Original Research

Differentially Expressed Proteins in the Serum of Elderly Patients Who Experienced Perioperative Neurocognitive Disorders Following Transurethral Resection of the Prostate

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Abstract

Objective: Perioperative neurocognitive disorders (PND) are a group of prevalent neurological complications that often occur in elderly individuals following major or emergency surgical procedures. The etiologies are not fully understood. This study endeavored to investigate novel targets and prediction methods for the occurrence of PND. Methods: A total of 229 elderly patients diagnosed with prostatic hyperplasia who underwent transurethral resection of the prostate (TURP) combined with spinal cord and epidural analgesia were included in this study. The patients were divided into two groups, the PND group and non-PND group, based on the Z-score method. According to the principle of maintaining consistency between preoperative and intraoperative conditions, three patients from each group were randomly chosen for serum sample collection. Isobaric tags for relative and absolute quantification (iTRAQ) proteomics technology was employed to analyze and identify the proteins that exhibited differential expression in the serum samples from the two groups. Bioinformatics analysis was performed on the proteins that exhibited differential expression. Results: Among the 1101 serum proteins analyzed in the PND and non-PND groups, eight differentially expressed proteins were identified in PND patients. Of these, six proteins showed up-regulation, while two proteins showed down-regulation. Further bioinformatics analysis of the proteins that exhibited differential expression revealed their predominant involvement in cellular biological processes, cellular component formation, as well as endocytosis and phagocytosis. Additionally, these proteins were found to possess the RING domain of E3 ubiquitin ligase. Conclusion: This study successfully identified eight proteins that exhibited differential expression levels between the two groups. Bioinformatics analysis indicates that proteins exhibiting differential expression are primarily implicated in the biological processes associated with microtubules. Investigating the microtubule formation process as it relates to neuroplasticity and synaptic formation may offer valuable insights for enhancing our comprehension and potential prevention of PND. Clinical Trial Registration: Registered (ChiCTR2000028836). Date (20190306).

Keywords: elderly patients; perioperative neurocognitive dysfunction; transurethral prostatectomy; iTRAQ technology; differentially expressed protein; surgery

1. Introduction

Perioperative neurocognitive disorders (PND), also known as postoperative neurocognitive dysfunction, refer to the deterioration in a range of cognitive functions that occurs during the perioperative period. These abnormalities encompass two distinct conditions, postoperative delirium (POD) and postoperative cognitive dysfunction (POCD) [1,2]. PND primarily presents as a range of neurocognitive impairments, including attention deficits, memory problems, executive dysfunction, and reduced information processing speed. The primary characteristics observed in patients following anesthesia and surgery are the persistent and progressive deterioration of their cognitive function. PND not only diminishes the overall quality of life for patients following surgery, but also extends the duration of hospital stay and even elevates patient mortality rates [3,4]. Extensive research has demonstrated that the occurrence of POCD in adults aged 65 years and above is reported to range from 9% to 54% within 1 week after surgery, 10% to 17% within 3 months after surgery, and 3% within 1 year [5–7]. Given the significant prevalence of this issue, it is therefore crucial to investigate more effective strategies for proactive prevention and intervention. The focus of research on PND currently primarily lies within the domains of major orthopedic surgery and cardiac surgery. There is a limited amount of literature available on PND following transurethral resection of the prostate (TURP). The majority of patients diagnosed with PND are elderly indi-
Individuals with compromised immune systems and poor physical fitness. Additionally, they often have comorbidities such as cardiovascular and cerebrovascular diseases. Consequently, postoperative infections can lead to the development of complications. The occurrence of PND frequently presents challenges to the patient’s postoperative care, consequently leading to an extended duration of hospitalization and escalated healthcare expenses.

As the fundamental element of human physiology, blood has the ability to effectively reflect an individual’s physiological condition and phenotype. The concentrations of different plasma proteins in clinical settings can act as molecular markers or highlight potentially pathogenic molecules that can aid in the diagnosis and treatment of various diseases. Nevertheless, given the vast array of protein types present in serum, precisely targeting and identifying important proteins poses a significant challenge. However, the field of proteomics offers the potential to effectively address this technical challenge. In recent years, there has been significant progress in proteomics technology, particularly with the advent of high-resolution mass spectrometry. This has enabled researchers to conduct in-depth, high-throughput, and rapid proteomic analysis. In the field of protein marker research, the utilization and application of isobaric tags for relative and absolute quantification (iTRAQ) have gained significant prominence. Through the utilization of rapid and high-throughput quantitative analysis techniques, it has become possible to compare proteome differences between samples [8–10]. The current dearth of high-throughput research on PND has hindered the identification of potential biomarkers. In the investigation of various neurological disorders, iTRAQ proteomics has been utilized and has demonstrated significant contributions. In the study of Alzheimer’s disease (AD), researchers initially employed iTRAQ to identify proteins that are differentially expressed in AD. Subsequently, through validation using expression quantitative detection technology, they identified potential molecular markers of AD, such as tau protein [11]. Therefore, in the field of PND research, our objective is to utilize iTRAQ proteomics technology to identify and isolate potentially significant protein molecules. This approach aims to identify target molecules or biological markers that can be further investigated in subsequent research endeavors.

The present study utilized a group of patients undergoing elective transurethral prostatectomy, along with their general information, blood samples, and cerebrospinal fluid samples. A description of the patient’s general information and analysis of cerebrospinal fluid proteomic data has been previously published [12]. In the present study, iTRAQ proteomics was employed to identify differentially expressed proteins in serum between patients with PND and patients without PND. Additionally, the functional annotation of these proteins was examined to investigate the potential functions of these differentially expressed proteins, as well as the underlying mechanisms in PND.

2. Materials and Methods

2.1 Participants

This study was conducted on a cohort of 229 patients who were scheduled to undergo elective transurethral resection of the prostate at The Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University. This study was registered with the Chinese Clinical Trial Registry (ChiCTR2000028836) and received approval from the ethics committee of Sichuan Tianfu New District People’s Hospital (20190306-05). Further details can be found in our previous publication [12]. The process is described in Fig. 1.

The inclusion criteria for this study were as follows: (a) Patients undergoing elective electroprostatectomy in Chengdu Tianfu New District People’s Hospital between January 2020 to January 2021; (b) elderly patients over 65 years of age; (c) American society of Anesthesiologists (ASA) classification I-III; (d) General anesthesia; (e) No previous history of cerebrovascular accidents; (f) No his-
Fig. 2. Quality control analysis of iTRAQ proteomics. (A) Distribution of mass error of all identified peptides. The abscissa represents protein mass delta (PPM) and the ordinate represents peptide score. (B) The length distribution of all identified peptides. The abscissa represents peptide length, the ordinate represents the number of peptides. (C) Peptide quantitative ratio distribution of all identified peptides. The abscissa represents peptide ratio and the ordinate represents the number of peptides.

tery of substance abuse; and (g) preoperative Mini Men-
tal State Examination (MMSE) score was greater than the
minimum score of the corresponding education level, such
as 17 points for illiteracy, 20 points for elementary school
education, 23 points for middle school and above [13].

The exclusion criteria for this study were as follows: (a)
Patients unwilling to cooperate with the cognitive func-
tion scale; (b) Patients with dementia and neurological
diseases; (c) Patients with severe respiratory, circulatory
or other systemic dysfunction; (d) Patients unwilling to coo-
perate with blood drawing; (f) Patients with low compliance,
severe hearing and visual impairment, reading and compre-
hension impairment and who did not cooperate with MMSE
testing and blood draw.

2.2 Grouping

According to the criteria for PND, patients were cat-
ergized into two groups: the PND group and the non-
perioperative neurocognitive disorders (non-PND) group,
based on their cognitive function in the 3 days following
surgery. In order to conduct iTRAQ proteomics analy-
sis, subjects were selected from both the PND group and
the non-PND group, ensuring no differences in intraoper-
ative indicators such as ASA classification, body mass in-
dex (BMI) index, MMSE score, and comorbidities. Addition-
ally, intraoperative indicators including operation time,
anesthesia time, intraoperative fluid volume, and blood
loss, were also considered for analysis.

2.3 PND Criteria

The MMSE score was employed as an assessment tool
for PND. The MMSE is a straightforward cognitive func-
tion screening tool (https://www.parine.com/Products/Pkey
/237) [14]. However, it is important to note that the MMSE
has limitations, including a capping effect and learning ef-
fect, which can reduce its sensitivity to detecting subtle
changes in cognitive function following surgery. The Inter-
national Study of Postoperative Dysfunction (ISPOCD)
has introduced a novel assessment approach known as the “Z-
score” scoring method, which is currently recognized as the
standard for evaluating POCD [15]. The cognitive function
of patients was assessed with MMSE by a senior Psychia-
trist 1 day before and 3 days after surgery. Presence of PND was determined using a Z-score based method, where 20 healthy people (age > 65 years) who did not receive surgery and had normal cognitive function during the same period was used as reference group. Z-score was defined as: $Z = (\Delta X - \Delta X_{\text{Reference}})/SD(\Delta X)$, where $\Delta X$ denotes the difference of two MMSE measurements before and after surgery in TURP patients, $\Delta X_{\text{Reference}}$ denotes the difference of two MMSE measurements of reference group individuals (this value was subtracted to adjust for practice effect), and $SD(\Delta X)$ Reference denotes the standard deviation of the difference of two MMSE measurements of reference group individuals. PND was diagnosed if $|Z| > 1.96$. According to the Z-score, TURP patients were classified as belonging to the PND group or the non-PND group.

2.4 Anesthesia and the Surgical Procedure

The demographic and clinical characteristics of all patients were recorded prior to surgery, including age, duration of illness, level of education, presence of other comorbidities, ASA classification, BMI index, preoperative MMSE score, and past medical history.

Routine preoperative visits were conducted to evaluate patients’ vital organ functions and their ability to tolerate anesthesia. These visits also aimed to identify any contraindications to anesthesia and to provide patients with information regarding the standard preoperative fasting and drinking protocols. Venous access was established upon the patient’s arrival in the room. Oxygen was administered via a mask, and the patient’s vital signs were closely monitored. Additionally, rescue medication was prepared as a precautionary measure to mitigate the risk of severe hypotension after anesthesia. Anesthesia induction was performed using intravenous bolus injection of propofol (Sichuan Guorui Pharmaceutical Co., Ltd., Chengdu, Sichuan, China) at a dose of 1.5–2 mg/kg or etomidate (Jiangsu Nhwa Pharmaceutical Co., Ltd., Xuzhou, Jiangsu, China) at a dose of 0.3–0.4 mg/kg, sufentanil (Yichang Humanwell Pharmaceuticals Co., Ltd., Yichang, Hubei, China) at a dose of 0.3–0.4 μg/kg, and cis-atracurium (Jiangsu Hengrui Pharmaceuticals Co., Ltd., Xuzhou, Jiangsu, China) at a dose of 0.2–0.4 mg/kg, sfufenital (Yichang Humanwell Pharmaceuticals Co., Ltd., Yichang, Hubei, China) at a dose of 0.3–0.4 μg/kg, and cis-atracurium (Jiangsu Hengrui Pharmaceuticals Co., Ltd., Lianyunang, Jiangsu, China) at a dose of 0.2 mg/kg. The induction continued until the bispectral index (BIS) value reached approximately 40–45, at which point a suitable laryngeal mask was employed. Anesthesia was sustained through the intravenous pump administration of cis-atracurium (Jiangsu Hengrui Pharmaceuticals Co., Ltd.) at a rate of 1–2 μg·kg⁻¹·min⁻¹, remifentanil (Yichang Humanwell Pharmaceuticals Co., Ltd.) at a rate of 0.1–0.2 μg·kg⁻¹·min⁻¹, and sevoflurane (Hengrui Pharmaceuticals Co., Ltd., Shanghai, China) at a concentration of 1.5–3%, while maintaining the BIS value within the range of 40–50. During the surgical procedure, several indices were closely observed, such as heart rate, blood pressure, variations in anesthesia depth, volume of bleeding, and fluid intake. If the heart rate was below 50 beats per minute, intravenous atropine at a dosage of 0.3–0.5 mg was administered. In the event that the blood pressure decreased by more than 30% from the baseline value, a single intravenous dose of dopamine (Yabang Medicine Co., Ltd., Shanghai, China) ranging from 2–4 mg was administered. Following the surgical procedure, neostigmine (Shanghai Xinyi Medicine Co., Ltd., Shanghai, China) 1 mg and atropine (Henan Hongrun Medicine Co., Ltd., Zhengzhou, Henan, China) 0.5 mg were administered to counteract any remaining muscle relaxation once spontaneous breathing had resumed. Following the procedure, the laryngeal mask was extracted, and the patient was relocated to the anesthesia recovery room for 30–60 minutes to undergo observation.

2.5 Serum Specimen Collection

Following the completion of the MMSE at 8 am on the third day post-surgery, the patients underwent blood collection using a coagulation tube. This procedure involved drawing 2 mL of blood from the medial aspect of the elbow, while the patients were in a fasting state. The tube was subsequently placed in a centrifuge following a 30-minute incubation period to allow for blood coagulation. The centrifugation process was carried out at a speed of 3000 rpm for a duration of 10 minutes. Serum was acquired from the supernatant. A pipette was employed to facilitate the transfer of the serum into the eppendorf (EP) tube. The EP tubes were labelled, the relevant patient details documented, and the samples were subsequently stored at –80 °C for preservation.

2.6 GO Function Annotation and Classification

The Gene Ontology (GO) database, available at http://www.geneontology.org/, serves as a comprehensive repository of gene research findings worldwide. It is curated and organized by the Gene Ontology Federation, providing a standardized framework for defining and describing the functions of genes and protein molecules. Firstly, the UniProtKB database (https://www.uniprot.org/) was utilized to ascertain the identification number of the differentially expressed protein. Subsequently, the identified protein identification number was used to retrieve the relevant GO annotation information from UniProt-GOA (http://www.ebi.ac.uk/GOA/) for further analysis. In the present study, analysis of the eight differentially expressed proteins was conducted using the GO database. The proteins were categorized based on their involvement in various biological processes (BP), cellular components (CC), and molecular functions (MF). All molecular aspects of these proteins have been previously classified and summarized in order to investigate their potential functions [16].

2.7 KEGG Pathway Analysis for Annotation Purposes

The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a publicly accessible database that provides spe-
Fig. 3. Differentially expressed proteins in the serum of patients with PND compared with non-PND patients on the third day after the operation. (A) A heat map depicting the distribution of 1101 proteins in two distinct groups of patients. Each individual square within the grid corresponds to a specific protein, with the intensity of color indicating the level of PND. The intensity of the color corresponds to the level of gene expression, with a higher expression level indicated by a redder color and a lower expression level indicated by a bluer color. Each row in the dataset represents varying levels of a specific protein across six different cases, while each column represents individual cases. (B) Volcano plot illustrating the differential expression of proteins between the two groups. The x-axis represents the fold change of the expression, while the y-axis represents the p value. The identification of differentially expressed proteins was conducted based on the criteria of statistical significance (p < 0.05) and fold change (>1.2 or <0.8). Every dot within the diagram symbolizes an individual protein molecule. In the PND group, down-regulated protein expression is represented by green dots, while red dots represent up-regulated protein expression. (C) Heat maps depicting the expression levels of eight differentially expressed proteins between the two groups were generated. PND, perioperative neurocognitive disorders.

Specialized information on genomes (http://www.genome.jp/kegg/). Genomic information was obtained from databases such as NCBI, followed by a systematic analysis of gene functions, genomic interactions, and functional information. This analysis was performed using a comprehensive knowledge base, which integrates and stores the information in the KEGG GENES database (https://www.genome.jp/kegg/genes.html). Furthermore, the KEGGPATHWAY database (https://www.genome.jp/kegg/pathway.htm) stores a wide range of information related to cellular processes, including imagery depicting metabolism, high-level information on membrane transport, signal transmission, the cell cycle, and other conserved sub-pathways. The primary focus of the KEGG LIGAND database (https://www.kegg.jp/dbget-bin/www_bfind?ligand) is to store comprehensive information pertaining to chemical substances, enzyme molecules, and enzymatic reactions. The extensive collection of pathway information available in the KEGG
Fig. 4. GO analysis of up-regulated proteins. (A) Gene Ontology (GO) enrichment analysis for cellular component (CC), molecular function (MF), and biological process (BP) functional classification of six up-regulated proteins in PND. The abscissa represents the number of clustered proteins, the ordinate represents the entries for GO analysis. The red bar chart represents BP; the green bar chart represents CC; the blue bar chart represents MF. (B) Distribution of the level 2 GO terms of up-regulated proteins.

database can be utilized to facilitate researchers in understanding the potential biological functions of target genes or proteins at a more comprehensive and systematic level. In order to obtain the results of gene or protein pathway analysis, the KEGG orthology (KO) number of the annotated protein was acquired using the KEGG online service tool KEGG Automatic Annotation Server (KAAS). Subsequently, this KO number was utilized in the KEGG mapper to identify the metabolic pathway to which the target gene or protein is mapped.

2.8 Domain Function Annotation Analysis

A protein domain refers to a highly conserved protein sequence that exhibits a specific structure and function throughout the evolutionary history of organisms. Therefore, it was of significant importance to conduct an analysis of the biological functions of proteins that were differentially expressed, with a particular focus on their structural domains. The protein domains were analyzed using the InterProScan (https://www.ebi.ac.uk/interpro/) domain function annotation software.

2.9 COG/KOG Classification

Clusters of orthologous groups of proteins (COG) are determined through a comparative analysis of the encoded proteins from fully sequenced genomes, performed on an individual basis. When evaluating proteins derived from a specific genome, this comparative analysis identifies the protein with the highest similarity in each respective genome. If a mutual best match is identified between these proteins, the resulting mutual best matches will constitute a cluster of orthologous groups. The EuKaryotic orthologous groups (KOG) annotation method is identical to the COG method.

2.10 Subcellular Structure Location Annotation

Subcellular structure location analysis was conducted to ascertain the precise cellular localization of the protein, thereby inferring its functional role in systems biology based on its spatial distribution within the cell. The subcellular localization of each protein within the cell can be determined using Worfpsort (https://wolfpsort.hgc.jp)/Cello (http://cello.life.nctu.edu.tw/).

2.11 Statistical Analysis

SPSS version 20.0 (IBM Corp., Armonk, NY, USA) was utilized for data analysis. Measurement data was characterized using the mean ± standard deviation (mean ± standard deviation (SD)). The comparison of two sample means was conducted using an independent sample t-test. The comparison of count data was performed using a chi-squared test. A significance level of $p < 0.05$ was used to determine statistical significance.
3. Results

3.1 The iTRAQ Proteomics Technique was Used to Analyze the General Information and Surgical Data of Patients’ Serum

According to the principle of maintaining consistency in preoperative and intraoperative conditions, we collected three serum samples from each group of patients for subsequent proteomic detection and data analysis. The purpose of this study was to compare the general data and cognitive function of the two patient groups before and during surgery.

Between the two groups, there were no statistically significant differences observed in ASA grade, BMI index, and preoperative MMSE score 1 day prior to surgery \( (p > 0.05) \) (Table 1). Furthermore, there were no statistically significant differences observed in operation time, anesthesia time, intraoperative fluid intake, and bleeding volume between the patients in both groups \( (p > 0.05) \) (Table 2).

3.2 iTRAQ Proteomics Quality Control

In the present investigation, the discrepancy in peptide mass between the measured mass of the peptide as determined by mass spectrometry and the protein mass deviation was found to be less than 10 parts per million \( (10 \times 10^{-6} \text{ Da}) \). The peptide score of the corresponding peptide was analyzed using the mass spectrometry software Mascot/sequst. The peptide mass error distribution chart was constructed by plotting the peptide mass error on the abscissa and the peptide score on the ordinate. All peptides exhibited a normal distribution with a mean centered around 0. This observation suggests that the mass spectrometer was functioning properly (Fig. 2A). In addition, another facet of quality control involved assessing the efficiency of enzymatic digestion in the samples. The peptide length distribution was constructed by plotting the length of the obtained peptides on the x-axis and the number of peptides corresponding to each length on the y-axis. The peptides analyzed in this study exhibited a peak length ranging from 7 to 13 amino acids. Furthermore, over 90% of the peptides fell within the range of the peptides were less than 24 amino acids in length suggesting a high enzymatic hydrolysis efficiency (Fig. 2B). The distribution of peptide quantitative ratios for all identified peptides exhibited a normal distribution, suggesting that the mass spectrometer was functioning properly (Fig. 2C).

3.3 Differentially Expressed Proteins in the Serum between the Two Groups

The study involved the detection and comparison of 1101 serum proteins from six patients. The differentially expressed proteins between three patients with PND...
and three patients without PND were identified (Fig. 3A). Screening was conducted based on the criteria of statistical significance ($p < 0.05$) and fold change in relative expression ($>1.2$ or $<0.8$). As a result, a total of eight proteins exhibiting differential expression were identified and visualized in the volcano plot (Fig. 3B). In the PND group, a total of six proteins were found to be up-regulated. These proteins include tektin-2, hephaestin, methylcytosine dioxygenase tet methylcytosine dioxygenase 2 (TET2), human leukocyte antigen (HLA) class I Histocompatibility antigen, tubulin beta-2A chain, and Rubicon (RUN) and contains four proteins-Fab1 (fatty acid biosynthesis I) (FYVE) domain-containing protein 1. Furthermore, there were two down-regulated proteins in the PND group, namely E3 ubiquitin-protein ligase RNF213 and Rho guanine nucleotide exchange factor 9 (Fig. 3C).

### 3.4 GO Analysis of Differentially Expressed Proteins

Functional annotation analyses were conducted on six proteins that exhibited relatively higher expression levels, as well as two proteins that showed down-regulation, in the context of PND. After conducting GO analysis, it was found that the up-regulated proteins are associated with a total of 17 BP. These processes were arranged in descending order based on the number of differentially expressed proteins involved. The top ten categories in biological processes included cellular processes, single-organism processes, biological regulation, localization, cellular component organization or biogenesis, regulation of biological processes, response to stimuli, immune system processes, developmental processes, and metabolic processes. Furthermore, the analysis of CC for the up-regulated proteins revealed the involvement of 10 cellular components. These components were also categorized based on the number of distinct proteins they contained, and included cell, cell part, organelle, organelle part, membrane, supramolecular fiber, membrane part, extracellular region part, extracellular region, and macromolecular complex. MF annotation analysis of the up-regulated proteins revealed their involvement in four distinct molecular functions, namely binding, catalytic activity, structural molecule activity, and transporter activity (Fig. 4A). The distribution of the level 2 GO terms of up-regulated proteins is shown in Fig. 4B. In comparison with non-PND patients, this study revealed a limited down-regulation of two proteins in PND patients. However, it is noteworthy that a total of 13 biological processes were implicated in the pathogenesis of PND. The biological processes associated with both proteins included cellular processes, single-organism processes, response to stimuli, regulation of biological processes, signaling, and biological regulation. The cellular components of the two proteins were identified as cell, cell part, organelle, membrane, membrane-enclosed lumen, and organelle part. In addition, the MF involved in this study were found to be binding, molecular function regulation, and catalytic activity, as depicted in Fig. 5A. The distribution of the level 2 GO terms of down-regulated proteins is shown in Fig. 5B.

### 3.5 KEGG Pathway Analysis of Differentially Expressed Proteins

After conducting KEGG PATHWAY analysis, it was determined that the eight differentially expressed proteins

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**Table 1. General information and cognitive function of patients whose serum underwent iTRAQ proteomics analysis.**

<table>
<thead>
<tr>
<th>General information</th>
<th>PND group</th>
<th>Non-PND group</th>
<th>$\chi^2/t$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases (n)</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years) (mean ± SD)</td>
<td>74.33 ± 3.79</td>
<td>77.00 ± 3.00</td>
<td>0.96</td>
<td>0.39</td>
</tr>
<tr>
<td>ASA grade (I/II/III)</td>
<td>0/2/1</td>
<td>0/2/1</td>
<td>0.000</td>
<td>1</td>
</tr>
<tr>
<td>BMI (cm/kg$^2$) (mean ± SD)</td>
<td>21.67 ± 1.53</td>
<td>23.33 ± 1.53</td>
<td>1.34</td>
<td>0.25</td>
</tr>
<tr>
<td>Hypertension (yes/no)</td>
<td>0/3</td>
<td>1/2</td>
<td>1.20</td>
<td>0.27</td>
</tr>
<tr>
<td>Diabetes (yes/no)</td>
<td>1/2</td>
<td>0/3</td>
<td>1.20</td>
<td>0.27</td>
</tr>
<tr>
<td>MMSE score one day before surgery (mean ± SD)</td>
<td>24.33 ± 1.53</td>
<td>25.33 ± 0.58</td>
<td>1.06</td>
<td>0.35</td>
</tr>
</tbody>
</table>

**Table 2. Surgical information of patients whose serum underwent iTRAQ proteomics analysis.**

<table>
<thead>
<tr>
<th>Surgical information</th>
<th>PND group</th>
<th>Non-PND group</th>
<th>$t$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases (n)</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical time (min) (mean ± SD)</td>
<td>79.67 ± 18.82</td>
<td>70.67 ± 19.01</td>
<td>0.84</td>
<td>0.58</td>
</tr>
<tr>
<td>Anesthesia time (min) (mean ± SD)</td>
<td>91.67 ± 18.23</td>
<td>88.67 ± 18.90</td>
<td>0.20</td>
<td>0.85</td>
</tr>
<tr>
<td>Fluid intake (mL) (mean ± SD)</td>
<td>683.33 ± 160.73</td>
<td>616.67 ± 76.38</td>
<td>0.65</td>
<td>0.55</td>
</tr>
<tr>
<td>Bleeding volume (mL) (mean ± SD)</td>
<td>58.33 ± 23.63</td>
<td>63.33 ± 7.64</td>
<td>0.35</td>
<td>0.75</td>
</tr>
</tbody>
</table>

PND, perioperative neurocognitive disorders; BMI, body mass index; MMSE, Mini Mental State Examination; n, number; SD, Standard deviation; ASA, American society of Anesthesiologists.
primarily contributed to the processes of endocytosis (https://www.genome.jp/entry/map04144) and phagosome (https://www.genome.jp/entry/map04145), as depicted in Fig. 6A. The specific signal pathways associated with these processes are illustrated in Fig. 6B,C.

3.6 Domain Enrichment Analysis of Differentially Expressed Proteins

Protein domains are the primary structural units of proteins, exhibiting a relatively high level of conservation and possessing distinct and autonomous structures and functions; therefore, analysis of shared protein domains may provide insights into the inherent biological functions of proteins. The software InterProScan uses sequence alignment to identify protein domains by searching the InterPro database. We conducted an enrichment analysis on the domains of the eight differentially expressed proteins, revealing that all of them exhibited the RING domain (Fig. 7).

3.7 COG/KOG Classification of Differentially Expressed Proteins

The differentially expressed proteins were analyzed for each COG/KOG term. The findings indicated that the up-regulated proteins were distributed across various COG terms, including secondary metabolites biosynthesis, transport and catabolism (25%), general function prediction only (25%), and cytoskeleton (50%) (Fig. 8A). In addition, the distribution of COG terms for down-regulated proteins revealed a significant presence of signal transduction mechanisms, accounting for 100% of the observed proteins (Fig. 8B).

3.8 Subcellular Localization Classification of Differential Proteins

The subcellular location classification of the up-regulated proteins revealed that 50% were located in the cytosol, 33.33% in the endoplasmic reticulum, and 16.67% in the extracellular region (Fig. 9A). Additionally, the distribution of down-regulated proteins in each subcellular location comprised of cytosol (50%) and plasma membrane (50%), as depicted in Fig. 9B.

4. Discussion

In this study, a total of six sera samples were selected for iTRAQ proteomics analysis. These samples were obtained from patients diagnosed with PND as well as patients without PND. The analysis was conducted 3 days after the surgical operation. The differential expression profiles of patients with PND were identified for the first time, thereby establishing a basis for future investigations. Following the screening process, a total of eight proteins exhibiting differential expression were identified. Subsequent bioinformatics analysis was conducted based on these differentially expressed proteins. We performed a similar study during the initial phase and identified the distinct expression profile of non-coding RNAs in individuals with PND [17].

Hephaestin is a protein that functions as an auxiliary for membrane iron transport. It is primarily expressed in the small intestine. The expression profile of the gene exhibits clear tissue specificity. The physiological function of this entity is to actively engage in the process of iron metabolism and facilitate the release of iron from intestinal epithelial cells into the bloodstream. In the context of the central nervous system, the presence of iron is essential for the process of synthesizing myelin and neurotransmitters. Excessive iron accumulation in the brain is also considered detrimental. In certain neurodegenerative diseases, such as AD and Huntington’s disease, elevated levels of iron have been observed in specific regions of the brain. This excessive iron accumulation is associated with intracellular overload. The activity of hydrogen oxide leads to the production of reactive oxygen free radicals, which subsequently cause harm to the phospholipid membrane and other components of nerve cells, leading to cellular damage and potential fatality [18]. The occurrence of neurological diseases may therefore be attributed to abnormal iron metabolism in the brain. Studies have indicated that hephaestin exhibits high expression in various regions of the brain, including the cerebral cortex, hippocampus, choroid plexus, striatum, and substantia nigra, as observed in experimental animal models. This regional variation in the expression of the central nervous system may be associated with abnormal iron accumulation in specific areas, leading to regional neuronal damage. The significant increase in the expression of hephaestin found in this study may therefore indicate a potential association between the occurrence of PND and disrupted iron metabolism, as well as iron overload in the brain.

The present study revealed a significant down-regulation of E3 ubiquitin-protein ligase RNF213 in the serum of patients with PND compared with those without PND. E3 ubiquitin-protein ligase RNF213 belongs to the E3 ubiquitin ligase superfamily, which is known for its specificity in ubiquitinating and degrading substrates involved in blood vessel formation [19–21]. The pathogenesis of PND is currently understood to involve the increased permeability of the blood-brain barrier, which plays a crucial role in its development. Vascular endothelial cells are recognized as a significant component in the formation of the blood-brain barrier. Furthermore, previous research has demonstrated that this protein possesses E3 ubiquitin ligase activity and contains the RING domain. It has been shown to degrade regulatory proteins involved in the activation of the non-classical Wnt signaling pathway, such as nuclear factor of activated T-cells (NFAT-1) and FILAMIN-A, through the process of ubiquitination. This degradation mechanism effectively inhibits the activation of the non-classical Wnt signaling pathway, thereby impeding the pathological progression of tau protein and offering protection against the devel-
Fig. 6. KEGG pathway analysis of differentially expressed protein. (A) The signaling pathways involved in the clustering of differentially expressed proteins. The color intensity represents the protein expression level; red represents higher expression and blue represents lower expression. (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling map of 7 differentially expressed proteins involved in endocytosis (https://www.genome.jp/entry/map04144). (C) KEGG signaling map of differentially expressed proteins involved in phagosome (https://www.genome.jp/entry/map04145).

Development of neurodegenerative diseases [22–25]. After tau protein loses its ability to properly interact with tubulin, the stability of microtubules is compromised, leading to an impaired process of synaptic plasticity and ultimately resulting in neurological degeneration [26–28]. The reduction of the E3 ubiquitin-protein ligase RNF213 protein following the operation therefore leads to the alleviation of the physiological inhibition of the tau pathological process, which is dependent on the non-canonical Wnt signaling pathway. Consequently, postoperative cognitive dysfunction may occur.

Tubulin beta-2A chain is a constituent of the protein complex known as tubulin, which plays a crucial role in cellular processes [29]. Tubulin is a highly conserved protein that forms microtubules, essential structures involved in various cellular functions, including cell division, in-
Fig. 7. **Domain enrichment analysis of differentially expressed proteins.** Each solid circle represents an enrichment entry, and the abscissa represents Fisher’s exact test $p$ values. The $p$ value was set as $-\log_{10}$. The ordinate represents the protein domains. Larger values represent a greater degree of enrichment.

Fig. 8. **COG/KOG classification of terms.**

(A) Distribution of proteins in the COG terms, including secondary metabolites biosynthesis, transport and catabolism, general function prediction only, and cytoskeleton. (B) Distribution of COG terms, including signal transduction mechanisms, for down-regulated proteins. COG, clusters of orthologous groups; KOG, EuKaryotic orthologous groups.

In the present study, the expression of tubulin beta-2A chain was up-regulated in patients with PND. The potential causes primarily stem from the following factor: the degradation of tubulin itself. Given that the participants in this study were elderly patients, it is plausible that the elevation of tubulin beta-2A chain in their serum could be attributed to aberrant tubulin functionality. This phenomenon may therefore indirectly indicate characteristic degenerative alterations in the nervous system, such as tubulin breakdown and the loss of nerve synapses. In the present study, tubulin beta-2A chain was up-regulated in patients with PND and this protein may be significantly correlated with the occurrence of PND, indicating its potential for further research.

In the present study, the expression of Rho guanine nucleotide exchange factor 9 (RhoGEF9) was significantly down-regulated in patients with PND compared with non-PND patients. RhoGEF9 belongs to the superfamily of Rho guanine nucleotide exchange factors. These molecules are known for their common biological activity of activating Rho guanosine triphosphatase (GTPase) to facilitate cytoskeleton production. Studies have demonstrated that the activation of Rho guanine nucleotide exchange factor leads to the activation of Rac-1, a small GTPase belonging to
the Rho family. Rac-1 plays a crucial role in actin cytoskeleton assembly, neuronal dendrite growth and development, and the modulation of synaptic structural plasticity changes [30]. Alterations in synaptic plasticity are widely regarded as the underlying structural foundation for cognitive processes, including learning and memory. When the expression of Rho guanine nucleotide exchange factors is down-regulated, it hinders the synaptic plasticity of central neurons, thereby impeding the normal functioning of the nervous system and potentially contributing to the development of neurodegenerative diseases. The likelihood of experiencing cognitive impairment is therefore elevated following surgical procedures [31].

Our analysis of biological processes revealed that the differentially expressed proteins primarily participate in microtubule-based processes. Parkinson’s disease is a neurodegenerative disorder that shares similarities with AD. A characteristic pathological alteration in PND is the inhibition of neuroplasticity, as evidenced by previous studies [32]. Neuroplasticity is contingent upon the generation of the neuronal cytoskeleton, with microtubules serving as a crucial component of neuronal cells and playing a vital role in their growth and development [33]. Recent studies have indicated that alterations in tubulin or microtubule-associated proteins occur during both neurodevelopment and neurodegenerative diseases [34]. In the present study, our findings indicated differential expression of tubulin beta-2A chain and RhoGEF9 proteins. In order to understand the pathogenesis of PND, it may therefore be beneficial to focus on the formation of microtubules as a starting point and investigate the role of neuroplasticity and synapse formation. This approach has the potential to provide new insights and breakthroughs in the understanding of PND.

5. Conclusion

The iTRAQ proteomics technique was employed to investigate variations in protein expression in the serum of patients with PND compared with non-PND patients. This analysis successfully identified a total of eight proteins that exhibited differential expression levels. Among the identified proteins, six were found to be up-regulated, namely tektin-2, hephaestin, methylcytosine dioxygenase TET2, HLA class I histocompatibility antigen, tubulin beta-2A chain, and RUN and FYVE domain-containing protein 1. Additionally, two proteins, E3 ubiquitin-protein ligase RNF213 and RhoGEF9, were found to be down-regulated. The subsequent biological data pertaining to these differentially expressed proteins provides insight into their underlying relationships.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.
Author Contributions
ZSW analyzed the data, designed and drew the figures and tables. YFT, QXX, TL, QYL collected the data and conducted the experiments. QYL and HYL collected and sorted references. YFT and TL wrote the original article. HYL and TL revised the article. YZ and QL designed the study and supervised it. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate
This study was registered with the Chinese Clinical Trial Registry (ChiCTR2000028836). All experimental protocols were approved by the ethics committee of Sichuan Tianfu New District People’s Hospital (20190306-05). Informed consent was obtained from all subjects or their legal guardians, and all methods were carried out in accordance with relevant guidelines and regulations.

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Conflict of Interest
The authors declare no conflict of interest.

References


