29.40 ± 0.70	15.70 ± 5.03	<0.01
Barthel	–	70.00 ± 15.28	–
Morse Fall Seale	–	46.50 ± 10.81	–
Braden Scale	–	20.50 ± 2.32	–
Padua Prediction Score	–	2.70 ± 2.06	–

Abbreviations: MMSE: Mini-Mental Status Examination; MoCA: Montreal Cognitive Assessment; Barthel: The Barthel index and data are presented as mean ± SD or number of patients.

Table 2. The blood marker detection results.

	Control group (n = 10)	PSCI group (n = 10)	P-value
Blood coagulation function			
PT%	102.13 ± 9.67	91.90 ± 7.58	0.027*
INR ratio	1.00 ± 0.05	1.06 ± 0.06	0.045*
Activated partial thromboplastin time	30.14 ± 3.99	31.33 ± 5.19	0.619
Fibrinogen content	2.61 ± 0.26	2.80 ± 0.62	0.470
Thrombin time	15.37 ± 1.16	14.86 ± 1.33	0.424
D-dimer	62.43 ± 27.65	118.6 ± 73.42	0.075
Blood biochemistry			
Serum creatinine	61.40 ± 14.89	60.60 ± 21.26	0.923
Glucose	6.54 ± 2.35	8.87 ± 3.68	0.109
Albumin	39.07 ± 4.57	38.44 ± 4.52	0.760
Calcium	2.29 ± 0.09	2.34 ± 0.13	0.368
Total bilirubin	13.69 ± 3.55	19.93 ± 8.57	0.047*
Direct bilirubin	3.23 ± 1.10	4.54 ± 2.47	0.143
Indirect bilirubin	10.46 ± 3.32	15.39 ± 6.43	0.045*
Alanine aminotransferase	17.30 ± 6.45	24.90 ± 13.16	0.118
Aspartate aminotransferase	19.40 ± 4.27	34.40 ± 35.08	0.196
Lactate dehydrogenase	152.67 ± 29.82	210.90 ± 47.47	0.006**
Hydroxybutyric acid dehydrogenase	114.33 ± 22.87	154.50 ± 40.21	0.017*
γ-glutamyltransferase	17.70 ± 6.63	29.50 ± 18.85	0.078
Alkaline phosphatase	60.89 ± 8.62	69.70 ± 20.31	0.245
Adenylate dehydrogenase	10.00 ± 2.87	10.70 ± 5.44	0.735
Serum lipid			
Total cholesterol	4.38 ± 0.56	4.18 ± 1.46	0.691
Apolipoprotein A1	1.17 ± 0.11	1.10 ± 0.17	0.401
Apolipoprotein B	0.83 ± 0.23	0.74 ± 0.26	0.501
High-density lipoprotein	0.98 ± 0.15	0.84 ± 0.13	0.045*
Low density lipoprotein	2.62 ± 0.55	2.42 ± 1.25	0.662
Lipoprotein (a)	182.00 ± 115.42	267.78 ± 176.09	0.285
Homocysteine	13.63 ± 3.75	15.78 ± 7.62	0.512

Values are mean ± SD. *P < 0.05, **P < 0.01.

classification results indicated that 28.2%, 24.8%, 5.1%, 2.6% and 5.1% of these down-regulated proteins were binding, catalytic activity, molecular function regulator, molecular transducer activity and transporter activity, respectively (Figure 6B). Throughout the biological process classification, the majority of the proteins were identified to be contributed in biological regulation (20.3%), cellular composition organization or biogenesis (18.6%), cellular process (39.0%), immune system process (6.8%), localization (13.6%), metabolic

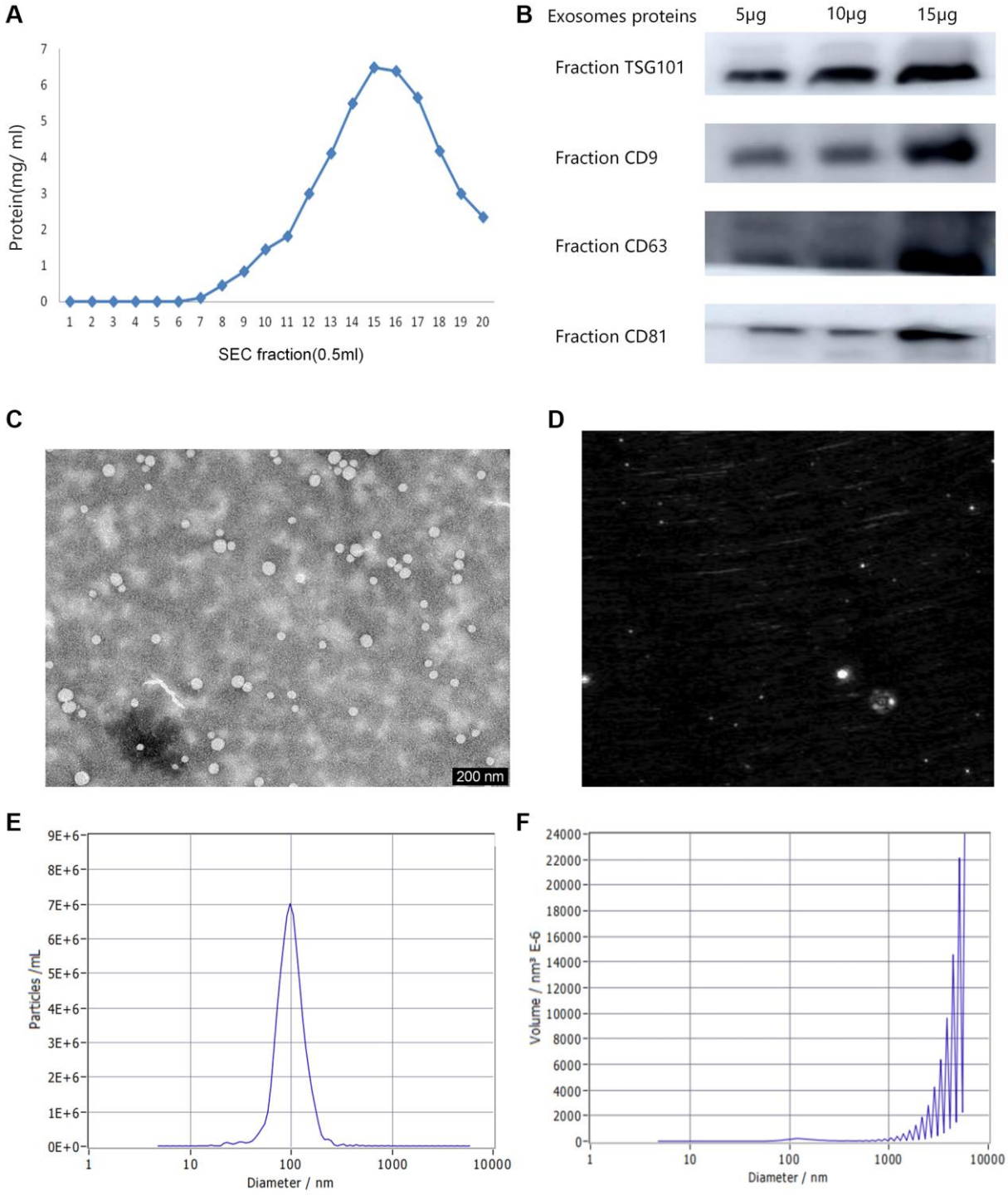

Figure 1. Various characterizations of plasma exosomes. (**A**) The content of plasma proteins in 0.5 mL fraction sample. (**B**) Western blot analysis of the typical exosomal proteins, TSG 101, CD9, CD63 and CD81. (**C**) Transmission electron microscopy (TEM) image indicating exosome morphology. (**D**) A representative laser scattering microscopy image of isolated exosomes. (**E**) The Nanoparticle tracking analysis (NTA) result of the particle size distribution for isolated exosomes. (**F**) The size distribution of volume consistent with the size range of exosomes.

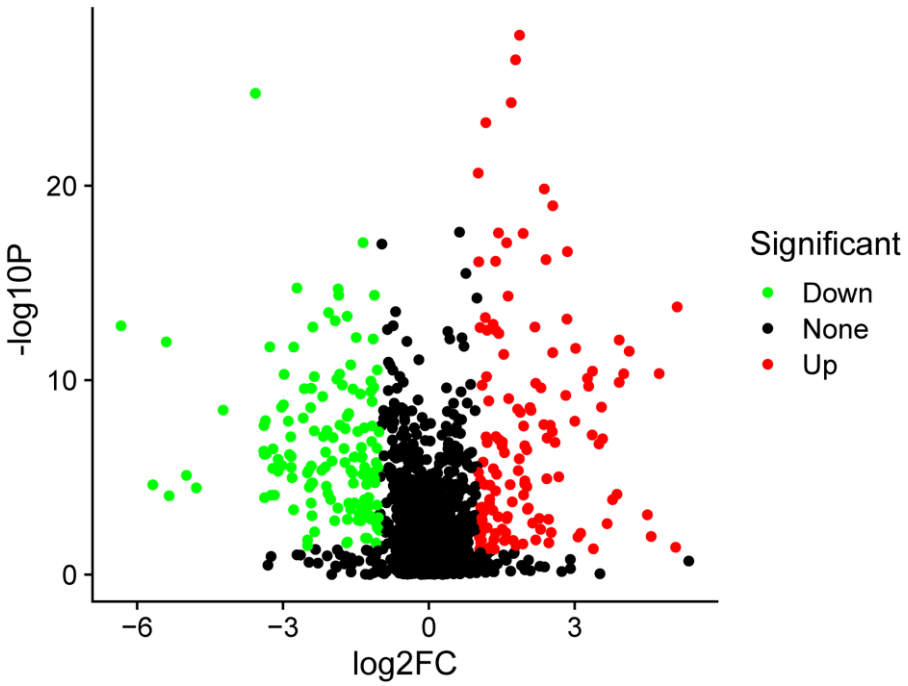

Figure 2. The volcano plot of differentially expressed proteins. The red points represented up-regulated proteins and green points represented down-regulated proteins between the PSCI and control groups.

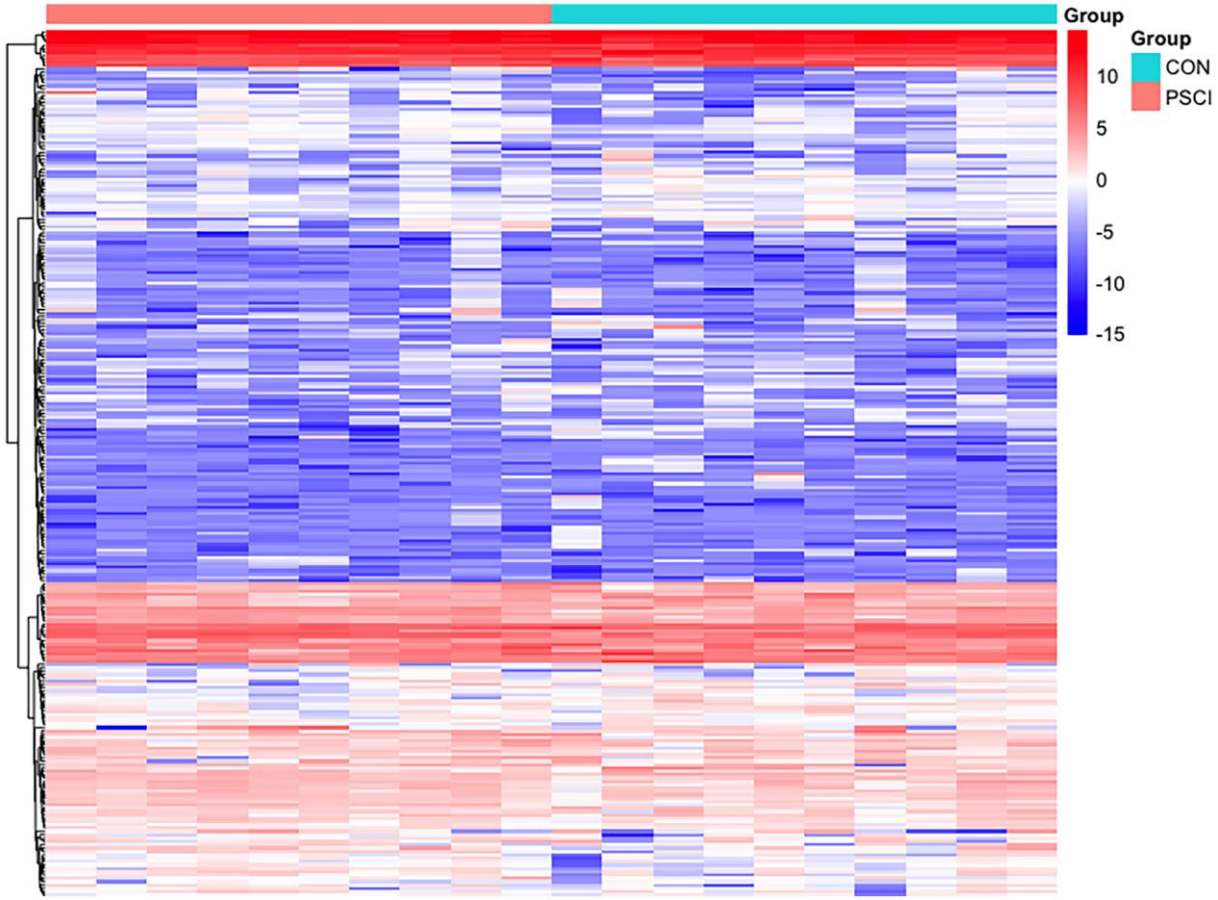

Figure 3. Hierarchical clustering of plasma exosome proteomes. The heat map represented the Z scores of all proteins quantified in Label-free quantitative proteomics.

Table 3. The identification results of main 30 up-regulated proteins between the PSCI group and control group.

No.	Accession No.	Protein name	Symbol	Exp. Mr (KDa)	Protein score	Fold change (PSCI/ Control)	*P*-value
1	P63104	14-3-3 protein zeta/delta	YWHAZ	27.75	177.81	2.13	<0.001
2	P31947	14-3-3 protein sigma	SFN	27.77	8.19	3.04	<0.01
3	Q9UQB8	Brain-specific angiogenesis inhibitor 1-associated protein 2	BAIAP2	60.87	43.47	4.95	<0.01
4	P78417	Glutathione S-transferase omega-1	GSTO1	25.90	27.57	3.00	<0.01
5	P61421	V-type proton ATPase subunit d 1	ATP6V0D1	40.33	8.42	2.91	<0.01
6	P34932	Heat shock 70 kDa protein 4	HSPA4	94.33	7.37	7.05	<0.01
7	P40197	Platelet glycoprotein V	GP5	60.96	49.41	5.19	<0.01
8	P37235	Hippocalcin-like protein 1	HPCAL1	22.31	5.46	5.82	
9	Q9Y6B6	GTP-binding protein SAR1b	SAR1B	22.41	5.08	2.28	<0.01
10	P54920	Alpha-soluble NSF attachment protein	NAPA	17.26	33.23	34.53	<0.01
11	P13746	Class I histocompatibility antigen, A-11 alpha chain	HLA-A	15.44	40.94	33.79	<0.05
12	P51575	P2X purinoceptor 1	P2RX1	13.33	44.98	15.11	<0.01
13	O76074	cGMP-specific 3,5-cyclic phosphodiesterase	PDE5A	17.60	99.98	11.74	<0.01
14	Q13642	Four and a half LIM domains protein 1	FHL1	16.00	36.26	8.72	<0.01
15	Q9BQE5	Apolipoprotein L2	APOL2	2.9676	37.092	5.85	<0.01
16	P08648	Integrin alpha-5	ITGA5	10.36	114.54	5.73	<0.01
17	O94804	Serine/threonine-protein kinase 10	STK10	11.78	112.13	3.86	<0.01
18	P11169	Solute carrier family 2, facilitated glucose transporter member 3	SLC2A3	76.41	53.92	3.65	<0.01
19	Q15762	CD226 antigen	CD226	23.75	38.61	3.45	<0.01
20	Q93084	Sarcoplasmic/endoplasmic reticulum calcium ATPase 3	ATP2A3	17.16	113.98	3.04	<0.01
21	O00194	Ras-related protein Rab-27B	RAB27B	21.19	24.61	2.69	<0.01
22	Q9NP79	Vacuolar protein sorting- associated protein VTA1 homolog	VTA1	28.89	33.88	2.63	<0.01
23	P14770	Platelet glycoprotein IX	GP9	71.37	19.05	2.61	<0.01
24	Q9UN37	Vacuolar protein sorting- associated protein 4A	VPS4A	11.56	48.897	2.37	<0.001
25	Q08830	Fibrinogen-like protein 1	FGL1	2.9253	36.379	9.61	<0.001
26	P02763	Alpha-1-acid glycoprotein 1	ORM1	13.49	23.51	2.12	<0.01
27	P02749	Beta-2-glycoprotein 1	APOH	233.67	38.30	2.11	<0.01
28	P55058	Phospholipid transfer protein	PLTP	20.08	54.74	2.10	<0.01
29	P30273	High affinity immunoglobulin epsilon receptor subunit gamma	FCER1G	10.82	9.67	2.04	<0.01
30	P01624	Ig kappa chain V-III region POM	IGKV3-15	17.61	11.92	2.03	<0.01

In the title line, Exp. Mr represented the experimental molecular weight of the proteins.

Table 4. The identification results of main 30 down-regulated proteins between the PSCI group and control group.

No.	Accession No.	Target protein	Symbol	Exp. Mr (KDa)	Protein score	Fold change (PSCI/ Control)	*P*-value
1	P06276	Cholinesterase	BCHE	12.41	68.42	0.41	<0.05
2	P01880	Ig delta chain C region	IGHD	42.25	17.46	0.02	<0.01
3	P04438	Ig heavy chain V-II region SESS		16.32	4.15	0.18	<0.01
4	P01771	Ig heavy chain V-III region HIL		13.44	39.48	0.27	<0.01
5	P01778	Ig heavy chain V-III region ZAP		12.34	6.03	0.10	<0.01

6	P07357	Complement component C8 alpha chain	C8A	65.16	22.61	0.14	<0.01
7	P20618	Proteasome subunit beta type-1	PSMB1	26.49	12.65	0.11	<0.01
8	Q9P289	Serine/threonine-protein kinase 26	STK26	46.53	2.66	0.13	<0.01
9	Q99536	Synaptic vesicle membrane protein VAT-1 homolog	VAT1	8.1909	41.92	0.48	<0.01
10	P03952	Plasma kallikrein	KLKB1	71.37	7.30	0.20	<0.01
11	P53396	ATP-citrate synthase	ACLY	120.84	21.57	0.41	<0.01
12	Q9NP58	ATP-binding cassette sub-family B member 6, mitochondrial	ABCB6	93.88	4.74	0.24	<0.01
13	P62330	ADP-ribosylation factor 6	ARF6	20.08	11.40	0.26	<0.05
14	P10809	60 kDa heat shock protein, mitochondrial	HSPD1	61.05	88.42	0.47	<0.01
15	O15162	Phospholipid scramblase 1	PLSCR1	35.05	10.23	0.11	<0.01
16	Q99828	Calcium and integrin-binding protein 1	CIB1	21.70	8.13	0.11	<0.01
17	Q5D862	Filaggrin-2	FLG2	248.07	81.40	0.12	<0.01
18	Q8WXI7	Mucin-16	MUC16	2284.30	8.38	0.13	<0.01
19	P56199	Integrin alpha-1	CFHR1	130.85	4.11	0.14	<0.01
20	P25311	Zinc-alpha-2-glycoprotein	AZGP1	34.26	8.18	0.15	<0.01
21	P02545	Prelamin-A/C	LMNA	74.14	26.39	0.01	<0.01
22	P30486	Class I histocompatibility antigen, B-48 alpha chain	HLA-B	40.36	29.55	0.03	<0.01
23	Q9NP59	Solute carrier family 40-member 1	SLC40A1	62.54	9.51	0.10	<0.01
24	P13164	Interferon-induced transmembrane protein 1	IFITM1	13.96	6.85	0.10	<0.01
25	O14818	Proteasome subunit alpha type-7	PSMA7	27.89	17.47	0.17	<0.01
26	P22234	Multifunctional protein ADE2	PAICS	47.08	10.28	0.17	<0.01
27	P30153	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	PPP2R1A	65.31	10.92	0.18	<0.01
28	Q9HCM2	Plexin-A4	PLXNA4	212.45	5.19	0.18	<0.05
29	Q8NG06	E3 ubiquitin-protein ligase TRIM58	TRIM58	54.77	29.55	0.18	<0.05
30	Q9BS26	Endoplasmic reticulum resident protein 44	ERP44	46.97	3.45	0.19	<0.01

In the title line, Exp. Mr represented the experimental molecular weight of the proteins.

Table 5. The molecular function and biological process category of main 30 up-regulated expressed proteins in PSCI.

No.	Target protein	Molecular function	Biological process
1	14-3-3 protein zeta/delta	Cadherin binding; ion channel binding; protein domain specific binding; protein kinase binding	Establishment of Golgi localization; protein insertion into mitochondrial membrane involved in apoptotic signaling pathway; synaptic target recognition
2	14-3-3 protein sigma	Cadherin and phosphoprotein binding; protein kinase C inhibitor activity	Intrinsic apoptotic signaling pathway; regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway; release of cytochrome c from mitochondria
3	Brain-specific angiogenesis inhibitor 1-associated protein 2	Cadherin binding involved in cell-cell adhesion; identical protein binding; proline-rich region binding; scaffold protein binding; transcription cofactor binding	Axonogenesis; cellular response to L-glutamate; modification of synaptic structure, modulating synaptic transmission; regulation of synaptic plasticity; vascular endothelial growth factor receptor signaling pathway
4	Glutathione S-transferase omega-1	Glutathione dehydrogenase (ascorbate) activity; glutathione transferase activity; methylarsonate reductase activity; oxidoreductase activity	L-ascorbic acid metabolic process; cellular response to arsenic-containing substance; glutathione derivative biosynthetic process; interleukin-12 mediated signaling pathway
5	V-type proton ATPase subunit d 1	Proton-exporting ATPase activity, phosphorylative mechanism proton-transporting ATPase activity	IRE1-mediated unfolded protein response; cellular iron ion homeostasis; cellular response to increased oxygen levels; phagosome acidification; proton transmembrane transport

6	Heat shock 70 kDa protein 4	ATP binding	Chaperone-mediated protein complex assembly; protein insertion into mitochondrial outer membrane; response to unfolded protein
7	Platelet glycoprotein V	Mediates vWF-dependent platelet adhesion to blood vessels	Blood coagulation, intrinsic pathway; cell adhesion; platelet activation
8	Hippocalcin-like protein 1	Calcium ion binding	
9	GTP-binding protein SAR1b	GTP binding; GTPase activity; metal ion binding	Antigen processing and presentation of exogenous peptide antigen via MHC class I and II; endoplasmic reticulum to Golgi vesicle-mediated transport; intracellular protein transport;
10	Alpha-soluble NSF attachment protein	Protein containing complex binding; soluble NSF attachment protein activity; syntaxin binding	Endoplasmic reticulum to Golgi vesicle mediated transport; synaptic vesicle priming; synaptic transmission, glutamatergic
11	P2X purinoceptor 1	ATP binding; ATP-gated ion channel activity; extracellularly ATP-gated cation channel activity; purinergic nucleotide receptor activity; zinc ion binding	Apoptotic process; calcium ion transport; neuronal action potential; Regulation of presynaptic cytosolic calcium ion concentration; synaptic transmission
12	cGMP-specific 3,5-cyclic phosphodiesterase	3′,5′-cyclic-GMP phosphodiesterase activity; cGMP binding; metal ion binding	cGMP catabolic process; MAP kinase activity; regulation of nitric oxide mediated signal transduction
13	Four and a half LIM domains protein 1	Ion channel binding; metal ion binding	Cell differentiation; potassium ion transport; membrane depolarization; regulation of potassium ion transmembrane transporter activity
14	Apolipoprotein L2	High density lipoprotein particle binding; lipid binding; signaling receptor binding	Cholesterol metabolic process; lipid metabolic process; lipid transport; lipoprotein metabolic process
15	Tetraspanin-32	Cytoskeleton organization	Cell-cell signaling; defense response to protozoan; integrin mediated signaling pathway; platelet aggregation;
16	Integrin alpha-5	Epidermal growth factor receptor binding; metal ion binding; platelet derived growth factor receptor binding; vascular endothelial growth factor receptor 2 binding	Angiogenesis; cell adhesion; cell substrate junction assembly; endodermal cell differentiation; cell migration; peptidyl tyrosine phosphorylation;
17	Serine/threonine-protein kinase 10	ATP binding; identical protein binding; protein homodimerization activity; protein serine/threonine kinase activity	Activation of protein kinase activity; lymphocyte aggregation and migration; neutrophil degranulation; protein autophosphorylation;
18	Solute carrier family 2, facilitated glucose transporter member 3	Glucose binding; glucose transmembrane transporter activity	L-ascorbic acid metabolic process; carbohydrate metabolic process; glucose transmembrane transport; neutrophil degranulation
19	CD226 antigen	Cell adhesion molecule binding; integrin binding; protein kinase binding	Cell recognition; cytokine production; T cell receptor signaling pathway; immunoglobulin mediated immune response; interferon-gamma production
20	Sarcoplasmic/endoplasmic reticulum calcium ATPase 3	ATP binding; calcium transmembrane transporter activity; metal ion binding; proton exporting ATPase activity	Calcium ion transmembrane transport; calcium ion transport; cellular calcium ion homeostasis; ion transmembrane transport
21	Ras-related protein Rab-27B	GDP binding; GTP binding; GTPase activity; myosin V binding; protein domain specific binding	Rab protein signal transduction; anterograde axonal protein transport; intracellular protein transport; multivesicular body sorting pathway; synaptic vesicle endocytosis
22	Vacuolar protein sorting-associated protein VTA1 homolog	Protein C-terminus binding	ESCRT III complex disassembly; endosomal transport; macroautophagy; multivesicular body assembly; multivesicular body sorting pathway
23	Platelet glycoprotein IX	Platelet activation apparently involves disruption of the macromolecular complex of GP-Ib with the platelet glycoprotein IX	Blood coagulation, intrinsic pathway; cell adhesion; platelet activation
24	Vacuolar protein sorting-associated protein 4A	ATP binding; ATPase activity; protein C-terminus binding; protein domain specific binding; protein containing complex binding	Ubiquitin-dependent protein catabolic process via the multivesicular body sorting pathway; exosomal secretion; multivesicular body assembly

25	Fibrinogen-like protein 1	Inhibiting inflammatory immune responses and metabolic function	Adaptive immune response
26	Alpha-1-acid glycoprotein 1	Functions as transport protein in the blood stream	Inflammatory response; neutrophil degranulation; platelet degranulation; interleukin-1 beta secretion
27	Beta-2-glycoprotein 1	Heparin binding; lipid binding; lipoprotein lipase activator activity; phospholipid binding	Angiogenesis; plasminogen activation; platelet degranulation; blood coagulation; regulation of fibrinolysis; triglyceride metabolic process
28	Phospholipid transfer protein	Ceramide binding and transfer activity; lipid transporter activity; phosphatidic acid binding and transfer activity;	Ceramide transport; high-density lipoprotein particle remodeling; lipid metabolic process; phospholipid transport; cholesterol efflux; vitamin E biosynthetic process
29	High affinity immunoglobulin epsilon receptor subunit gamma	IgE binding, IgE receptor activity, IgG binding, protein homodimerization activity	Immunoglobulin mediated immune response, innate immune response, neutrophil chemotaxis, T cell differentiation
30	Ig kappa chain V-III region POM	Antigen binding	Fc-gamma receptor signaling pathway involved in phagocytosis; complement activation, classical pathway; immune response; leukocyte migration;

Table 6. The molecular function and biological process category of main 27 down-regulated proteins in PSCI.

No.	Target protein	Molecular function	Biological process
1	Cholinesterase	Acetylcholinesterase activity; amyloid-beta binding; choline binding; cholinesterase activity; enzyme binding	Choline metabolic process; cocaine metabolic process; neuroblast differentiation; response to alkaloid and folic acid; response to glucocorticoid
2	Ig delta chain C region	Antigen binding; immunoglobulin receptor binding	B cell receptor signaling pathway; complement activation, classical pathway; innate immune response; positive regulation of interleukin-1 secretion
3	Complement component C8 alpha chain	Complement binding; protein-containing complex binding	Complement activation, alternative or classical pathway; immune response; regulation of complement activation
4	Proteasome subunit beta type-1	Endopeptidase activity; threonine type endopeptidase activity	Proteasomal ubiquitin dependent protein catabolic process; Wnt signaling pathway; post translational protein modification; protein polyubiquitination;
5	Serine/threonine-protein kinase 26	ATP binding; magnesium ion binding; protein homodimerization activity; protein kinase activity; protein serine/threonine kinase activity	Activation of protein kinase activity; neuron projection morphogenesis; protein phosphorylation; signal transduction by protein phosphorylation
6	Angiopoietin-like protein 8	Hormone activity	cEll maturation; cellular lipid metabolic process; lipid metabolic process; lipoprotein lipase activity;
7	Plasma kallikrein	Serine-type endopeptidase activity	Factor XII activation; blood coagulation, intrinsic pathway; extracellular matrix disassembly; fibrinolysis; plasminogen activation
8	ATP-citrate synthase	ATP binding; ATP citrate synthase activity; cofactor binding; metal ion binding	Acetyl-CoA and cholesterol biosynthetic process; citrate metabolic process; coenzyme A metabolic process
9	ATP-binding cassette sub-family B member 6, mitochondrial	ATP binding; ATPase activity; ATPase-coupled heme transmembrane transporter activity; heme binding	Cellular iron ion homeostasis; heme transport; transmembrane transport
10	ADP-ribosylation factor 6	GTP binding; GTPase activity; protein N-terminus binding; thioesterase binding	Intracellular protein transport; maintenance of postsynaptic density structure; protein localization to endosome; synaptic vesicle endocytosis
11	60 kDa heat shock protein, mitochondrial	ATP binding; ATPase activity; apolipoprotein binding; chaperone binding	Chaperone-mediated protein complex assembly; B cell activation and cytokine production; protein import into mitochondrial intermembrane space

12	Phospholipid scramblase 1	CD4 receptor binding; DNA binding transcription activator activity, calcium ion binding; phospholipid scramblase activity	Apoptotic process; phosphatidylserine biosynthetic process; plasma membrane phospholipid scrambling
13	Calcium and integrin-binding protein 1	Ras GTPase binding; calcium ion binding; calcium dependent protein kinase inhibitor activity; protein C-terminus binding; protein kinase binding	Angiogenesis; apoptotic process; cell adhesion; cellular response to DNA damage stimulus and growth factor stimulus; cytoplasmic microtubule organization
14	Filaggrin-2	Calcium ion binding; structural constituent of epidermis; transition metal ion binding	Cell adhesion; epidermis morphogenesis; neutrophil degranulation
15	Mucin-16	Thought to provide a protective, lubricating barrier against particles and infectious agents at mucosal surfaces	O-glycan processing; cell adhesion; stimulatory C-type lectin receptor signaling pathway
16	Integrin alpha-1	Collagen binding; collagen binding involved in cell-matrix adhesion; metal ion binding; protein phosphatase binding; signaling receptor binding	Activation of MAPK activity; neuron projection morphogenesis; neutrophil chemotaxis; neuron apoptotic process; phosphoprotein phosphatase activity; vasodilation
17	Zinc-alpha-2-glycoprotein	Protein transmembrane transporter activity; ribonuclease activity	Cell adhesion; detection of chemical stimulus involved in sensory perception of bitter taste; retina homeostasis; transmembrane transport
18	Prelamin-A/C	Identical protein binding; structural molecule activity	Protein localization to nucleus and protein stability; cellular response to hypoxia; nuclear envelope organization; telomere maintenance
19	class I histocompatibility antigen, B-48 alpha chain	Peptide antigen binding	Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent; antigen processing and presentation of exogenous peptide antigen
20	Solute carrier family 40-member 1	Iron ion transmembrane transporter activity; peptide hormone binding	Cellular iron ion homeostasis; iron ion export across plasma membrane; iron ion transmembrane transport; lymphocyte homeostasis; spleen trabecula formation
21	Interferon-induced transmembrane protein 1	Inhibits the entry of viruses to the host cell cytoplasm, permitting endocytosis, but preventing subsequent viral fusion	Cell surface receptor signaling pathway; response to interferon alpha or beta; type I interferon signaling pathway
22	Proteasome subunit alpha type-7	Endopeptidase activity; identical protein binding; threonine-type endopeptidase activity	Ubiquitin dependent protein catabolic process; post translational protein modification; protein deubiquitination; protein polyubiquitination
23	Multifunctional protein ADE2	Cadherin binding; phosphoribosylaminoimidazole carboxylase synthase activity	Purine nucleobase biosynthetic process; purine ribonucleoside monophosphate biosynthetic process
24	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	Protein antigen binding; protein heterodimerization activity; protein phosphatase regulator activity; protein serine/threonine phosphatase activity	RNA splicing; ceramide metabolic process; peptidyl serine dephosphorylation; protein dephosphorylation
25	Plexin-A4	Semaphorin receptor activity	Chemorepulsion of branchiomotor axon; regulation of axonogenesis, axon extension and GTPase activity; semaphorin-plexin signaling pathway involved in axon guidance
26	E3 ubiquitin-protein ligase TRIM58	Dynein heavy chain binding; dynein intermediate chain binding; ubiquitin protein ligase activity; zinc ion binding	Protein autoubiquitination; protein polyubiquitination; regulation of nuclear migration along microtubule; ubiquitin dependent protein catabolic process
27	Endoplasmic reticulum resident protein 44	Protein disulfide isomerase activity	Cell redox homeostasis; glycoprotein metabolic process; response to endoplasmic reticulum stress and unfolded protein

process (21.2%), multicellular organismal process (9.3%), response to stimulus (16.1%) and signaling (9.3%) (Figure 6C). Based on the categorization of pathways, most of these proteins were connected to gonadotropin-releasing hormone receptor pathway (1.7%) and ubiquitin proteasome pathway (1.7%) (Figure 7). The chemokine and cytokine signaling pathway which induced the inflammation included rho-related GTP-binding protein, platelet factor 4 variant, C5a anaphylatoxin chemotactic receptor 1, and C-X-C

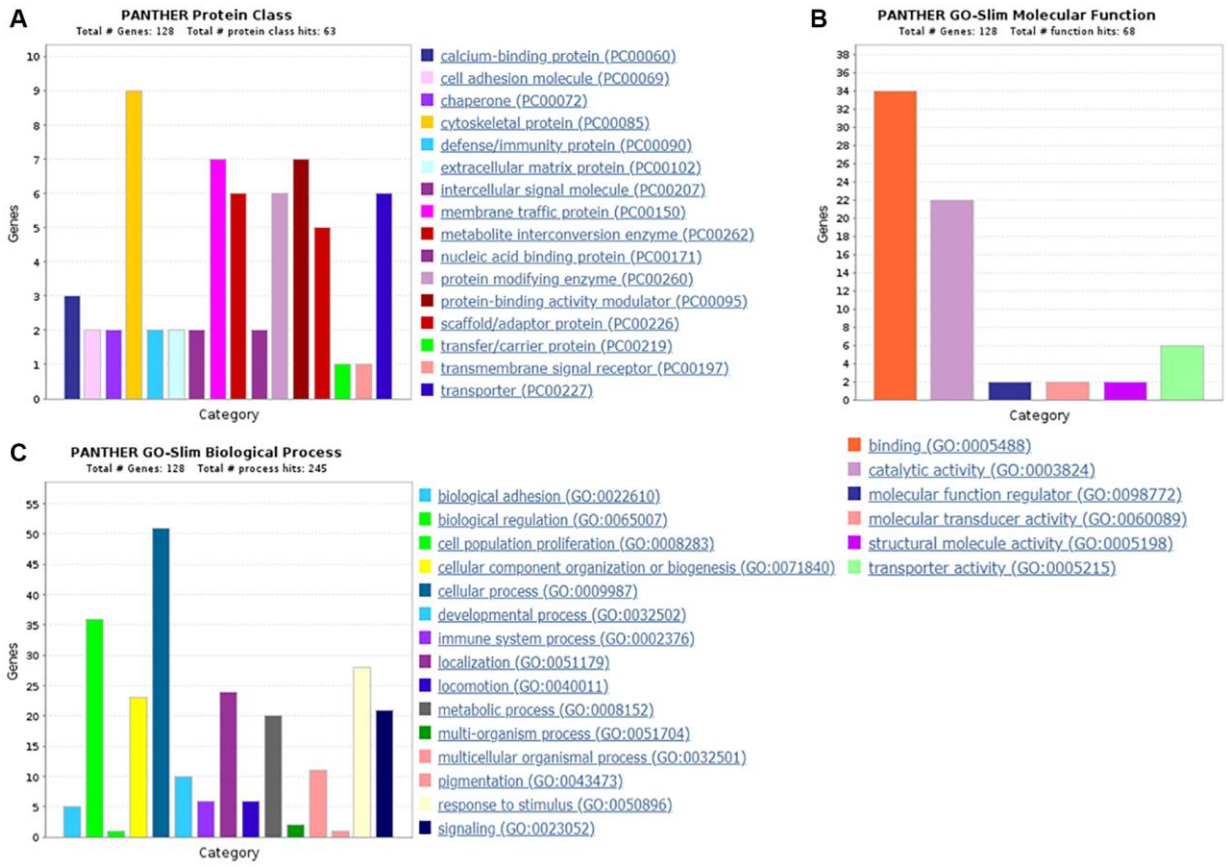

Figure 4. The GO analysis results of up-regulated proteins in the PSCI group. (**A**) The protein classification of up-regulated proteins. (**B**) The molecular function of up-regulated proteins. (**C**) The biological process of up-regulated proteins.

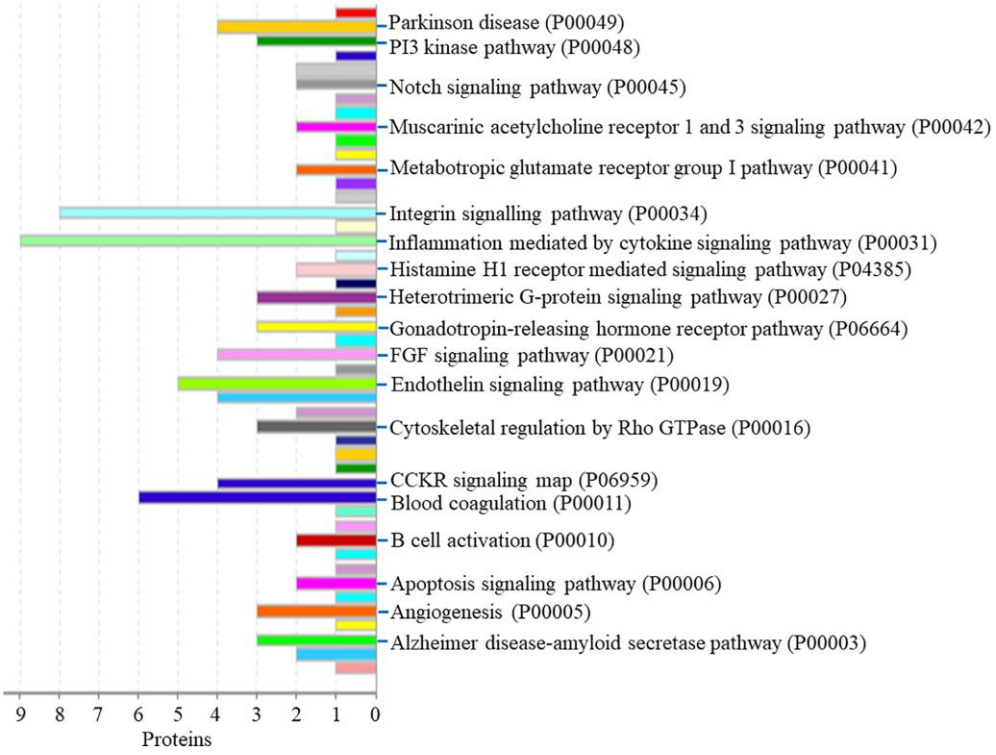

Figure 5. The pathway of up-regulated proteins in the PSCI group.

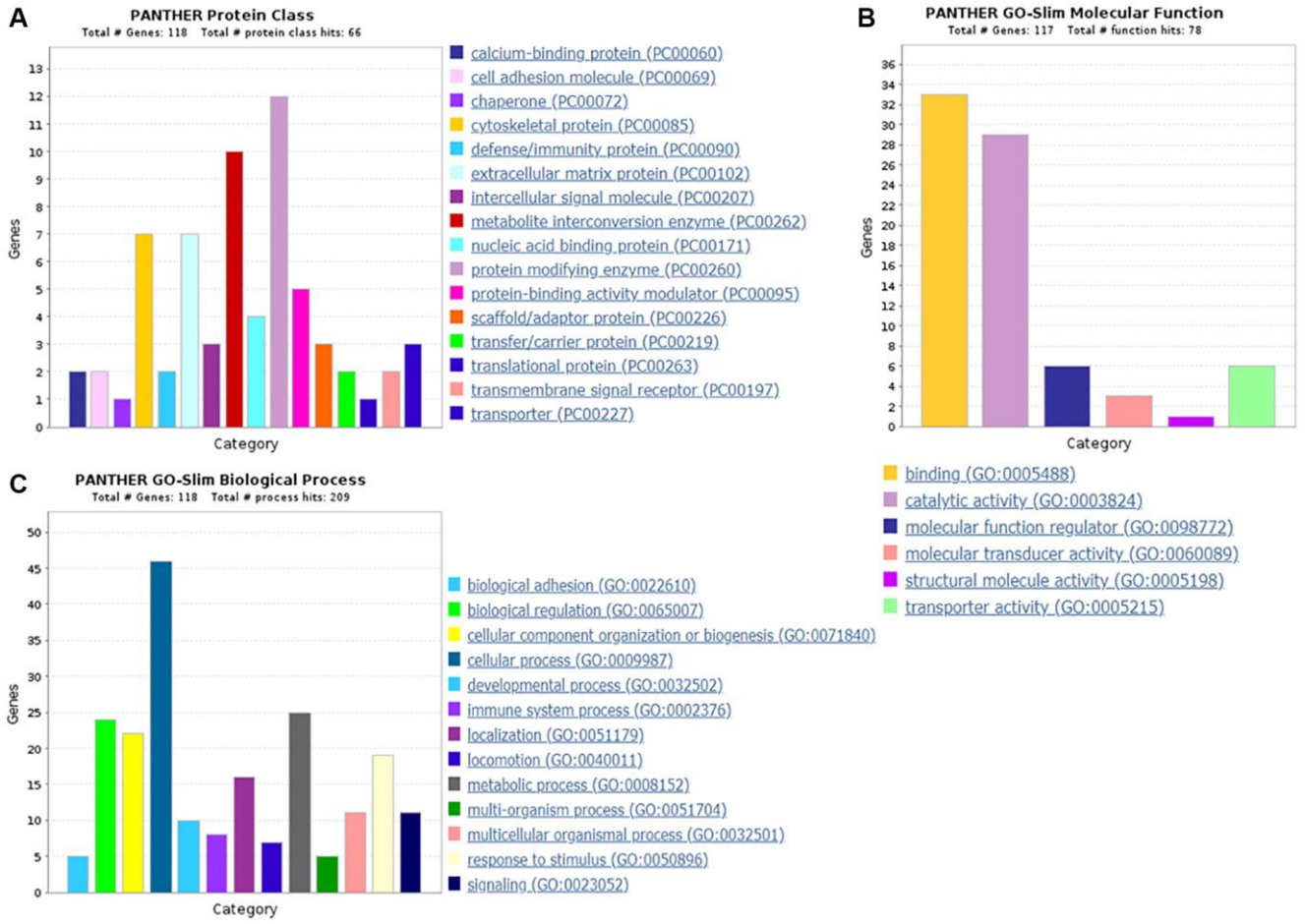

Figure 6. The GO analysis results of down-regulated proteins in the PSCI group. (**A**) The protein classification of down-regulated proteins. (**B**) The molecular function of down-regulated proteins. (**C**) The biological process of down-regulated proteins.

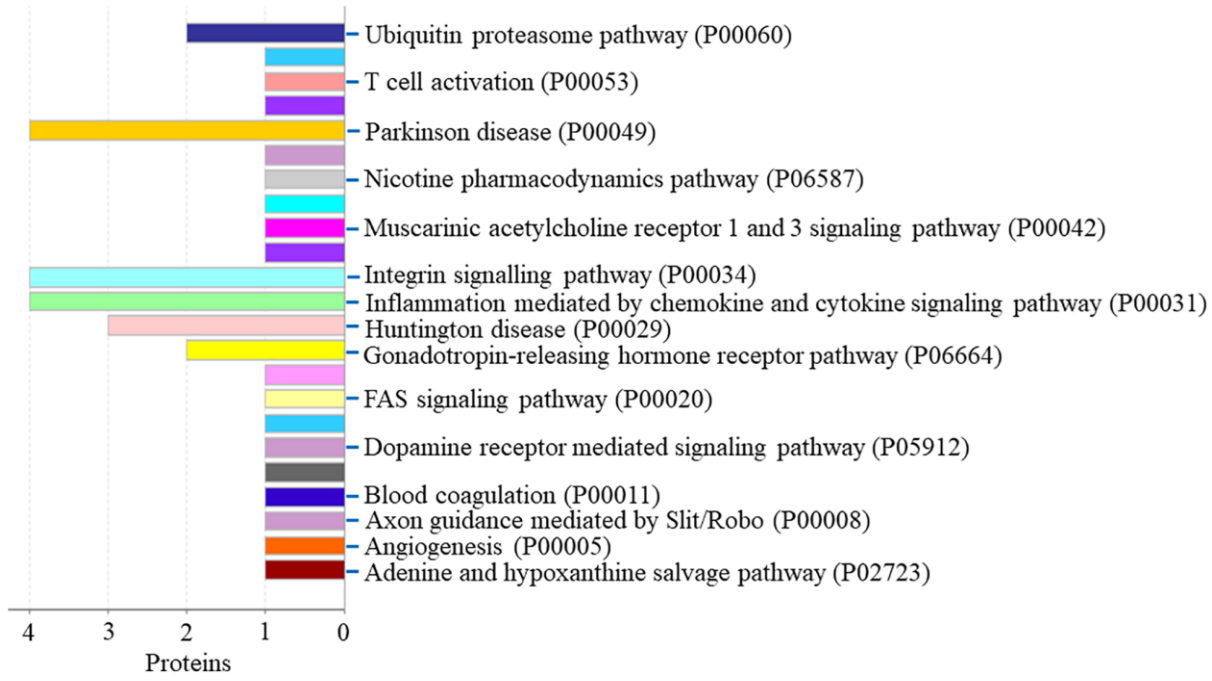

Figure 7. The pathway of down-regulated proteins in the PSCI group.

chemokine receptor type 2. The integrin signaling pathway included collagen alpha-1(I) chain, collagen alpha-2(I) chain, integrin alpha-1, and ADP-ribosylation factor 6.

PPI networks analysis results

PPI networks analysis results of the up-regulated proteins

Utilizing STRING analysis, a controlled PPI network with high-quality was constructed. 127 up-regulated proteins were suitable for PPI network analysis (focus molecule) and PPI networks with high-quality were built according to the STRING database. A complete PPI regulation network with 127 up-regulated proteins were presented in Figure 8. The network clustering results showed that the PPI network consists of six specified function clusters that comprise proteins with similar functions and are expressed by various colors (Figure 9). These six visualized interaction function clusters (sub-networks) were related to degradation of ubiquitinated proteins and folding of proteins (lime green), calcium-dependent protein binding and ESCRT III complex disassembly (yellow), cytoskeleton reorganization and platelet aggregation (green), phospholipid scrambling of phosphatidylserine in platelets and ATP mediates synaptic transmission (purple), lipid binding, signal transduction (red), and

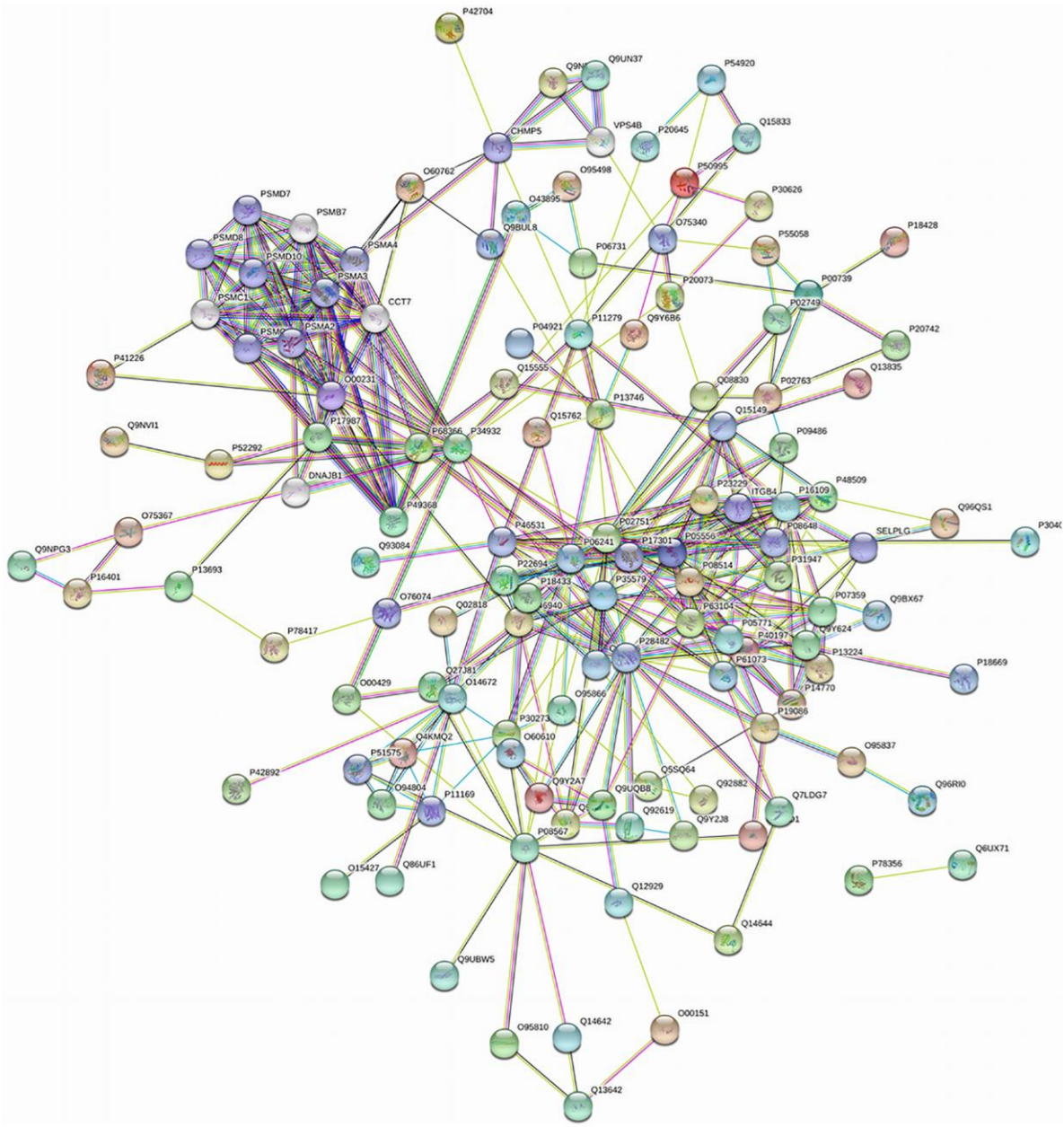

Figure 8. The PPI regulation network of up-regulated proteins in the PSCI group.

blood coagulation, intrinsic pathway (blue), respectively. The PPI network which included the anticipated functional intermediate partners are presented in Table 7.

PPI networks analysis results of the down-regulated proteins

118 down-regulated proteins were suitable for PPI network analysis (focus molecule) and a controlled PPI networks with high-quality were constructed according to the STRING database. A complete regulation of PPI network by down-regulated proteins were presented in Figure 10. The network clustering result showed that the PPI network consists of six specified function clusters that comprise proteins with similar functions and are expressed by various colors (Figure 11). These six visualized interaction function clusters

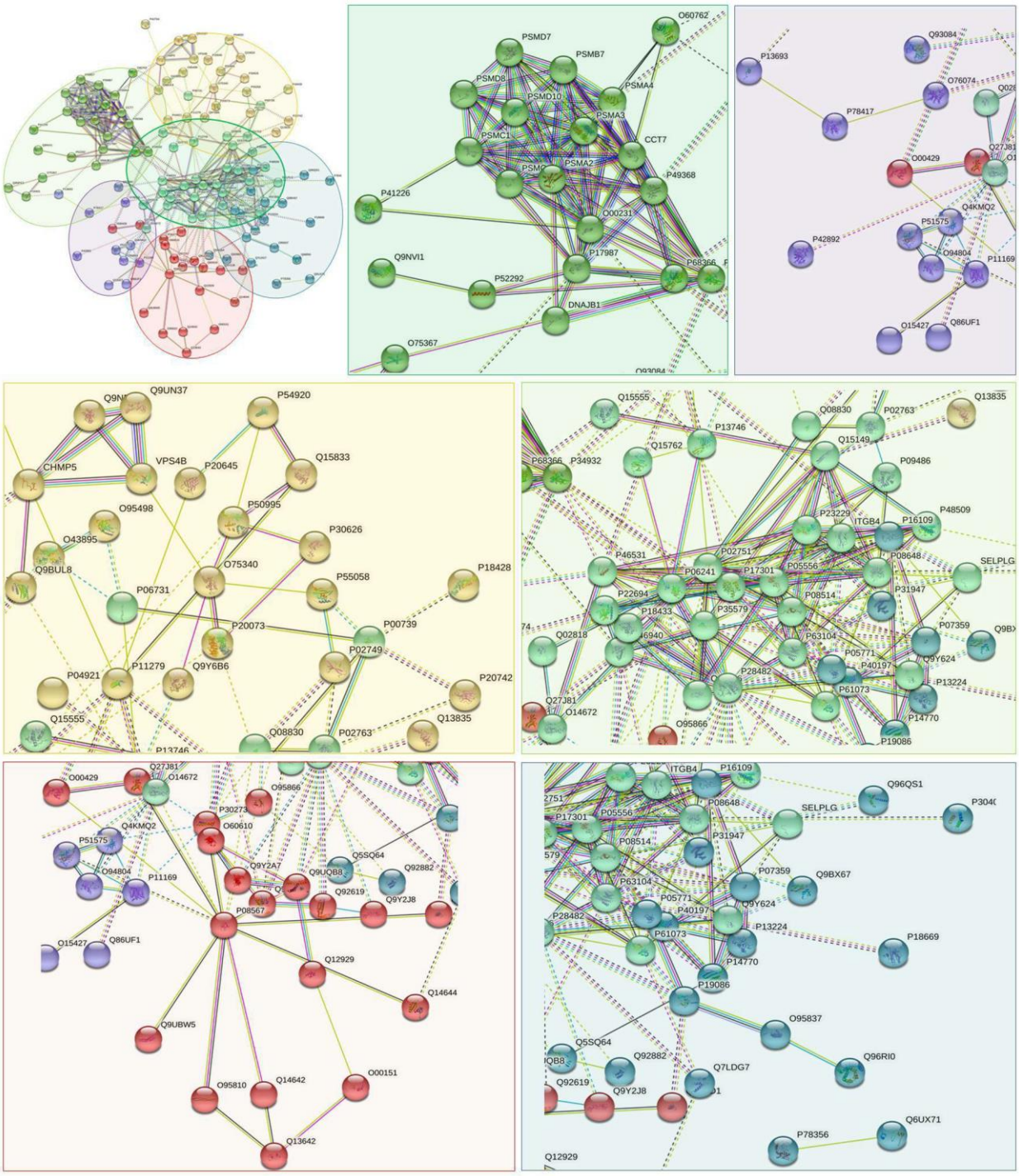

Figure 9. The PPI network means clustering of up-regulated proteins in the PSCI group. The PPI network is clustered to a specified number of clusters.

Table 7. The symbols and full names of the predicted functional intermediate partners in the PPI network of up-regulated expressed proteins shown in Figure 8.

No.	Accession No.	Symbol	Full name	Molecular function	Number of amino acids
1	P25787	PSMA2	Proteasome subunit alpha type-2	Component of the 20S core proteasome complex involved in the proteolytic degradation of most intracellular proteins.	234
2	P25789	PSMA4	proteasome subunit alpha type-4	Participates in the ATP-dependent degradation of ubiquitinated proteins and plays a key role in the maintenance of protein homeostasis by removing misfolded or damaged proteins.	261
3	P16144	ITGB4	Integrin beta-4	Binds to NRG1 (via EGF domain) and this binding is essential for NRG1-ERBB signaling	1822
4	P43686	PRS6B	26S proteasome regulatory subunit 6B	A multiprotein complex involved in the ATP-dependent degradation of ubiquitinated proteins.	418
5	P51665	PSMD7	26S proteasome non-ATPase regulatory subunit 7	Plays a key role in the maintenance of protein homeostasis by removing misfolded or damaged proteins.	324
6	O75832	PSMD10	26S proteasome non-ATPase regulatory subunit 10	Acts as a chaperone during the assembly of the 26S proteasome, specifically of the PA700/19S regulatory complex	226
7	Q9NZZ3	CHMP5	Charged multivesicular body protein 5	Peripherally associated component of the endosomal sorting required for transport complex III which is involved in multivesicular bodies formation and sorting.	219
8	Q14242	SELPLG	P-selectin glycoprotein ligand 1	A SLe(x)-type proteoglycan, which through high affinity, calcium-dependent interactions, mediates rapid rolling of leukocytes over vascular surfaces in inflammation.	428

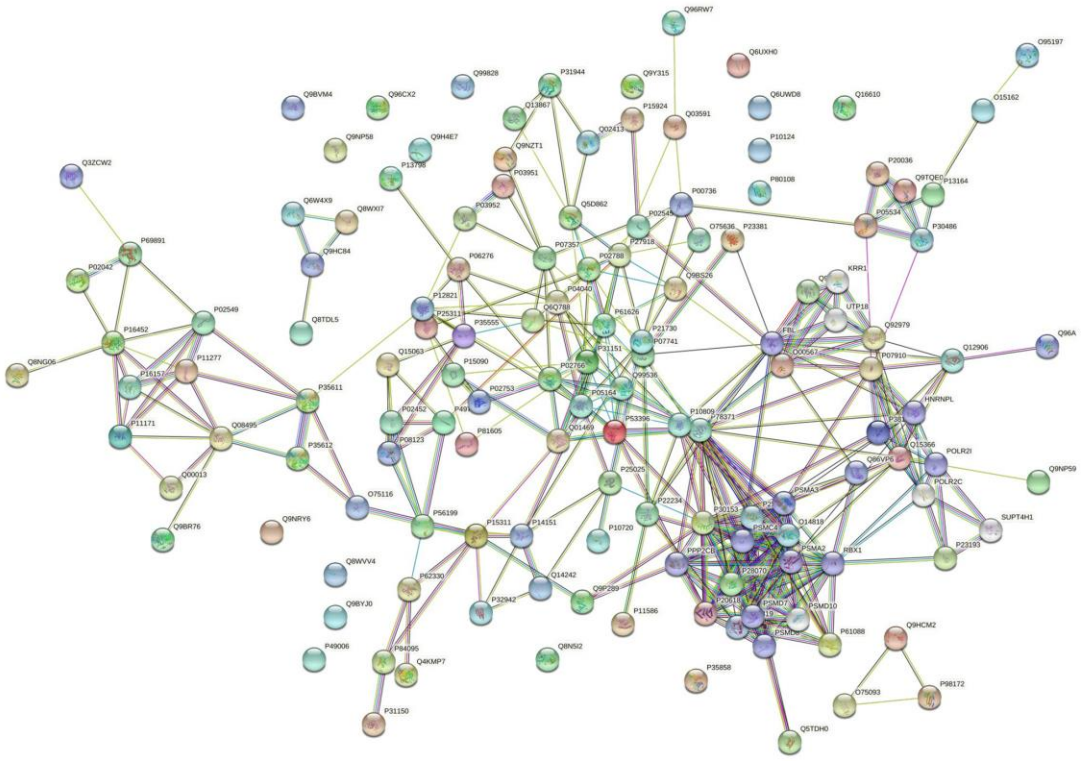

Figure 10. The PPI regulation network of down-regulated proteins in the PSCI group.

(sub-networks) were related to protein localization to juxtaparanode region of axon (yellow), cell adhesive protein binding, Fibrin Clot formation (green), mRNA splicing and RNA recognition (red), complement activation, lipid metabolism (purple), protein trafficking and cytoskeleton remodeling (lime green), and ATP-dependent degradation of ubiquitinated proteins (blue), respectively. The anticipated functional intermediate partners in the PPI network are presented in Table 8.

ELISA quantitative determination results of plasma proteins

Compared with control group, human 14-3-3 protein zeta/delta (YWHAZ) and human brain-specific angiogenesis suppressor 1 correlated protein 2 (BAIAP2) levels of plasma were significantly increased ($P < 0.01$) while that of human IgD (IGHD), human ATP binding cassette subfamily B member 6, mitochondrial (ABCB6) and human heat shock protein 60 (HSPD1) were significantly decreased in patients with and without PSCI ($P < 0.01$) (Figure 12).

DISCUSSION

General comments

To further explore the molecular mechanism of cognitive impairment, label-free quantitative proteomics were employed to analyse the differential expressed

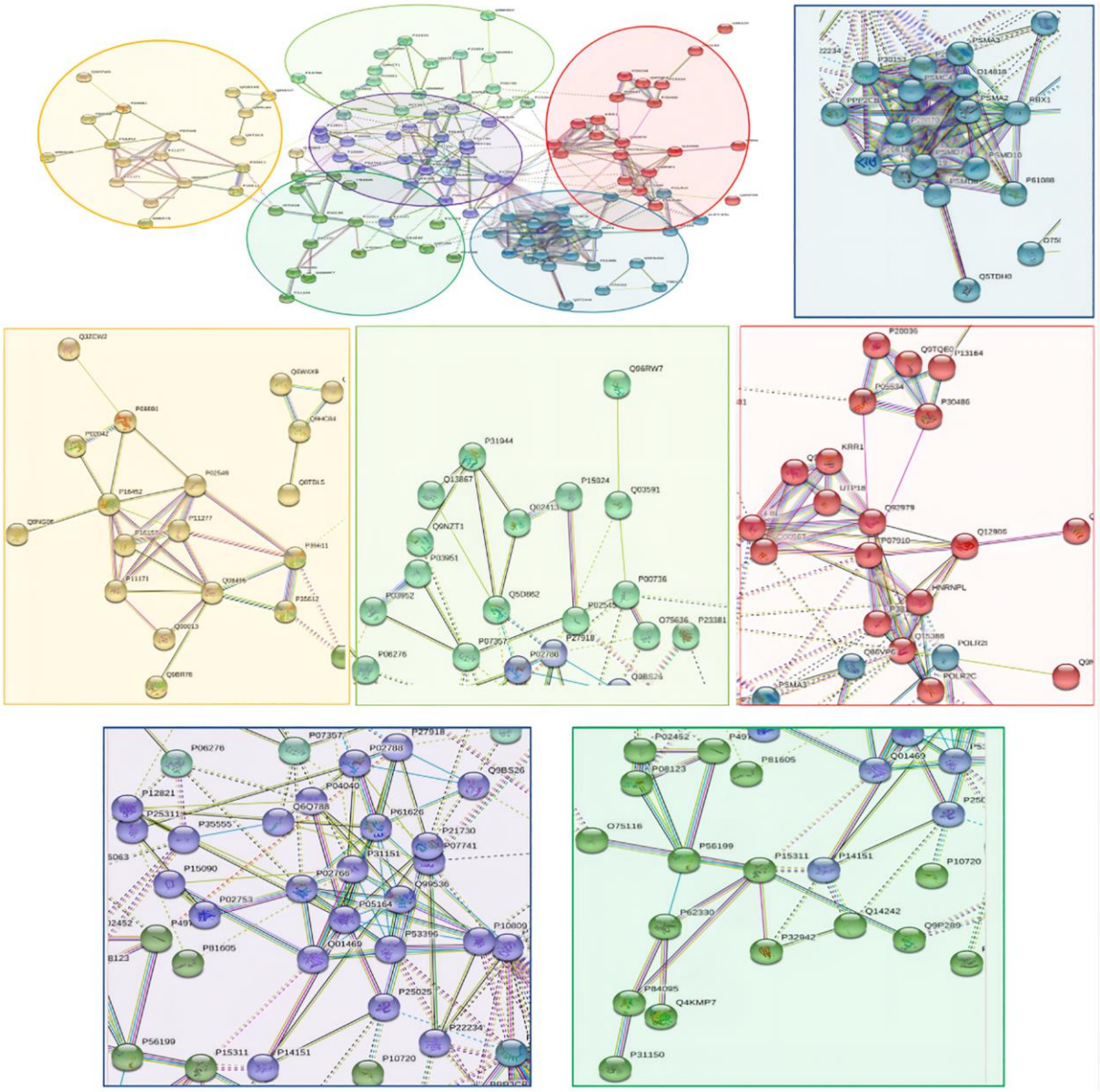

Figure 11. The PPI network means clustering of down-regulated proteins in the PSCI group. The PPI network is clustered to a specified number of clusters.

Table 8. The symbols and full names of the predicted functional intermediate partners in the PPI network of down-regulated expressed proteins shown in Figure 10.

No.	Accession No.	Symbol	Full name	Molecular function	Number of amino acids
1	P22087	FBL	rRNA 2′-O-methyltransferase fibrillarin	S-adenosyl-L-methionine-dependent methyltransferase that has the ability to methylate both RNAs and protein, catalyzing the site-specific 2′-hydroxyl methylation of ribose moieties in pre-ribosomal RNA.	321
2	P14866	HNRNPL	Heterogeneous nuclear ribonucleoprotein L	Splicing factor binding to exonic or intronic sites and acting as either an activator or repressor of exon inclusion, Exhibiting a binding preference for CA-rich elements.	589
3	P36954	POLR2I	DNA-directed RNA polymerase II subunit RPB9	DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates.	125
4	P62714	PPP2CB	Serine/threonine-protein phosphatase 2A catalytic subunit beta isoform	PP2A can modulate the activity of phosphorylase B kinase casein kinase 2, mitogen-stimulated S6 kinase, and MAP-2 kinase.	309
5	P25788	PSMA3	Proteasome subunit alpha type-3	Plays numerous essential roles within the cell by associating with different regulatory particles.	255
6	P48556	PSMD8	26S proteasome non-ATPase regulatory subunit 8	Component of the 26S proteasome and participates in apoptosis or DNA damage repair.	350
7	P62877	RBX1	E3 ubiquitin-protein ligase RBX1	E3 ubiquitin ligase component of multiple cullin-RING- based E3 ubiquitin-protein ligase (CRLs) complexes which mediate the ubiquitination and subsequent proteasomal degradation of target proteins.	108

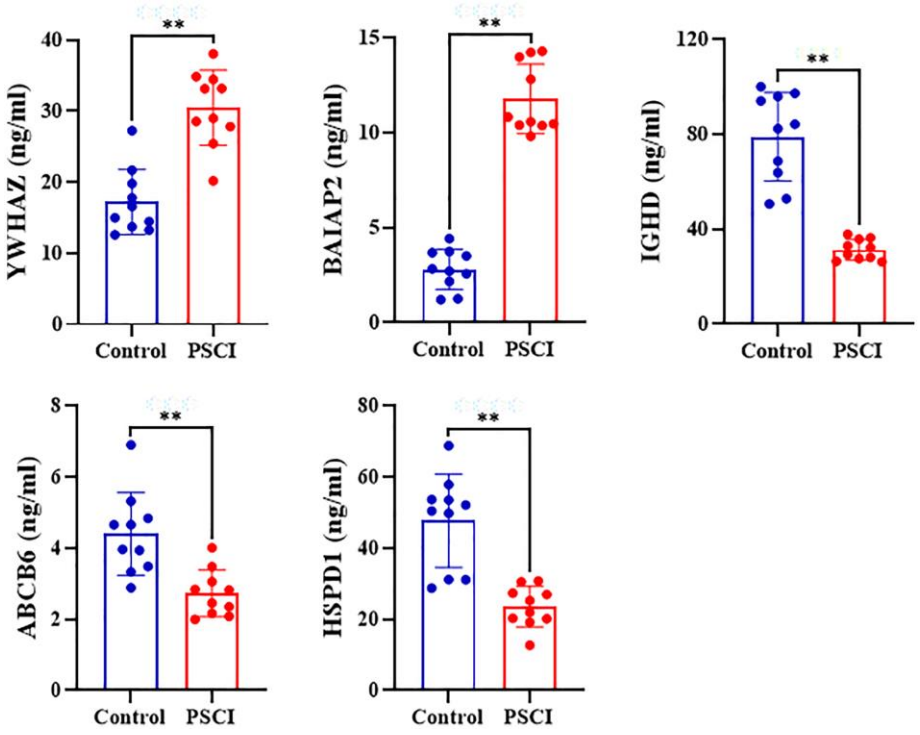

Figure 12. Plasma levels of human 14-3-3 protein zeta/delta (YWHAZ), human Brain-Specific Angiogenesis Inhibitor 1-Associated Protein 2 (BAIAP2), human IgD (IGHD), human ATP Binding Cassette Subfamily B Member 6, Mitochondrial (ABCB6), and human Heat Shock Protein 60 HSPD1 in patients with and without post stroke cognitive impairment.

proteins of plasma exosome in PSCI patients. Proteomics identified 259 differentially expressed proteins, containing 131 upregulated proteins and 128 downregulated proteins. These upregulated proteins are connected to ubiquitinated proteins degradation, calcium dependent protein binding, reorganization of cytoskeleton and platelet aggregation and blood coagulation. These downregulated proteins are related to protein localization to juxtaparanode region of axon, cell adhesive protein binding, fibrin clot formation, complement activation, lipid metabolism and ATP-dependent degradation of ubiquitinated proteins. The mechanisms of cognitive impairment of PSCI are related to blood flow regulation, energy metabolism, protein folding and degradation, cell apoptosis, synaptic plasticity. These were discussed in detail below.

Blood flow regulation associated proteins

Plasma kallikrein, a multifunctional serine protease associated with activation of contact coagulation [12]. Plasma kallikrein mechanisms of action can be utilized to support pro-thrombotic or anti-thrombotic characteristics. The kallikrein-kinin system suppresses thrombin-induced platelet activation, indicating an anti-thrombotic function [13]. Plasma kallikrein decreased collagen-induced platelet activation via binding collagen [14]. Whereas, the effect of pro-thrombotic is suggested by the plasma kallikrein critical role in contact activation by conversion of FXII to FXIIa. Additionally, plasma kallikrein converts prorenin to renin, which then converts angiotensinogen to angiotensin I [15]. Plasma kallikrein had been implicated in contributing to both hematoma expansion and thrombosis in stroke [16]. The outcomes of this investigation revealed that the plasma kallikrein expression was downregulated proteins. Plasma kallikrein may influence the occurrence and development of acute stroke through the activation and transformation pathway of FXII.

Platelet glycoprotein V (gpV), is a membrane constituent which containing an 82 kDa relative molecule mass, correlates with the leucine-rich proteins family. It is only expressed in platelets and megakaryocytes, and is non-covalently correlated with the gpIb-IX complex to develop a receptor for von Willebrand factor and thrombin [17, 18]. Hence, the GPIb-V-IX complex serve as the vWF receptor and modulates adhesion of vWF-dependent platelet to blood vessels. Platelet adhesion to damaged vascular surfaces in the arterial circulation is a crucial initiating event in hemostasis [19]. Platelet glycoprotein V may utilize as a platelet activation *in vivo* marker in thrombotic conditions. The expression of platelet-glycoprotein V in patients suffering from acute stroke is elevated, which is consistent with the study of Amin HM et al. [20]. Therefore, platelet glycoprotein V may play a protective role in the brain through blood coagulation. Fibrinogen like protein 1 (FGL1) is a released protein having mitogenic effect on primary hepatocytes. FGL1 includes an N-terminal signal recognition peptide, a potential N-terminal coil-coil domain, a C-terminal fibrinogen related domain (FReD) and multiple cysteines presumably utilized for inter and intra molecular disulfide bonds [21]. FGL1 may perform a potential function in these processes such as proliferation, angiogenesis, apoptosis and extracellular matrix modulation like structurally comparable proteins (angiopoietins, tenascins, fibrinogen) [22, 23]. Furthermore, the presence of FGL1 in the serum of rats after cytokine stimulation indicates that it could function as a significant biomarker for systemic inflammation [24]. Therefore, FGL1 may mediate the inflammatory response directly or indirectly in acute stroke. Our result of the increased expression of this protein just confirms this hypothesis.

Energy metabolism

ATP-binding cassette sub-family B member 6 (ABCB6), a member of adenosine triphosphate–binding cassette (ABC) transporter the family. It binds with heme and porphyrins and have a function in their ATP-dependent uptake into the mitochondria and plays a crucial role in the synthesis of heme [25]. Some researches have found that ABCB6 expression is protective against various results elevating oxidative stress, including exposure of arsenite [26, 27]. The outcomes of this investigation revealed that the expression level of ABCB6 reduced in patients suffering from acute stroke. The ABCB6 expression and function of closely related to the oxidation mechanism of mitochondria [25]. Therefore, ABCB6 may serve a protective function in the brain via antioxidant mechanisms.

Synaptic plasticity

Brain-specific angiogenesis inhibitor 1-associated protein 2 (BAIAP2), adapter protein that connects membrane-bound small G-proteins to cytoplasmic effector proteins. Subsequent researches have conclusively proven BAIAP2 serves as an essential regulator of membrane and actin dynamics at subcellular structures rich in actin, such as filopodia and lamellipodia [28–30]. Actin skeleton and its dynamics play an important role in the excitatory synaptic transmission and plasticity regulation [31, 32]. The results of this investigation revealed that the expression level of BAIAP2 increased in individuals with acute

stroke. In the brain, BAIAP2 may perform a neuroprotective function by improving synaptic function.

CONCLUSION

In conclusion, the present study found 259 differentially expressed proteins such as 131 upregulated proteins and 128 downregulated proteins using label-free quantitative proteomics approach in plasma exosome of PSCI patients. The findings suggested that the mechanism of cognitive impairment may be related to blood flow regulation, energy metabolism, protein folding and degradation, cell apoptosis, synaptic plasticity, stress response and protein phosphorylation in PSCI patients. Therefore, these proteins may be target-related proteins and shed light on pathogenesis mechanisms on a global scale of cognitive impairment at plasma exosome proteins level in PSCI patients. The disorders of plasma exosome proteomics may be explained the cognitive impairment in PSCI patients. Further association studies need to be clarified.

AUTHOR CONTRIBUTIONS

Qi BY and Wei DF designed the research; Qi BY, Wei DF, Kong LB, Lai XX, Wang LS, Fei Liu F and Ji WW performed research and analyzed data; Qi BY and Wei DF wrote the manuscript; Qi BY, Ji WW and Wei DF revised the manuscript. The final manuscript was reviewed and approved by all authors.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest related to this study.

ETHICAL STATEMENT AND CONSENT

The study was approved by the Ethics Committee of Dongzhimen Hospital affiliated with Beijing University of Chinese Medicine (approval number: DZMEC-KY-2019-04). The written consent of all participants or their legal guardians has been obtained.

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SUPPLEMENTARY MATERIALS

Supplementary Tables

Supplementary Table 1. The identification results of the other 101 up-regulated proteins between the PSCI and control groups.

No.	UniProt ID	Target protein	Gene symbol	Protein score	Mr (kDa)	Fold change (PSCI/ Control)	*P*-value
1	Q04828	Aldo-keto reductase family 1 member C1	AKR1C1	7.0284	36.788	4.13	<0.001
2	P50995	Annexin A11	ANXA11	55.951	54.389	3.55	<0.001
3	P20073	Annexin A7	ANXA7	60.69	52.739	4.08	<0.001
4	Q4KMQ2	Anoctamin-6	ANO6	16.419	106.16	2.94	0.018
5	Q96QS1	Tetraspanin-32	TSPAN32	17.05	34.63	6.05	<0.001
6	O75882	Attractin	ATRN	5.3994	158.54	2.06	0.008
7	P55957	BH3-interacting domain death agonist	BID	5.4515	21.994	3.07	0.001
8	Q9UBW5	Bridging integrator 2	BIN2	16.654	61.874	5.65	<0.001
9	Q9Y376	Calcium-binding protein 39	CAB39	6.2081	39.869	13.75	<0.001
10	P22694	cAMP-dependent protein kinase catalytic subunit beta	PRKACB	15.569	40.622	2.84	<0.001
11	P06731	Carcinoembryonic antigen-related cell adhesion molecule 5	CEACAM5	3.4781	76.794	5.52	0.001
12	P20645	Cation-dependent mannose-6-phosphate receptor	M6PR	4.1554	30.993	2.10	<0.001
13	P48509	CD151 antigen	CD151	22.583	28.295	2.24	<0.001
14	Q96DZ9	CKLF-like MARVEL transmembrane domain-containing protein 5	CMTM5	8.2909	24.652	8.11	<0.001
15	P30447	Class I histocompatibility antigen, A-23 alpha chain	HLA-A	17.273	40.732	11.92	<0.001
16	Q96FN4	Copine-2	CPNE2	4.6578	61.189	2.08	<0.001
17	O75367	Core histone macro-H2A.1	H2AFY	5.632	39.617	2.09	<0.001
18	P61073	C-X-C chemokine receptor type 4	CXCR4	10.04	39.745	11.32	<0.001
19	Q9Y4D1	Disheveled-associated activator of morphogenesis 1	DAAM1	25.077	123.47	3.47	0.032
20	O14672	Disintegrin and metalloproteinase domain-containing protein 10	ADAM10	87.302	84.141	2.26	<0.001
21	O60762	Dolichol-phosphate mannosyltransferase subunit 1	DPM1	3.0248	29.634	4.54	<0.001
22	O00429	Dynamin-1-like protein	DNM1L	4.8548	81.876	3.84	<0.001
23	P42892	Endothelin-converting enzyme 1	ECE1	8.9951	87.163	10.28	<0.001
24	Q12929	Epidermal growth factor receptor kinase substrate 8	EPS8	18.03	91.88	7.17	<0.001
25	Q96PL5	Erythroid membrane-associated protein	ERMAP	6.8807	52.604	3.93	<0.001
26	Q9NVI1	Fanconi anemia group I protein	FANCI	2.4495	149.32	4.29	<0.001
27	Q15149	Plectin	PLEC	19.19	531.78	2.12	0.003
28	Q86WI1	Fibrocystin-L	PKHD1L1	8.8806	465.73	3.29	<0.001
29	P02751	Fibronectin	FN1	323.31	262.62	2.03	<0.001
30	P19086	Guanine nucleotide-binding protein G(z) subunit alpha	GNAZ	36.541	40.923	4.24	<0.001
31	O95837	Guanine nucleotide-binding protein subunit alpha-14	GNA14	6.3116	41.57	3.93	<0.001
32	P51790	H(+)/Cl(−) exchange transporter 3	CLCN3	4.0367	90.965	6.38	<0.001
33	P00739	Haptoglobin-related protein	HPR	125.79	39.029	2.38	<0.001
34	P80422	Ig gamma lambda chain V-II region DOT		2.8868	11.787	3.33	0.018
35	P01604	Ig kappa chain V-I region Kue		3.2276	12.126	9.77	<0.001
36	P01610	Ig kappa chain V-I region WEA		4.7707	11.84	10.45	0.047
37	P18135	Ig kappa chain V-III region HAH		9.7585	14.073	12.72	0.002
38	P06311	Ig kappa chain V-III region IARC/BL41		27.922	14.07	22.59	0.001
39	P06889	Ig lambda chain V-IV region MOL		10.976	11.143	26.71	<0.001
40	P52292	Importin subunit alpha-1	KPNA2	5.1473	57.861	8.02	<0.001

41	P17301	Integrin alpha-2	ITGA2	93.857	129.29	2.26	<0.001
42	P23229	Integrin alpha-6	ITGA6	165.92	126.6	2.70	<0.001
43	P08514	Integrin alpha-IIb	ITGA2B	323.31	113.38	2.59	<0.001
44	P05556	Integrin beta-1	ITGB1	93.303	88.414	2.02	<0.001
45	Q27J81	Inverted formin-2	INF2	10.723	135.62	2.59	<0.001
46	Q9Y624	Junctional adhesion molecule A	F11R	37.373	32.583	3.10	<0.001
47	Q9BX67	Junctional adhesion molecule C	JAM3	7.2824	35.02	3.12	<0.001
48	P42704	Leucine-rich PPR motif-containing protein, mitochondrial	LRPPRC	2.5029	157.9	2.34	0.046
49	Q6ZUX7	Lipoma HMGIC fusion partner-like 2 proteins	LHFPL2	5.4873	24.486	16.11	<0.001
50	P18428	Lipopolysaccharide-binding protein	LBP	68.595	53.383	3.19	<0.001
51	Q5SQ64	Lymphocyte antigen 6 complex locus protein G6f	LY6G6F	9.1082	32.464	2.39	<0.001
52	P11279	Lysosome-associated membrane glycoprotein 1	LAMP1	8.3269	44.882	7.21	<0.001
53	Q15555	Microtubule-associated protein RP/EB family member 2	MAPRE2	7.2936	37.031	4.92	0.005
54	Q92619	Minor histocompatibility protein HA-1	HMHA1	9.453	124.61	10.33	<0.001
55	P28482	Mitogen-activated protein kinase 1	MAPK1	2.6431	41.389	2.53	0.016
56	O15427	Monocarboxylate transporter 4	SLC16A3	7.7629	49.469	4.83	0.001
57	Q15746	Myosin light chain kinase, smooth muscle	MYLK	8.0124	210.71	2.43	0.036
58	Q9Y2A7	Nck-associated protein 1	NCKAP1	18.888	128.79	2.21	<0.001
59	P35579	Myosin-9	MYH9	323.31	226.53	2.20	<0.001
60	Q02818	Nucleobindin-1	NUCB1	10.379	53.879	3.93	<0.001
61	Q92882	Osteoclast-stimulating factor 1	OSTF1	4.9459	23.787	2.49	<0.001
62	O00151	PDZ and LIM domain protein 1	PDLIM1	22.463	36.071	2.59	0.008
63	P78356	Phosphatidylinositol 5-phosphate 4-kinase type-2 beta	PIP4K2B	3.3849	47.377	2.61	<0.001
64	P18669	Phosphoglycerate mutase 1	PGAM1	4.9925	28.804	4.60	0.016
65	Q13835	Plakophilin-1	PKP1	3.0107	82.86	15.11	<0.001
66	P07359	Platelet glycoprotein Ib alpha chain	GP1BA	72.749	71.539	2.30	<0.001
67	P13224	Platelet glycoprotein Ib beta chain	GP1BB	34.808	21.717	2.50	<0.001
68	P08567	Pleckstrin	PLEK	96.786	40.124	3.68	<0.001
69	Q6UX71	Plexin domain-containing protein 2	PLXDC2	3.1051	59.582	5.86	<0.001
70	Q9BUL8	Programmed cell death protein 10	PDCD10	6.8355	24.701	2.55	0.049
71	O75340	Programmed cell death protein 6	PDCD6	11.365	21.868	2.14	<0.001
72	O00231	proteasome non-ATPase regulatory subunit 11	PSMD11	2.9363	47.463	2.14	0.002
73	O60610	Protein diaphanous homolog 1	DIAPH1	24.691	141.35	2.72	<0.001
74	O95866	Protein G6b	G6B	27.188	26.163	3.24	<0.001
75	P05771	Protein kinase C beta type	PRKCB	16.466	76.868	3.58	<0.001
76	Q9Y2J8	Protein-arginine deiminase type-2	PADI2	4.2012	75.563	2.30	0.004
77	Q96RI0	Proteinase-activated receptor 4	F2RL3	3.8785	41.133	5.32	<0.001
78	P16109	P-selectin	SELP	28.054	90.833	2.07	0.050
79	Q14644	Ras GTPase-activating protein 3	RASA3	65.234	95.698	2.73	<0.001
80	P46940	Ras GTPase-activating-like protein IQGAP1	IQGAP1	14.068	189.25	3.83	0.026
81	Q7LDG7	RAS guanyl-releasing protein 2	RASGRP2	13.93	69.248	2.51	<0.001
82	P18433	Receptor-type tyrosine-protein phosphatase alpha	PTPRA	11.171	90.599	2.12	0.017
83	Q9HBH0	Rho-related GTP-binding protein RhoF	RHOF	5.2317	23.625	4.04	<0.001
84	Q9BRU9	rRNA-processing protein UTP23 homolog	UTP23	2.6882	28.402	23.83	0.011
85	Q9NUV7	Serine palmitoyltransferase 3	SPTLC3	2.7378	62.049	2.30	<0.001
86	O95810	Serum deprivation-response protein	SDPR	59.51	47.173	2.86	<0.001
87	A6NMB1	Sialic acid-binding Ig-like lectin 16	SIGLEC16	2.8659	52.991	2.17	<0.001
88	P30626	Sorcin	SRI	14.391	21.676	5.40	<0.001
89	P09486	SPARC	SPARC	14.135	34.632	2.05	0.011
90	Q15833	Syntaxin-binding protein 2	STXBP2	94.853	66.452	2.94	<0.001
91	P17987	T-complex protein 1 subunit alpha	TCP1	9.5532	60.343	2.33	<0.001

92	P49368	T-complex protein 1 subunit gamma	CCT3	20.31	60.533	4.59	<0.001
93	Q86UF1	Tetraspanin-33	TSPAN33	23.298	31.538	3.87	<0.001
94	P13693	Translationally-controlled tumor protein	TPT1	2.975	19.595	2.85	0.005
95	P30408	Transmembrane 4 L6 family member 1	TM4SF1	3.2213	21.632	3.63	<0.001
96	P68366	Tubulin alpha-4A chain	TUBA4A	14.7	49.924	5.38	<0.001
77	Q14642	Type I inositol 1,4,5-trisphosphate 5-phosphatase	INPP5A	3.5125	47.819	2.14	<0.001
98	P06241	Tyrosine-protein kinase Fyn	FYN	4.4482	60.761	2.35	<0.001
99	Q9NPG3	Ubinuclein-1	UBN1	2.7915	121.52	4.40	0.002
100	P41226	Ubiquitin-like modifier-activating enzyme 7	UBA7	4.9628	111.69	5.16	<0.001
101	O95498	Vascular non-inflammatory molecule 2	VNN2	7.7606	58.502	2.10	0.024

In the title line, Exp. Mr represented the experimental molecular weight of the proteins.

Supplementary Table 2. The identification results of other 98 down-regulated proteins between the PSCI and control groups.

No.	UniProt ID	Target protein	Gene Symbol	Protein Score	Mr (kDa)	Fold change (PSCI/ Control)	*P*-value
1	Q00013	55 kDa erythrocyte membrane protein	MPP1	29.003	52.296	0.23	<0.001
2	P13798	Acylamino-acid-releasing enzyme	APEH	25.707	81.224	0.39	<0.001
3	P07741	Adenine phosphoribosyltransferase	APRT	5.640	19.608	0.23	<0.001
4	P35611	Alpha-adducin	ADD1	64.705	80.954	0.12	<0.001
5	P12821	Angiotensin-converting enzyme	ACE	47.608	149.710	0.45	<0.001
6	P16157	Ankyrin-1	ANK1	323.310	206.260	0.45	<0.001
7	Q6Q788	Apolipoprotein A-V	APOA5	26.542	41.212	0.38	<0.001
8	Q8N5I2	Arrestin domain-containing protein 1	ARRDC1	5.041	45.981	0.19	<0.001
9	P35612	Beta-adducin	ADD2	46.085	80.853	0.18	<0.001
10	Q13867	Bleomycin hydrolase	BLMH	3.605	52.562	0.15	<0.001
11	Q8TDL5	BPI fold-containing family B member 1	BPIFB1	11.262	52.441	0.05	<0.001
12	Q96CX2	BTB/POZ domain-containing protein KCTD12	KCTD12	6.607	35.700	0.12	<0.001
13	P11586	C-1-tetrahydrofolate synthase, cytoplasmic	MTHFD1	6.445	101.560	0.08	<0.001
14	P21730	C5a anaphylatoxin chemotactic receptor 1	C5AR1	13.851	39.335	0.36	<0.001
15	Q9NZT1	Calmodulin-like protein 5	CALML5	4.879	15.892	0.37	0.001
16	P49747	Cartilage oligomeric matrix protein	COMP	16.663	82.860	0.20	0.006
17	P31944	Caspase-14	CASP14	12.773	27.679	0.41	<0.001
18	P04040	Catalase	CAT	158.240	59.755	0.46	<0.001
19	Q9TQE0	Class II histocompatibility antigen, DRB1-9 beta chain	HLA-DRB1	35.992	29.826	0.41	<0.001
20	P03951	Coagulation factor XI	F11	28.429	70.108	0.39	<0.001
21	P02452	Collagen alpha-1(I) chain	COL1A1	3.211	138.940	0.13	<0.001
22	P08123	Collagen alpha-2(I) chain	COL1A2	6.514	129.310	0.47	<0.001
23	P00736	Complement C1r subcomponent	C1R	87.215	80.118	0.45	<0.001
24	Q03591	Complement factor H-related protein 1	CFHR1	3.435	37.650	0.46	<0.001
25	Q9BR76	Coronin-1B	CORO1B	3.423	54.234	0.40	<0.001
26	Q86VP6	Cullin-associated NEDD8-dissociated protein 1	CAND1	7.461	136.370	0.45	<0.001
27	P25025	C-X-C chemokine receptor type 2	CXCR2	5.076	40.759	0.19	0.001
28	Q08495	Dematin	DMTN	50.325	45.514	0.38	<0.001
29	Q9Y315	Deoxyribose-phosphate aldolase	DERA	11.727	35.230	0.31	0.001
30	P81605	Dermcidin	DCD	8.586	11.284	0.39	<0.001
31	Q02413	Desmoglein-1	DSG1	14.071	113.750	0.14	<0.001
32	P15924	Desmoplakin	DSP	107.470	331.770	0.02	<0.001

33	Q9H4E7	Differentially expressed in FDCP 6 homolog	DEF6	6.243	73.910	0.10	<0.001
34	P98172	Ephrin-B1	EFNB1	7.399	38.006	0.33	<0.001
35	P16452	Erythrocyte membrane protein band 4.2	EPB42	211.300	77.008	0.46	<0.001
36	Q16610	Extracellular matrix protein 1	ECM1	10.391	60.673	0.47	0.024
37	P15311	Ezrin	EZR	36.780	69.412	0.38	<0.001
38	P15090	Fatty acid-binding protein, adipocyte	FABP4	9.663	14.719	0.44	0.001
39	Q01469	Fatty acid-binding protein, epidermal	FABP5	13.170	15.164	0.40	<0.001
40	P35555	Fibrillin-1	FBN1	20.479	312.240	0.25	<0.001
41	Q9BYJ0	Fibroblast growth factor-binding protein 2	FGFBP2	6.010	24.581	0.34	<0.001
42	O75636	Ficolin-3	FCN3	130.590	32.903	0.48	<0.001
43	Q3ZCW2	Galectin-related protein	LGALSL	3.739	18.986	0.31	0.024
44	Q9BVM4	Gamma-glutamylaminecyclotransferase	GGACT	2.631	17.328	0.14	<0.001
45	Q96RW7	Hemicentin-1	HMCN1	9.912	613.380	0.25	<0.001
46	P02042	Hemoglobin subunit delta	HBD	42.271	16.055	0.28	<0.001
47	P69891	Hemoglobin subunit gamma-1	HBG1	11.663	16.140	0.33	<0.001
48	P07910	Heterogeneous nuclear ribonucleoproteins C1/C2	HNRNPCL4	11.389	33.670	0.11	<0.001
49	P05534	HLA class I histocompatibility antigen, A-24 alpha chain	HLA-A	57.269	40.688	0.49	0.003
50	P30450	HLA class I histocompatibility antigen, A-26 alpha chain	HLA-A	57.588	41.061	0.22	<0.001
51	P20036	HLA class II histocompatibility antigen, DP alpha 1 chain	HLA-DPA1	2.810	29.380	0.14	<0.001
52	P01621	Ig kappa chain V-III region NG9 (Fragment)		2.595	10.729	0.02	<0.001
53	P04209	Ig lambda chain V-II region NIG-84		3.539	11.581	0.50	<0.001
54	P35858	Insulin-like growth factor-binding protein complex acid labile subunit	IGFALS	10.043	66.034	0.34	<0.001
55	P32942	Intercellular adhesion molecule 3	ICAM3	11.777	59.540	0.31	<0.001
56	Q12906	Interleukin enhancer-binding factor 3	ILF3	3.242	95.337	0.32	<0.001
57	P02788	Lactotransferrin	LTF	43.636	78.181	0.31	<0.001
58	Q96AG4	Leucine-rich repeat-containing protein 59	LRRC59	5.097	34.930	0.49	0.001
59	P14151	L-selectin	SELL	34.681	42.187	0.29	<0.001
60	P61626	Lysozyme C	LYZ	7.020	16.537	0.22	<0.001
61	P49006	MARCKS-related protein	MARCKSL1	9.031	19.529	0.24	<0.001
62	Q02817	Mucin-2	MUC2	64.496	540.290	0.36	<0.001
63	Q9HC84	Mucin-5B	MUC5B	238.930	596.330	0.20	<0.001
64	Q6W4X9	Mucin-6	MUC6	9.907	257.050	0.26	0.002
65	P05164	Myeloperoxidase	MPO	5.226	83.868	0.10	<0.001
66	O00567	Nucleolar protein 56	NOP56	7.993	66.049	0.40	<0.001
67	Q15063	Periostin	POSTN	6.397	93.313	0.12	<0.001
68	P80108	Phosphatidylinositol-glycan-specific phospholipase D	GPLD1	4.472	92.335	0.33	<0.001
69	Q9NRY6	Phospholipid scramblase 3	PLSCR3	7.649	31.648	0.23	<0.001
70	P10720	Platelet factor 4 variant	PF4V1	7.572	11.553	0.26	<0.001
71	Q15366	Poly(rC)-binding protein 2	PCBP2	16.973	38.580	0.19	<0.001
72	Q9Y2R4	Probable ATP-dependent RNA helicase DDX52	DDX52	3.420	67.497	0.23	<0.001
73	P27918	Properdin	CFP	34.547	51.276	0.46	<0.001
74	P25789	Proteasome subunit alpha type-4	PSMA4	8.810	29.483	0.42	<0.001
75	P28070	Proteasome subunit beta type-4	PSMB4	5.199	29.204	0.48	<0.001
76	P11171	Protein 4.1	EPB41	192.910	97.016	0.30	<0.001
77	Q5TDH0	Protein DDI1 homolog 2	DDI2	4.051	44.522	0.49	<0.001

78	Q8WVV4	Protein POF1B	POF1B	21.398	68.064	0.38	0.002
79	P31151	Protein S100-A7	S100A7	3.630	11.471	0.39	<0.001
80	Q14242	P-selectin glycoprotein ligand 1	SELPLG	12.039	43.201	0.27	<0.001
81	P31150	Rab GDP dissociation inhibitor alpha	GDI1	15.814	50.582	0.35	<0.001
82	O95197	Reticulon-3	RTN3	3.396	112.610	0.43	0.001
83	P02753	Retinol-binding protein 4	RBP4	12.398	23.010	0.42	<0.001
84	O75116	Rho-associated protein kinase 2	ROCK2	10.343	160.900	0.19	<0.001
85	P84095	Rho-related GTP-binding protein	RHOG	24.350	21.308	0.30	<0.001
86	Q92979	Ribosomal RNA small subunit methyltransferase NEP1	EMG1	3.192	26.720	0.42	0.014
87	P38159	RNA-binding motif protein, X chromosome	RBMX	6.121	42.331	0.48	0.004
88	P10124	Serglycin	SRGN	13.192	17.652	0.28	<0.001
89	O75093	Slit homolog 1 protein	SLIT1	3.174	167.920	0.49	0.005
90	P02549	Spectrin alpha chain, erythrocytic 1	SPTA1	323.310	280.010	0.34	<0.001
91	P11277	Spectrin beta chain, erythrocytic	SPTB	323.310	246.470	0.32	<0.001
92	Q4KMP7	TBC1 domain family member 10B	TBC1D10B	2.593	87.198	0.41	0.001
93	P78371	T-complex protein 1 subunit beta	CCT2	27.382	57.488	0.34	<0.001
94	P23193	Transcription elongation factor A protein 1	TCEA1	35.693	33.969	0.31	0.021
95	Q6UWD8	Transmembrane protein C16orf54	C16orf54	24.211	24.359	0.44	<0.001
76	P02766	Transthyretin	TTR	4.849	15.887	0.46	<0.001
97	P23381	Tryptophan-tRNA ligase, cytoplasmic	WARS	5.162	53.165	0.30	<0.001
98	P61088	Ubiquitin-conjugating enzyme E2 N	UBE2N	3.957	17.138	0.22	<0.001

In the title line, Exp. Mr represented the experimental molecular weight of the proteins.