

Exploring the Role of SMPD3 in the lncRNA-miRNA-mRNA Regulatory Network in TBI Progression by Influencing Energy Metabolism

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Background: Traumatic brain injury (TBI) is associated with disturbances in energy metabolism. This study aimed to construct a lncRNA-miRNA-mRNA network through bioinformatics methods to explore energy metabolism-related genes in the pathogenesis of TBI.

Methods: Data from datasets GSE171718, GSE131695, and GSE223245 obtained from the Gene Expression Omnibus, were analyzed to identify differentially expressed (DE) genes. Regulatory relationships were investigated through miRDB, miRTarBase, and TargetScan, thereby forming a lncRNA-miRNA-mRNA network. The Molecular Signatures Database (MSigDB) was utilized to identify energy metabolism-related genes, and a protein-protein interaction (PPI) network was established through the STRING database. Functional annotation and enrichment analysis were conducted using GO and KEGG. The TBI mouse model was established to detect the expression levels of *GOLGA8B*, *ZNF367*, and *SMPD3* in brain tissues.

Results: *SMPD3* emerged as the key DE gene linked to energy metabolism in TBI, demonstrating a negative correlation with miR-218-5p and being associated with moderate unconsciousness and female patients. The PPI network revealed *SMPD3* interactions with proteins associated with cell death, sphingolipid metabolism, and neurodegenerative diseases such as Alzheimer's disease. *In vivo*, *GOLGA8B*, *ZNF367*, and *SMPD3* mRNA levels were significantly lower in TBI mice.

Conclusion: In summary, *SMPD3* represents a crucial metabolic gene in the progression of TBI. It potentially provides a new therapeutic target for metabolic disorders caused by traumatic brain injury (TBI) and holds significant theoretical value for further research.

Keywords: traumatic brain injury, bioinformatics analysis, lncRNA-miRNA-mRNA network, energy metabolism, *SMPD3*

Introduction

Traumatic brain injury (TBI), generally resulting from blunt and penetrating mechanisms, stands as one of the leading causes of disability and death in both developing and developed countries, representing a significant global public health challenge.¹ According to the TBI Model System National Database Statistics from 2017, gender differences exist in the epidemiology of TBI, with male cases accounting for more than 73% of all TBIs reported.² Each year, the incidence of TBI is estimated at 50 million cases worldwide, a figure that continues to grow rapidly.³ The global economy burden of TBI is approximately 400 billion dollars, accounting for 0.5% of the gross world product.⁴ Clinically, the severity of TBI can be stratified into mild (14–15), moderate (9–13) and severe (3–8) based on the Glasgow Coma Scale (GCS) score, and the corresponding disability rates are 10%, 60%, and 100%, respectively.^{5,6} For moderate to severe TBI, the pathological features vary during different stages of TBI progression, such as a series of metabolic changes take place, such as those in glucose, lipid, and acid-base balance.⁷ In addition, after TBI, especially after moderate to severe

TBI, the function of the blood-brain barrier is disrupted and inflammation infiltrates, which leads to neuronal damage in the hypothalamus region and subsequently results in a series of neurometabolic dysfunctions.⁸ These indications help to better distinguish the types of TBI patients and adopt more targeted treatment methods. In addition, global trends also indicate that there are gender differences in the incidence of traumatic brain injury, with males being more likely to experience it than females, but females have significant differences in the severity of initial injury and mortality rate.⁹ This gender-based difference might be attributed to the fact that women present more favorable brain physiological states subsequent to TBI, particularly better mitochondrial function and decreased excitotoxicity.¹⁰ Therefore, it is of great significance to conduct research using clinical samples from different genders and various TBI stages, with the aim of identifying specific targeted marker genes for the purpose of precise clinical diagnosis and treatment.

There are intricate mechanisms in TBI-induced brain energy metabolism disorders involving multiple factors and links. The uptake and utilization of glucose in the brains of patients are inhibited to varying degrees after TBI. GLENN et al conducted a study on 49 TBI patients and 31 normal volunteers. This research found that, compared with the normal volunteers, the CMRglc in TBI patients decreased and the oxygen to glucose ratio (OGR) also dropped.¹¹ Similarly, lactate and pyruvate are intermediate products of glucose metabolism. Clinically, LPR (lactate/pyruvate ratio) reflects anaerobic glucose metabolism. Its increase can be caused by cerebral ischemia-hypoxia or non-ischemic factors. LPR>25 indicates cerebral energy metabolism disorder, and LPR>40 indicates cerebral metabolic crisis.¹² Although metabolites contribute to clinical diagnosis, the specific regulatory mechanisms and key regulatory networks are still unclear. It's significant to explore the regulatory mechanisms related to energy metabolism and the connections between key genes, it's crucial for elucidating the progression mechanism of TBI disease and providing targets for clinical treatment and diagnosis.

Recent advancements in bioinformatics and high-throughput technologies have created unprecedented opportunities to delve into the molecular landscape of TBI.^{4,13} By integrating diverse omics data, ranging from transcriptomics to small non-coding RNA analyses, researchers have sought to decipher the intricate regulatory networks that govern the pathogenesis of TBI.^{14–16} Among these, the competing endogenous RNA (ceRNA) hypothesis has emerged as a prominent paradigm that postulated the crosstalk among long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and messenger RNAs (mRNAs) as key players in gene expression regulation.^{17,18} lncRNAs function as competing endogenous RNAs (ceRNAs) which compete with miRNAs in a sponge-like manner. This reduces the activity of miRNAs and eventually undermines the interaction between miRNAs and their target genes.^{19,20} Especially, some people believe that lncRNAs can affect neurological function and the progression of neurological related diseases and TBI through the ceRNA mechanism. For example, He et al discovered that Astrocyte derived lncRNA 4933431K23Rik exosome modules exhibit microglial phenotype and improve post-traumatic recovery via SMAD7 regulation.²¹ Astrocyte-derived extracellular vesicles carrying lncRNA NKILA can relieve neuronal injury in TBI. This is achieved by up-regulating nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 (NLRX1) and competitively binding to miR-195.²² Therefore, ceRNA is regarded as being closely associated with the progression of TBI, and has great research value.

Energy metabolism is a fundamental process in cellular homeostasis, which plays a critical role in various neurological disorders, including TBI.^{23–25} It is well-known that glutamatergic neurotransmission and ion homeostasis are disturbed following TBI, resulting in an increased energy demand.^{26,27} According to reports, the brain immediately enters a high metabolic state after TBI, during which glucose utilization increases for up to 30 minutes.²⁸ After this brief high metabolic state, the brain undergoes a prolonged period of low metabolism, lasting from several days to several weeks.^{29,30} Therefore, improving brain energy metabolism after TBI is a promising therapeutic area. Studies have shown that during TBI, the levels of interstitial hypoxanthine, inosine, adenosine and lactate are increased, while glucose content decreases.^{31–33} These alterations suggest that energy metabolism is disturbed following TBI, leading to neuronal cell loss and the increased ATP consumption.^{34,35} Dysregulation of energy metabolism genes may impact cellular responses to TBI-induced stress and plays a pivotal role in the pathogenesis of TBI.^{36,37} Given the important role of energy metabolism in the progression of TBI, it is of great significance to search for key energy metabolism genes related to TBI, explore the molecules involved in different stages of TBI disease progression and their dynamic changes at each stage, both for basic research and clinical treatment diagnosis.

In this study, we conducted a comprehensive bioinformatics analysis. First, by analyzing differentially expressed lncRNAs (DElncRNAs), DEMiRNAs, and DEMRNAs and utilizing the publicly available transcriptome and expression profile data in the Gene Expression Omnibus (GEO), this research revealed the functional role of energy metabolism genes in the progression of moderate to severe TBI through the lncRNA-miRNA-mRNA regulatory network. We investigated the relationship between energy-related miRNAs and clinical symptoms in TBI samples in order to reveal potential links between molecular subtypes and disease phenotypes. Additionally, functional enrichment analyses of core marker mRNAs within the regulatory network illuminated the biological processes and pathways involved in TBI, particularly highlighting energy metabolism-related pathways that could be crucial in TBI pathophysiology. The insights garnered from this research may significantly advance our understanding of TBI pathogenesis and provide a solid foundation for future experimental investigations.

Methods

Data Retrieval of TBI-Related Transcriptome, Expression Profiling and Energy Metabolism Genes

Data sets containing transcriptome and expression profiling data related to “traumatic brain injury” or “TBI” were acquired from the GEO database. According to Article 32 in Chapter 3 of the “Ethical Review Measures for Life Science and Medical Research Involving Human Subjects” (Reference: https://www.gov.cn/zhengce/zhengceku/2023-02/28/content_5743658.htm) issued by the National Science and Technology Ethics Committee of China, the use of legally obtained publicly available personal information data or biological samples for life science and medical research can be exempted from ethical review.

Clinical sample information of GSE171718, GSE131695, and GSE223245 is displayed in [Supplementary Table 1A-C](#) respectively. The lncRNA/mRNA expression profile dataset in serum samples is GSE171718, and the TBI patients have a GCS score ranging from 9–12, the samples collected from Affiliated Hospital of Zunyi Medical University from January 2019 to March 2019.³⁸ The miRNA expression profile dataset in blood samples is GSE131695, and the GCS score of 5.6–7.38 for TBI patients.³⁹ The miRNA expression profile dataset in blood samples is GSE223245, and the GCS score of 3–12 for TBI patients.⁴⁰

To identify energy metabolism-related genes, we searched the MSigDB (v7.4; <http://software.broadinstitute.org/gsea/msigdb>) with the keyword “energy” and selected gene sets annotated as “all collection”, then integrated them in [Supplementary Table 2](#). The bioinformatics analysis workflow is shown in [Figure 1](#).

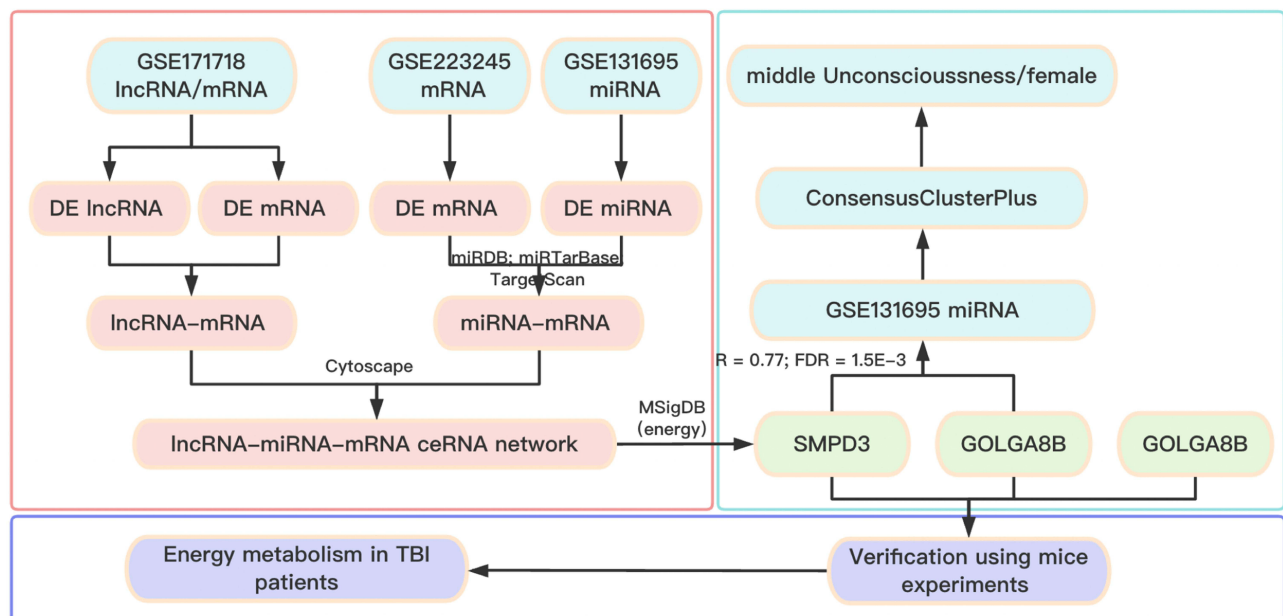


Figure 1 Flow chart of the overall analysis.

Screening of DElncRNAs, DEMiRNAs and DEMRNAs in TBI Samples

The factoextra package in R (<https://github.com/kassambara/factoextra>) was utilized for the principal components analysis (PCA) of gene expression and manifested as `fviz_pca_ind` function. Thereafter, differential expression analysis was conducted using the R package “limma” to screen the DElncRNAs, DEMiRNAs and DEMRNAs and construct the competing endogenous RNA (ceRNA) network. For the selection of DElncRNAs, DEMiRNAs and DEMRNAs, respectively, the following cutoff points were used: lncRNA (P value < 0.05 , $|\log_2(\text{Foldchange})| > 0.58$), miRNA (P value < 0.05 , $|\log_2(\text{Foldchange})| > 0.58$) and mRNA (P value < 0.05 , $|\log_2(\text{Foldchange})| > 0.58$).

Construction of the lncRNA-miRNA-mRNA ceRNA Network

The construction process of the lncRNA-mRNA-miRNA ceRNA regulatory network in TBI as follow: First, Spearman correlation analysis of DElncRNAs and DEMRNAs in serum samples was performed with the “`cor.test`” function in R, and the Benjamin & Hochberg method was used to calculate the FDR. $\text{FDR} < 0.05$ and $|\text{R}| > 0.2$ was used to determine significant lncRNA-mRNA association pairs. Then, we matched highly-expressed DEMiRNAs with lowly-expressed DEMRNAs in TBI samples, and vice versa. Subsequently, based on the miRDB (<http://mirdb.org/index.html>), miRTarBase (<https://mirtarbase.cuhk.edu.cn>) and TargetScan (https://www.targetscan.org/vert_80/) databases, we selected mRNA - miRNA pairs confirmed by at least two databases for further analysis. Finally, shared mRNAs between DElncRNA-DEmRNA pairs and DEMiRNA-DEmRNA pairs were used to construct the lncRNA-mRNA-miRNA ceRNA regulatory network, visualized with Cytoscape 3.8.1. (<https://cytoscape.org/>).

Identification of Marker mRNAs in the lncRNA-miRNA-mRNA ceRNA Network

Random Forest classifiers were constructed based on the expression levels of core marker mRNAs in the lncRNA-mRNA-miRNA ceRNA network of GSE171718 and GSE223245 datasets. The training set was selected at 80%, and 1000 validation cycles were done to determine the credibility of the AUC evaluation of core marker mRNA in the ceRNA network. Subsequently, intersections of core marker mRNAs and energy - metabolism-related genes were identified. Then, energy metabolism-related miRNAs interacting with these core marker mRNAs in the ceRNA network were extracted.

Identification of Relationships Between Energy Metabolism-Related miRNAs and Clinical Symptoms in TBI Patients

Using the expression data of energy metabolism-related miRNAs in the GSE131695 database, consistency clustering analysis was done for TBI samples using the R package Consensus Cluster Plus with parameters $\text{max K}=10$ (maximum cluster number to evaluate) and $\text{reps}=100$ (number of subsamples). The optimal number of clusters was determined and the samples were classified into subtypes. Then, PCA was used to verify classification reliability. Subsequently, TBI subtypes and clinical symptoms like coma degree and gender were analyzed for enrichment using the fisher test. To identify representative genes for subtypes with significantly enriched clinical indicators, differential analysis was done between subtype and non-subtype samples using the limma package of R. Only highly expressed genes (P value < 0.05 , $\log_2(\text{Foldchange}) > 1$) within the subtype were selected as representative miRNA of the subtype. Based on the miRDB, miRTarBase and TargetScan database, mRNA regulated by these representative miRNAs were selected, and then functional annotation and enrichment analysis were conducted using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). Term $P_{\text{adjust-value}} < 0.05$ was considered statistically significant difference.

Functional Enrichment Analysis and Protein-Protein Interaction (PPI) Network Construction

To investigate protein interactions involving core marker mRNAs within the lncRNA-miRNA-mRNA regulatory network, the STRING database (<https://string-db.org/>) was used to construct a PPI network. Functional annotation and enrichment analysis were performed using GO and KEGG.

Establishment of a TBI Mouse Model

Twelve C57BL/6J mice weighing 18–22 g (8 to 10-week-old) were obtained from Pengyue Laboratory Animal Breeding Co., Ltd. (Jinan, China). Housing facility were maintained at 21 ± 2 °C and 50–70% humidity, with a 12 hours light/12 hours dark cycle. All animal feeding and housing procedures followed the Guideline for the Care and Use of Laboratory Animals. These mice were randomly divided into two groups: control group and TBI model group, with six mice in each group. The experimental design adheres to the 3R principle of “Replacement, Reduction, and Refinement”. Throughout the entire experiment, strict compliance with the drug instructions is maintained, the surgical time is effectively shortened, and the pain endured by the experimental animals is thereby minimized. Mice were anesthetized with intraperitoneal injection of pentobarbital (50 mg/kg) and placed on a brain stereotaxic apparatus. A craniotomy was done on the right parietal cortex. The free-fall impact method was used to establish the TBI mouse model. A 40 g weight was dropped from 20 cm to impact the mouse’s head with 100 ms dwell time and 1 mm depth. TBI model was confirmed by observing apnea and limb convulsions. After hemostasis, the wound was sutured. Control group had the same procedures without impact injury. One week later, mice were euthanized with an overdose of pentobarbital (200 mg/kg) and brain tissues were collected. Two experienced operators blinded to the design performed these experiments. All experimental procedures complied with the ARRIVE guidelines and the NIH guide for laboratory animal care and use, and were approved by the ethical committee of Jining First People’s Hospital (JNRM-2023-DW-061).

Total RNA Isolation and RT-qPCR

Total RNA was isolated from 5 mg of brain tissue using the Total RNA Extraction Kit (Promega, Madison, WI, USA) following the manufacturer’s instructions. RT-qPCR analysis was performed using Hifair[®] II 1st Strand cDNA Synthesis SuperMix (Yeasen Biotechnology, Shanghai, China) and Hif[®] qPCR SYBR Green Master Mix (Yeasen Biotechnology). Gene expression levels were calculated with the $2^{-\Delta\Delta C_t}$ method with U6 as the normalization control. The primer sequences are listed in [Table 1](#).

Statistical Analysis

A quantile normalization scheme was applied to all samples. Following log₂ transformation of the data, the limma package was employed for differential expression analyses. Correlation analyses were conducted with Spearman correlation, applying the Benjamini & Hochberg (BH) correction to control for multiple comparisons. Fisher’s exact test to examine associations between TBI classifications and patient characteristics, including coma scale and gender. Data are presented as the mean \pm standard deviation (SD). $P < 0.05$ was considered statistically significant.

Result

The Data Integration and Analysis for lncRNA/mRNA and miRNA/mRNA Association Pairs

PCA was employed to analyze the expression differences of lncRNA/mRNA between control and TBI samples. As shown in [Supplementary Figure 1A](#) and [B](#), significant differences in lncRNA/mRNA expression were observed between

Table 1 Real-Time PCR Primer Synthesis List

Gene	Sequences	
ZNF367	Forward	5' - GCACTCAGCAAACACCAGTCTC -3'
	Reverse	5' - CAGCCTTCTGAACTAGCTTGCC-3'
SMPD3	Forward	5' - CAGCAGCACTCGCTCTTTAC -3'
	Reverse	5' - CTCGCTCTCCAACACCTTTTGA -3'
GOLGA2D	Forward	5' - GTCGGAAGAAACCAGGCAGA-3'
	Reverse	5' - TGGGCACATTCTCTGGTGAC -3'
GAPDH	Forward	5 - GGCATGGACTGTGGTCATGAG -3
	Reverse	5' - TGCACCACCAACTGCTTAGC -3'

control and TBI samples. The R package “limma” was used to perform differential expression analysis for GSE171718 dataset. Using a cutoff of P value < 0.05 and $|\log_2(\text{Foldchange})| > 0.58$, 229 DElncRNAs were identified, with 160 lncRNAs upregulated and 69 downregulated in TBI samples (Figure 2A and Supplementary Table 3A). Additionally, 683 DEmRNAs were identified, with 381 mRNAs upregulated and 302 downregulated in TBI samples (Figure 2B and Supplementary Table 3B). The Spearman correlation coefficient between DElncRNAs and DEmRNAs was calculated using the `cor.test` function, yielding 48985 lncRNA/mRNA association pairs with a cutoff of P value < 0.05 and $|R| > 0.6$. PCA was then applied to the mRNA data from the GSE223245 dataset and the miRNA data from the GSE131695 dataset (Supplementary Figure 1C and D), revealing significant differences between control and TBI samples. In the GSE223245 dataset, using the R package “limma” (P value < 0.05 and $|\log_2(\text{Foldchange})| > 0.58$), 744 DEmRNAs were identified, including 251 upregulated and 493 downregulated in TBI samples (Figure 2C and Supplementary Table 3C). Moreover, 144 DEMiRNAs were identified in the GSE131695 dataset. Among these, 96 mRNAs were upregulated, while 48 were downregulated in TBI samples (Figure 2D and Supplementary Table 3D). Based on the miRDB, miRTarBase and TargetScan databases, we identified miRNA/mRNA association pairs with opposite expression trend in TBI samples.

Construction of lncRNA-miRNA-mRNA Network

Using the lncRNA/mRNA association pairs from the GSE171718 dataset and miRNA/mRNA association pairs from the GSE223245 and GSE131695 datasets, we constructed the lncRNA-miRNA-mRNA ceRNA network (Figure 3A and Supplementary Table 4). Furthermore, the expression levels of the three marker genes (*GOLGA8B*, *ZNF367* and *SMPD3*) in the GSE171718 and GSE223245 datasets were utilized to construct a random forest classifier. As illustrated in Figure 3B and C, both datasets exhibited high AUC density curves, suggesting that these mRNAs play a critical role in the pathogenesis of TBI.

Relationships Between Energy Metabolism-Related Genes and TBI Pathogenesis

As illustrated in Figure 4A, only one overlapping gene was identified between DEmRNAs and energy metabolism-related gene sets: *SMPD3*. Additionally, significant co-expression was observed among the three marker genes (*SMPD3*, *GOLGA8B*, and *ZNF367*) in the GSE223245 dataset, with a notable interaction between *SMPD3* and *GOLGA8B* (Table 2). Consequently, the significantly interacting miRNAs with *GOLGA8B* and *SMPD3* in the ceRNA network were selected (Table 3), and consensus clustering analysis was performed on the GSE131695 dataset, resulting in well-defined clustering into four classes (Supplementary Figure 2A-L). PCA analysis further demonstrated significant separation among the clusters (Figure 4B). Among the various clinical symptoms, cluster 4 was significantly associated with moderate unconsciousness (Figure 4C), while cluster 1 was significant associated with female patients (Figure 4D).

Functional Analysis of mRNA Enriched in Cluster 1 and 4

Subsequently, representative miRNAs for cluster 1 and 4 were identified based on their expression levels. Gender difference-related miRNAs are presented in Supplementary Table 5A, and moderate unconsciousness-related miRNAs are presented in Supplementary Table 5B. The regulatory relationships between miRNAs and mRNAs were determined using the miRDB, miRTarBase, and TargetScan databases for cluster 1-regulated mRNAs (Supplementary Table 5C) and cluster 4-regulated mRNAs (Supplementary Table 5D). GO and KEGG enrichment analyses revealed that mRNAs in cluster 1 were primarily associated with lactose metabolism, response to parathyroid hormone, and long-term depression, whereas mRNAs in cluster 4 were primarily involved in the regulation of heart and organ growth and mesenchyme morphogenesis (Supplementary Table 5E and F).

PPI Analysis of *SMPD3*, *GOLGA8B*, and *ZNF367*

The PPI network of the three core marker mRNAs was constructed using the STRING database (Figure 5A). As shown in Supplementary Table 6A and B, functional enrichment analysis of the interacting proteins revealed their primary involvement in programmed necrotic cell death, glycosphingolipid metabolic process, Alzheimer’s disease, and insulin resistance. Additionally, a TBI mouse model was established to validate the expression levels of *SMPD3*, *GOLGA2D* and *ZNF367* in brain tissues. Because the *GOLGA8B* gene does not exist in murine, we measured the expression levels of

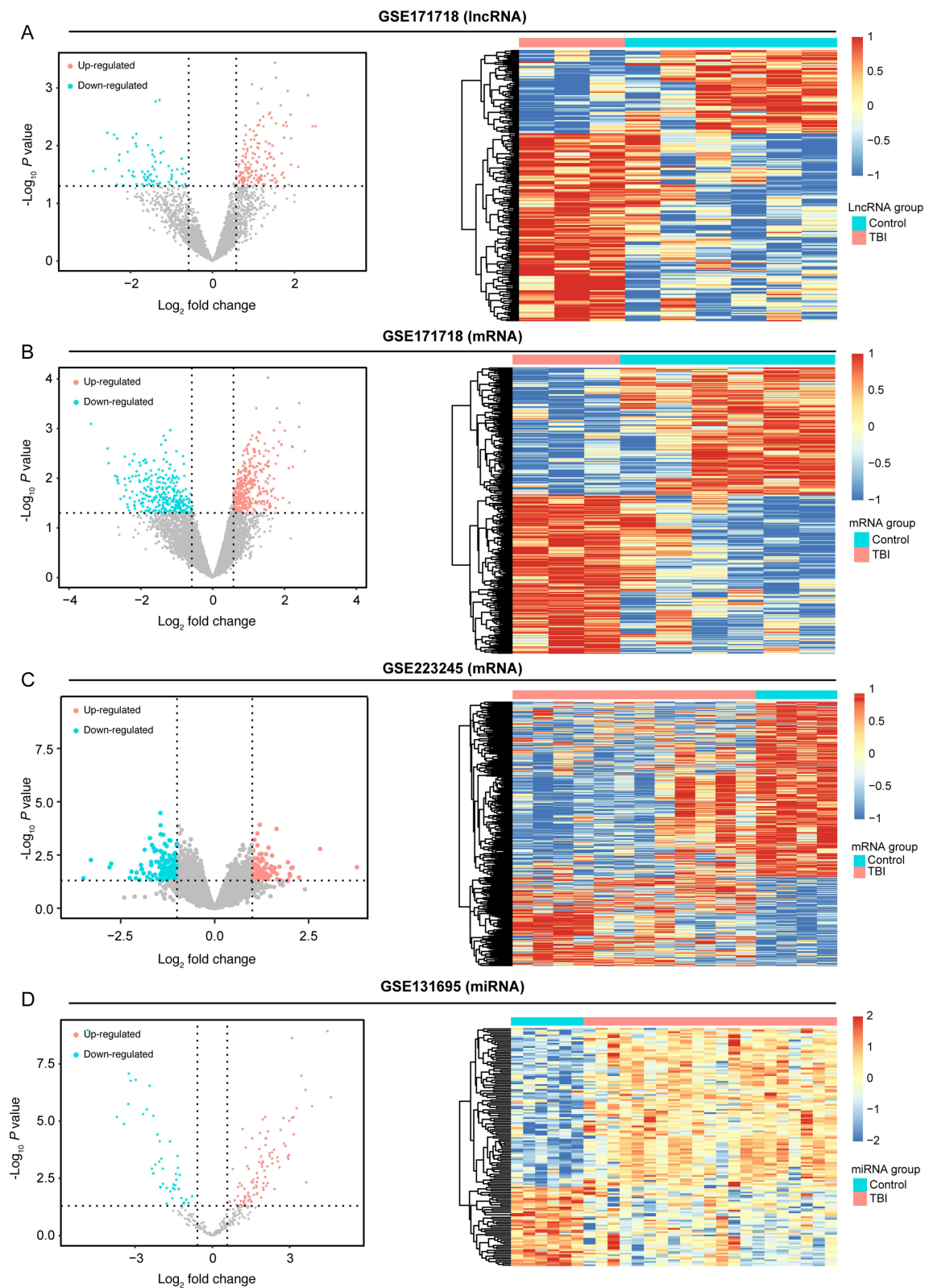


Figure 2 The data integration and analysis for lncRNA/mRNA and miRNA/mRNA association pairs.

Notes: (A) Volcano plots and heat map of DE-lncRNAs in the GSE171718 dataset. (B) Volcano plots and heat map of DE-mRNAs in the GSE171718 dataset. (C) Volcano plots and heat map of DE-mRNAs in the GSE223245 dataset. (D) Volcano plots and heat map of DE-miRNAs in the GSE131695 dataset.

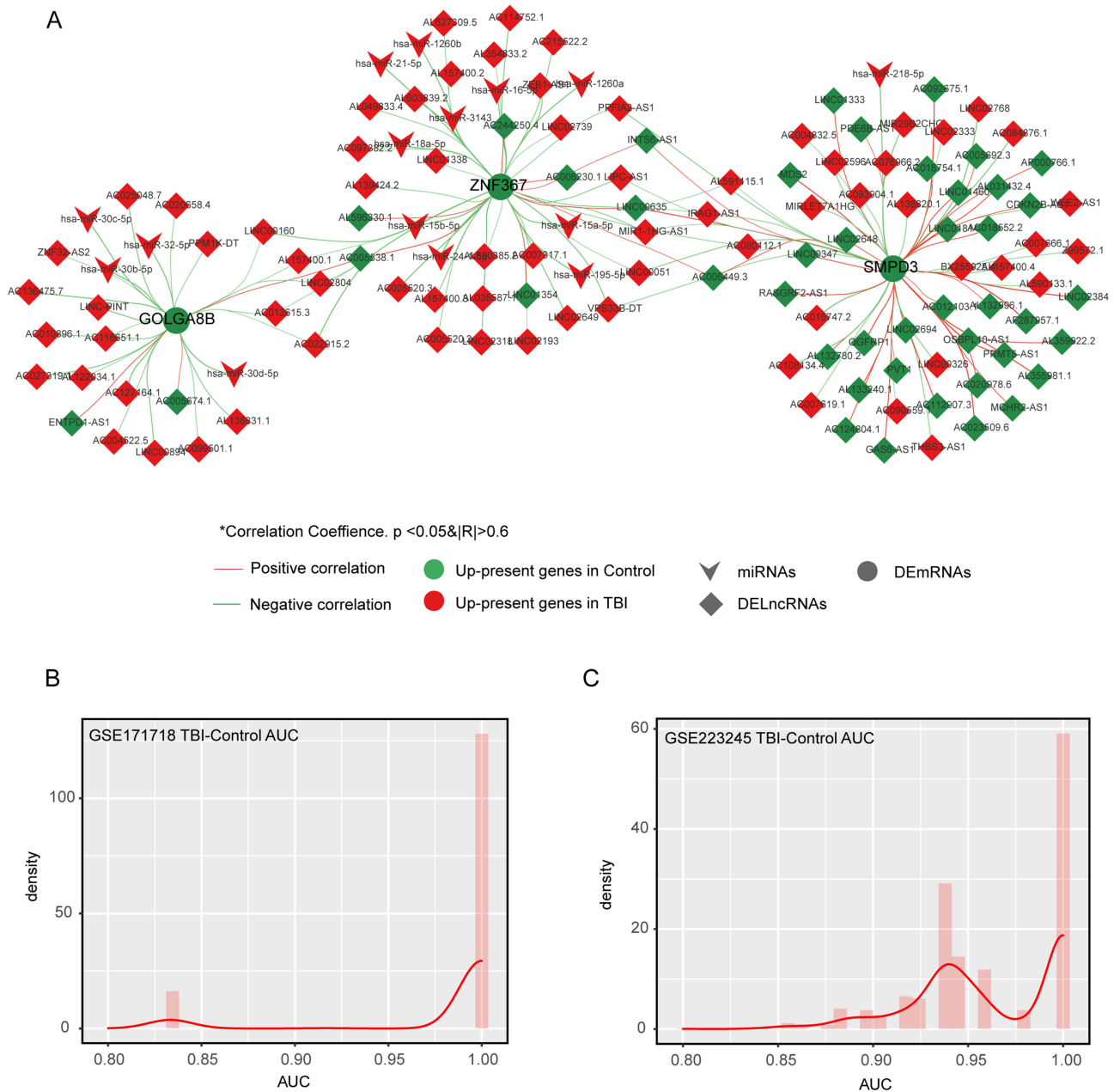


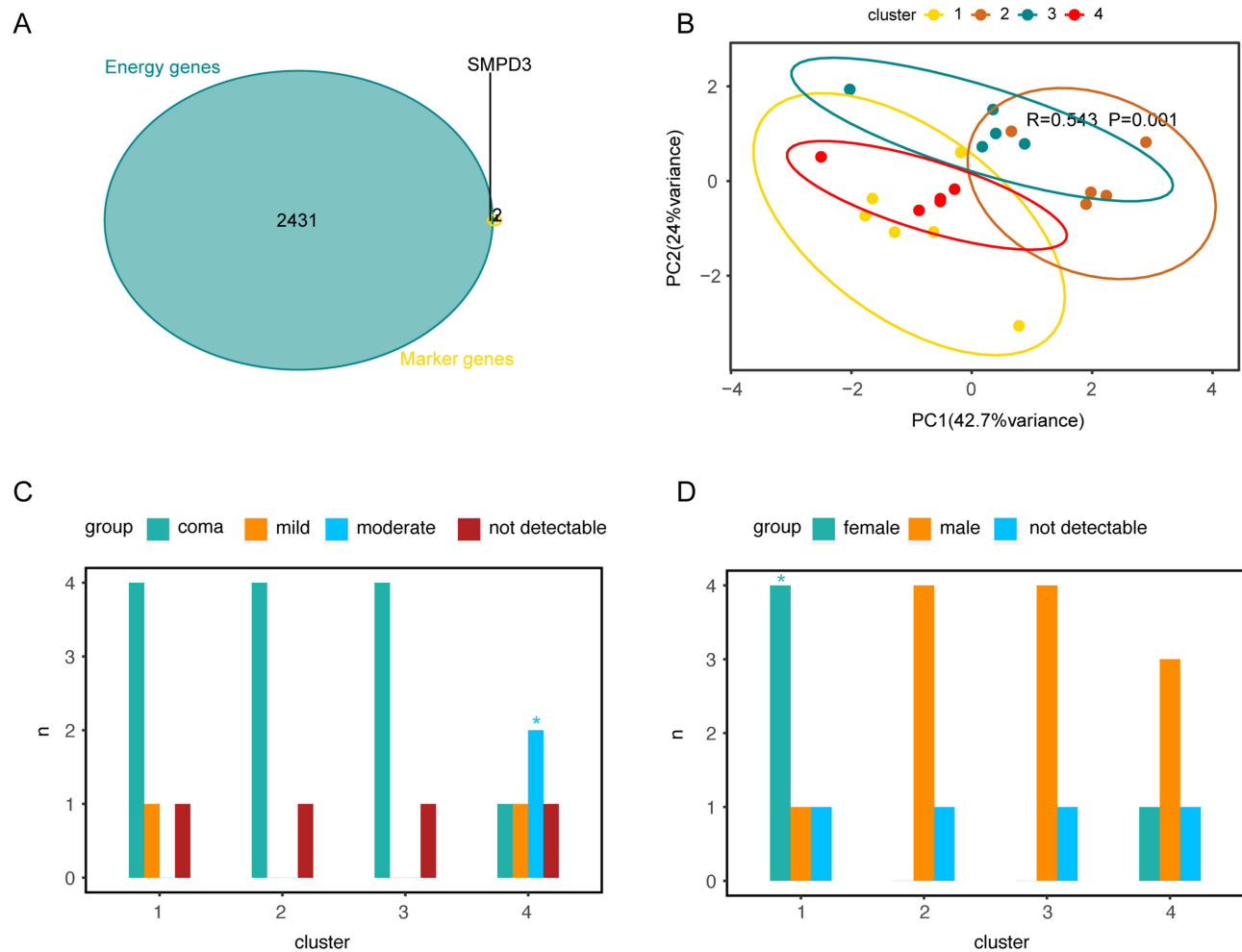
Figure 3 Establishment of ceRNAs regulatory network.

Notes: (A) The lncRNA-miRNA-mRNA ceRNAs regulatory network. (B) AUC and accuracy density distributions of GSE171718. (C) AUC and accuracy density distributions of GSE223245.

GOLGA2D, which has the highest homology to *GOLGA8B*. As illustrated in Figure 5B-D, the expression of *SMPD3*, *GOLGA2D*, and *ZNF367* was significantly downregulated in TBI samples compared to control samples ($P < 0.05$), consistent with our analysis results (Supplementary Table 7A-C).

Discussion

TBI can be defined as the disruption in brain function caused by external physical forces.⁴¹ Glucose aerobic metabolism is the primary energy source for the normal brain. Adequate cerebral blood flow delivers glucose, oxygen, and other nutrients to sustain normal neurologic function.³⁰ During TBI progression, multiple pathological alterations occur, including impaired cerebral blood flow regulation, brain tissue damage, brain metabolism disturbance, and inflammatory



responses, resulting in neuronal death.⁴² Preclinical animal studies consistently reported that energy metabolic stress occurs following TBI.^{43,44}

In the current study, we identified DElncRNAs, DEMiRNAs, and DEMRNAs in TBI and control samples from the GSE171718, GSE131695 and GSE223245 datasets. Using lncRNA/mRNA association pairs from the GSE171718 dataset and miRNA/mRNA association pairs from the GSE223245 and GSE131695 datasets, we constructed a lncRNA-miRNA-mRNA ceRNA network that includes three core marker mRNAs: *GOLGA8B*, *ZNF367* and *SMPD3*. *GOLGA8B*, located on chromosome 15, belongs to the Golgin family and is primarily expressed in brain tissues, particularly in the cerebellum and cerebellar hemisphere tissues.^{45,46} Previous studies have linked *GOLGA8B* to various neurological

Table 2 Three Marker Genes (*SMPD3*, *GOLGA8B*, and *ZNF367*) Were Co-Expressed in the GSE223245 Dataset

Node1	Node2	R	Pvalue	Fdr
SMPD3	ZNF367	0.596707	0.014683	0.044049
SMPD3	GOLGA8B	0.768601	0.000504	0.001511
ZNF367	GOLGA8B	0.449524	0.080655	0.241966

Table 3 The Significantly Interacting miRNAs with *GOLGA8B* and *SMPD3* in the ceRNA Network

Node 1	Node 2 R	R
Hsa-mir-30b-5p	GOLGA8B	-1
Hsa-mir-30c-5p	GOLGA8B	-1
Hsa-mir-30d-5p	GOLGA8B	-1
Hsa-mir-32-5p	GOLGA8B	-1
Hsa-mir-218-5p	SMPD3	-1

diseases, including dementia and amyotrophic lateral sclerosis.⁴⁷ In this study, *GOLGA8B* was identified as a differentially expressed marker gene in TBI samples from the GSE171718 and GSE223245 datasets. Furthermore, our animal experiments confirmed that *GOLGA2D*, the gene with the highest homology to *GOLGA8B* in mice, was expressed at lower levels in TBI mice compared to control mice. Additionally, miRNAs such as miR-30b-5p, miR-30c-5p, miR-30d-5p, and miR-32-5p, which interact with *GOLGA8B*, were associated with moderate unconsciousness and female patients in TBI, suggesting that *GOLGA8B* was a pathogenic role in in TBI.

ZNF367, located on chromosome 9q22.32, is part of the zinc finger protein family.⁴⁸ It is differentially expressed in cancers such as breast cancer, glioma and adrenocortical carcinoma, and papillary thyroid cancer.^{49–51} *ZNF367* has been reported to be an important modulator of neuroblast proliferation and plays a key role in DNA replication and cell cycle regulation.⁵² In our study, *ZNF367* was found to be downregulated in TBI samples from the GSE171718 and GSE223245 datasets and in the brain tissues of TBI mice. This suggests that, similar to *GOLGA8B*, *ZNF367* may also contribute to the pathogenic of TBI. However, PPI analysis did not reveal any interacting proteins with *GOLGA8B* or *ZNF367*, indicating that while these genes may be involved in TBI pathogenesis, they are likely not related to energy metabolism. The precise functions of *GOLGA8B* and *ZNF367* in TBI pathogenesis require further investigation.

Sphingomyelin is the major component of subcellular membranes in the central nervous system, can be hydrolyzed into phosphorylcholine and ceramide by *SMPD1*, *SMPD2* and *SMPD3*. Ceramide, among these hydrolysis products, plays a crucial role in cell growth, differentiation, senescence, and apoptosis.^{53,54} Previous research has demonstrated that *SMPD3* deficiency can disrupt the hypothalamus–pituitary growth axis and promote the progressive depositions of neuronal proteins, ultimately leading to neurodegenerative and cognitive decline-related diseases such as Alzheimer’s disease.^{55,56} The translation product of *SMPD3* is nSMase2. It’s strongly expressed in the brain and generates ceramide, a bioactive lipid involved in many cellular processes like gene expression control.⁵⁷ nSMase2 is a target for treating cancer and neurological disorders.⁵⁸ In the cerebral ischemia model, inhibiting nSMase2 to stop ceramide production and accumulation mainly happens in hippocampal astrocytes, lessening neuronal damage. This indicates that targeting nSMase2 activity to inhibit ceramide production might be a good way to prevent pathological ceramide accumulation in neurological diseases.⁵⁹ The analysis and prediction of RNA seq data from the cerebral cortex of mice lacking nSMase2 showed enhanced neural plasticity and stronger resistance to some neurodegenerative diseases.⁶⁰ In addition, there have been reports indicating that nSMase2 can regulate insulin resistance, which is similar to our findings on energy metabolism.⁶¹ Multiple studies have shown that the activity of nSMase2 is closely related to the progression of neurological diseases such as cerebral ischemia and Alzheimer’s disease. In the nervous system, *SMPD3* has been shown to regulate neuronal survival and differentiation.⁶² Therefore, we screened *SMPD3* from the TBI dataset and speculated that it also plays a key role in the progression of TBI.

Consistent with this, our study identified *SMPD3* as the only differentially expressed gene associated with energy metabolism in TBI progression. The predicted miR-218-5p, which interacts with *SMPD3*, was also linked to moderate unconsciousness and female patients in TBI. Furthermore, our animal experiments revealed significant downregulation of *SMPD3* expression in TBI mice compared to control mice. These results strongly suggest that *SMPD3* functions as an energy metabolism gene involved in TBI progression. To further substantiate this hypothesis, we analyzed the PPI network for the *SMPD3* protein. The analysis indicated that *SMPD3*-interacting protein are primarily involved in programmed cell death, apoptosis, sphingolipid metabolism, Alzheimer’s disease, and insulin resistance. Therefore, we

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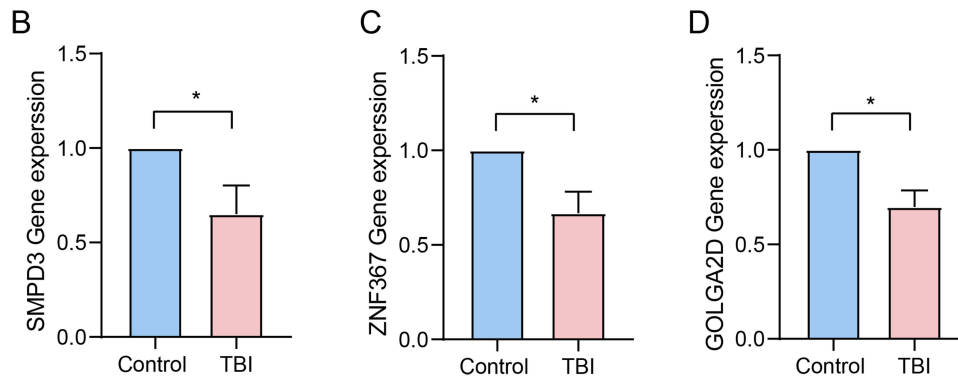
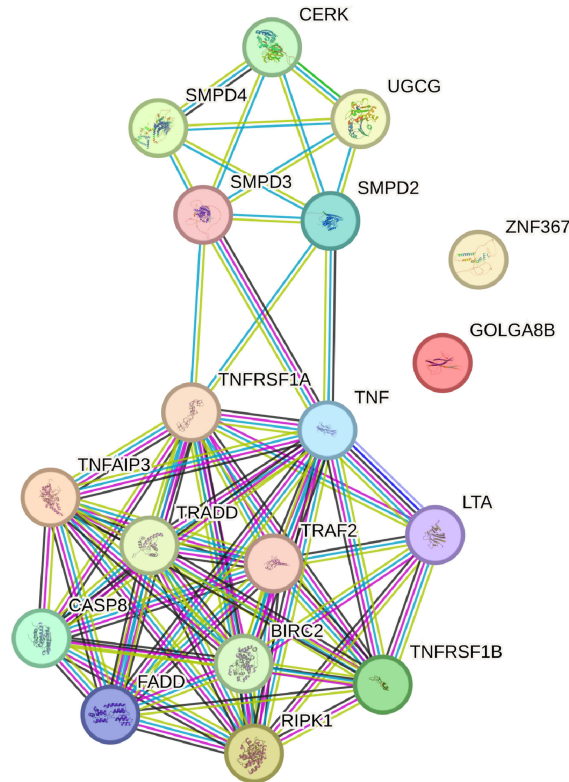


Figure 5 PPI analysis of *SMPD3*, *GOLGA8B*, and *ZNF367*.

Notes: (A) The PPI network of the three core marker mRNAs was constructed using the STRING database. The expression of *SMPD3* (B), *ZNF367* (C) and *GOLGA2D* (D) in different groups. * $P < 0.05$.

conclude that *SMPD3*, acts as an energy metabolism gene, contributes to programmed cell death, apoptosis, and sphingolipid metabolism, ultimately influencing the progression of TBI.

Limitations and Strengths

Our study also has certain limitations, such as not distinguishing age, gender, and strict TBI staging. In future research, we will refine clinical samples of TBI of different classifications, actively explore the regulatory role and mechanism of *SMPD3* in the progression of TBI by regulating energy metabolism, detect dynamic expression profiles, and construct a complete TBI energy metabolism regulatory network. Furthermore, we will also actively utilize basic experiments to verify and explore the underlying mechanism of action at a deeper level. Nevertheless, our research findings can provide insights for TBI energy metabolism related research and clinical diagnosis and treatment, valuable theoretical basis for

TBI related research and offer new reference biomarkers and therapeutic targets for the diagnosis, staging, and treatment of TBI in clinical practice.

Conclusion

In summary, a lncRNA-miRNA-mRNA network centered on three core marker mRNAs—*GOLGA8B*, *ZNF367* and *SMPD3*—was constructed. Among these, *SMPD3* emerged as the only differentially expressed gene associated with energy metabolism in TBI progression, particularly linked to moderate unconsciousness and female TBI patients. This study highlights potential targets for future experimental validation and offers valuable insights for further research on the role of energy metabolism in TBI.

Data Sharing Statement

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interest.

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