Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Exploring the pathogenesis of traumatic brain injury and traumatic epilepsy based on bioinformatics analysis

limei Liu
Qinghai University
jiameng Jia
Qinghai University
yu Pan
panyu@btch.edu.cn

Department of Rehabilitation Medicine Tsinghua Chang Gung Memorial Hospital

Research Article

Keywords: Traumatic brain injury, post-traumatic epilepsy, bioinformatics, neuroinflammation, neuroplasticity

Posted Date: June 29th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9544103/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Exploring the pathogenesis of traumatic brain injury and traumatic epilepsy based on bioinformatics analysis

Limei Liu[1] Jiameng[1] Jia Yu Pan[2]

1. Qinghai University 251 Ningda Road, Chengbei District, Xining， Qinghai Province, China ,Postcode：810000

2. Department of Rehabilitation Medicine Tsinghua Chang Gung Memorial Hospital

Limei Liu：E-mail Address:yb231002150146@qhu.edu.cn Orcid：0009-0005-3377-3266

Jiameng Jia：E-mail Address:yb241002150200@qhu.edu.cn

Orcid：0009-0001-9737-4994

Yu Pan:panyu@btch.edu.cn Orcid： 0000-0002-5703-5322

Background: Traumatic brain injury (TBI) is a well-established risk factor for the development of post-traumatic epilepsy (PTE). However, the molecular mechanisms linking TBI to PTE remain incompletely understood. This study aimed to explore the shared molecular signatures and underlying pathogenic mechanisms between TBI and PTE, thereby providing novel insights into the prevention and treatment of PTE.**Materials and Methods:**Publicly available transcriptomic datasets from patients with TBI and PTE were analyzed to identify common differentially expressed genes (DEGs). Functional enrichment analyses, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses, were performed to elucidate the biological functions and signaling pathways associated with the shared DEGs. Protein–protein interaction (PPI) networks were subsequently constructed to identify key hub genes. Candidate hub genes were further validated using the Comparative Toxicogenomics Database (CTD). In addition, the expression levels of the identified core genes were experimentally validated in a controlled cortical impact (CCI)-induced mouse model of TBI.**Results:**Integrated bioinformatics analyses revealed significant transcriptomic similarities between TBI and PTE, highlighting shared molecular pathways and regulatory networks involved in disease progression. Among the identified hub genes, DUSP1 and EGR1 emerged as pivotal regulators,

exhibiting significant differential expression across both TBI and PTE datasets. Experimental validation in the CCI-induced TBI mouse model further confirmed the dysregulated expression of these genes.**Conclusion:**Our findings demonstrate that DUSP1 and EGR1 represent key molecular links between TBI and PTE and may serve as promising therapeutic targets for the prevention and treatment of TBI-associated PTE. These results enhance our understanding of the molecular mechanisms underlying epileptogenesis following TBI and provide a foundation for the development of novel targeted therapeutic strategies.

Keywords: Traumatic brain injury; post-traumatic epilepsy; bioinformatics; neuroinflammation; neuroplasticity.

Introduction：

Traumatic brain injury (TBI) is a major global cause of mortality and long-term disability, affecting more than 55 million individuals worldwide each year [1]. TBI induces immediate structural damage, including cerebral contusion, intracranial hemorrhage, and neuronal death, which subsequently initiates a cascade of secondary injury processes characterized by neuroinflammation, oxidative stress, mitochondrial dysfunction, and blood–brain barrier disruption [2–5]. These pathological events contribute to persistent neurological deficits and substantially increase the risk of chronic neurological complications.Post-traumatic epilepsy (PTE) is one of the most severe and debilitating long-term consequences of TBI, occurring in approximately 20–50% of patients following moderate-to-severe brain injury [6]. PTE is characterized by recurrent unprovoked seizures that arise secondary to trauma-induced epileptogenic changes within the brain. Despite advances in antiepileptic therapies, nearly 30% of patients with PTE develop drug-resistant epilepsy, resulting in progressive cognitive decline, functional impairment, and psychological distress [7,8]. Consequently, PTE imposes a considerable socioeconomic burden on patients, caregivers, and healthcare systems.Accumulating evidence has established a close pathological association between TBI and PTE. Mechanisms

implicated in post-traumatic epileptogenesis include neuronal hyperexcitability, synaptic reorganization, glial activation, chronic neuroinflammation, and disruption of the excitatory–inhibitory balance within neural circuits [9,10]. However, most previous investigations have focused on either TBI or PTE independently, limiting our understanding of the molecular mechanisms that drive the transition from traumatic injury to chronic epilepsy. In particular, the shared gene regulatory networks, signaling pathways, and molecular mediators linking these two conditions remain incompletely characterized. Furthermore, comprehensive studies exploring the common pathogenic mechanisms of TBI and PTE from a systems biology perspective are still scarce.Recent advances in bioinformatics and high-throughput sequencing technologies have enabled the integration of multi-omics data, including genomic, transcriptomic, and proteomic information, thereby providing powerful tools for identifying key molecular regulators and disease-associated pathways [11]. Through the analysis of publicly available datasets, common differentially expressed genes (DEGs) associated with both TBI and PTE can be identified and further characterized using functional enrichment analyses, pathway mapping, and protein–protein interaction (PPI) network construction. Such integrative approaches facilitate the systematic exploration of shared molecular mechanisms and may reveal critical biological processes involved in the pathogenesis of both disorders.Among the candidate molecular regulators, Dual Specificity Phosphatase 1 (DUSP1) and Early Growth Response 1 (EGR1) have attracted increasing attention because of their important roles in neuroinflammation, neuronal plasticity, neural repair, and epileptogenesis [12]. DUSP1 functions as a negative regulator of mitogen-activated protein kinase (MAPK) signaling and has been shown to attenuate neuroinflammatory responses and promote neuroprotection following TBI [13,14]. By suppressing excessive inflammatory activation, DUSP1 may reduce secondary neuronal injury and potentially influence the development of PTE. EGR1, an immediate-early transcription factor involved in neuronal activity-dependent gene regulation, plays a crucial role in neuronal plasticity, injury responses, and neural circuit remodeling [15]. Previous studies have demonstrated that EGR1 expression is rapidly induced after

TBI and contributes to neurorepair through the regulation of neurotrophic signaling pathways [16,17]. Nevertheless, aberrant or sustained EGR1 activation may alter neuronal excitability and facilitate epileptogenic network remodeling, thereby promoting seizure susceptibility [18]. Collectively, these findings suggest that DUSP1 and EGR1 may represent key molecular nodes at the intersection of post-traumatic recovery and epileptogenesis.

In the present study, we employed an integrated bioinformatics strategy to systematically investigate the shared molecular mechanisms underlying TBI and PTE. By identifying common DEGs, characterizing enriched biological functions and signaling pathways, and constructing regulatory interaction networks, we sought to elucidate the molecular events that contribute to the progression from TBI to PTE. Particular emphasis was placed on the identification and functional characterization of hub genes, including DUSP1 and EGR1, to clarify their potential roles in the TBI–PTE continuum. The findings of this study may provide novel insights into the molecular basis of post-traumatic epileptogenesis and offer potential therapeutic targets and biomarkers for the prevention and treatment of PTE.

Materials and Methods

Data Acquisition and Processing

Gene expression datasets pertaining to traumatic brain injury (TBI) and post-traumatic epilepsy (PTE) were obtained from the Gene Expression Omnibus (GEO) database by searching the keywords "Traumatic brain injury" and "Post-Traumatic Epilepsy." Two relevant datasets, GSE242025 (Siddiqui N et al., 2022) [19] and GSE271985 (Lee Y et al., 2024; Lefton M et al., 2025) [20], were selected for subsequent analysis. Quality control and batch effect correction were performed using principal component analysis (PCA) and the ComBat function within the sva R package (version 3.52.0). Subsequently, differentially expressed genes (DEGs) were identified via the limma R package (version 3.60.4), applying thresholds of p-value < 0.05 and $|\log2 \text{ fold change}| \geq 1$. The overall analytical workflow is schematically summarized in Figure 1.

Identification of Crosstalk Genes

To identify molecular intersections between TBI and PTE, differentially expressed genes (DEGs) were first extracted from the GSE242025 and GSE271985 datasets using the limma R package. Subsequently, an online Venn analysis tool was employed to delineate the overlapping DEGs between both conditions. Genes exhibiting consistent differential expression across the two datasets were defined as crosstalk genes for further investigation.

Functional and Pathway Enrichment Analysis

The biological relevance of the identified crosstalk genes was examined through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. These analyses aimed to elucidate the involvement of these genes in disease-associated biological processes and signaling cascades. Statistical significance was assessed using false discovery rate (FDR) correction, with an adjusted p-value < 0.05 considered significant.

Protein–Protein Interaction Network and Hub Gene Identification

A protein–protein interaction (PPI) network was constructed using the STRING database (https://string-db.org/) based on the common DEGs. Topological parameters, including degree, closeness centrality, and betweenness centrality, were calculated to identify hub genes and key functional modules within the network.

Validation Using the Comparative Toxicogenomics Database

To corroborate the relevance of the identified hub genes in traumatic epilepsy, their expression and disease associations were further interrogated using the Comparative Toxicogenomics Database (CTD). This resource facilitated the validation of gene–disease relationships and provided mechanistic insights into the potential roles of these genes in TBI and PTE progression.

Single-Cell Transcriptomic Analysis

Single-cell RNA sequencing data from TBI (GSE290150) and PTE (GSE274303) datasets were processed and integrated using the Seurat package (v5.1.0). Following quality control and normalization, t-distributed stochastic neighbor embedding (t-SNE) was applied to visualize cellular heterogeneity. Clustering was performed at a resolution of 0.4 after evaluating stability and marker gene expression. Cell subtypes were annotated with the GPTCelltype R package (v1.0.1), and subtype-specific marker genes were identified using the FindAllMarkers function.

Animal Model of Traumatic Brain Injury

Adult male C57BL/6J mice (6–8 weeks old) were maintained under specific pathogen-free conditions with controlled temperature, humidity, and a 12-hour light/dark cycle. After anesthesia with isoflurane (2% induction, 1.5% maintenance), a moderate TBI was induced using the Feeney weight-drop method. Briefly, a 4 mm craniotomy was performed 2 mm lateral to the midline and 3 mm posterior to the bregma. A 40 g weight was dropped from a height of 7.5 cm onto the exposed dura. Mice were randomly assigned to Sham (n=6) or TBI (n=6) groups.

Animal Behavioural Assessment

Rotarod Test: Prior to the initial test, mice underwent a three-day training period on the rotarod apparatus . During the test, the rotarod speed was uniformly increased from 4 rpm to 40 rpm over a period of 3 minutes, and the time taken for the mouse to fall from the rotating rod was recorded.

Balance Beam Test: Testing was conducted on a narrow balance beam (approximately 80 cm long, 1.5 cm wide, and 100 cm above the ground), with the mice's motor coordination quantified by counting the time taken to traverse the beam.

Both the balance beam and rotarod tests involved a three-day training period for the mice prior to modelling. Following traumatic brain injury (TBI) modelling, behavioural assessments were conducted on day 7.

HE staining

On day 7 following TBI, brain tissue from mice in each group was subjected to H&E staining, with healthy mice serving as the control group

Immunofluorescence Staining

Following transcardial perfusion with PBS and 4% paraformaldehyde, brains were post-fixed, dehydrated in sucrose gradients (20% and 30%), and sectioned at 25 μm thickness. Sections were blocked with 10% donkey serum, then incubated overnight at 4°C with primary antibodies against Iba1 (abcam ab283346), DUSP1 (Bioss bsm-52770R，), and EGR1 (Bioss bs-23707R). After washing, fluorescent secondary antibodies were applied for 1 hour at room temperature. The concentrations of primary and secondary antibodies were 1:800 and 1:1000, respectively.Nuclei were counterstained with DAPI. Images were acquired using fluorescence microscopy, and fluorescence intensity was quantified with ImageJ software, normalized to the Sham group.

Statistical Analysis

All statistical analyses were conducted using R and GraphPad Prism (v3.10.1). Data distribution and variance homogeneity were assessed to select appropriate tests: unpaired t-test or Mann–Whitney U test for two-group comparisons, and one-way ANOVA or Kruskal–Wallis test for multi-group analyses. A two-sided p-value < 0.05 was considered statistically significant.

Results

Identification of Differentially Expressed Genes

The analytical workflow is schematically presented in Figure 1. Gene expression profiles associated with traumatic brain injury (TBI) and post-traumatic epilepsy (PTE) were retrieved from the NCBI GEO database using the series matrix files of datasets GSE242025 and GSE271985. The GSE242025 dataset comprises five TBI and five control mouse samples, whereas the GSE271985 dataset includes ten seizure-induced

epilepsy samples and nine controls. Differentially expressed genes (DEGs) between experimental and control groups were identified using the limma package, with thresholds set at $p < 0.05$ and $|\log_2FC| > 1$.

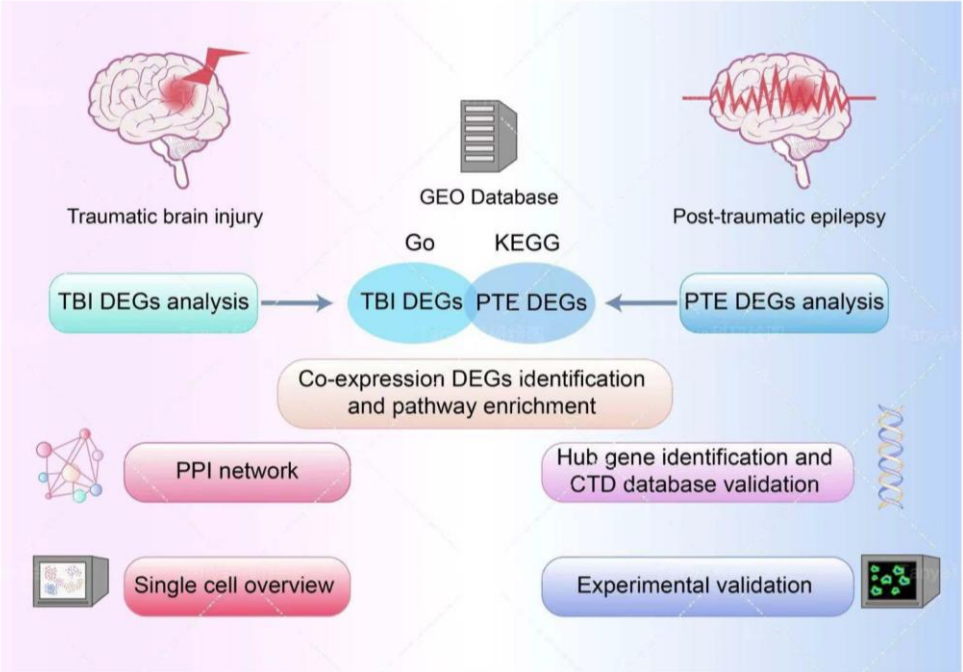


Figure 1 The overall research flowchart for this paper.

GO/KEGG Enrichment Analysis of Differentially Expressed Genes in Traumatic Brain Injury and Post-Traumatic Epilepsy

Using a threshold of $p < 0.05$ and $|\log_2FC| > 0.5$, a total of 983 upregulated and 855 downregulated differentially expressed genes (DEGs) were identified in the GSE242025 dataset for traumatic brain injury (TBI). In the post-traumatic epilepsy (PTE) dataset GSE271985, 483 upregulated and 374 downregulated DEGs were similarly detected (Figure 2A and B).

In the GSE242025 dataset, the 1,838 DEGs were significantly enriched in biological processes related to synaptic structure regulation, membrane potential modulation, and learning and memory. Pathway analysis further indicated notable involvement in neurodegenerative disease-associated pathways, particularly the MAPK signaling cascade (Figure 3A, 3B). In contrast, the 857 DEGs from the

GSE271985 dataset were predominantly associated with oxidative phosphorylation, mitochondrial respiratory chain complex assembly, and the PI3K-Akt signaling pathway (Figure 3C, 3D).

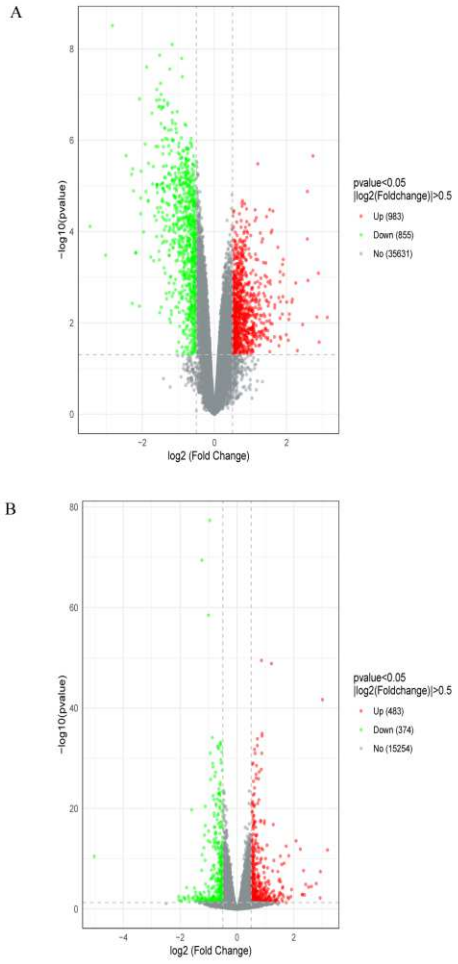


Figure2：Identification of Differentially Expressed Genes (DEGs) in Traumatic Brain Injury (GSE242025) and Post-Traumatic Epilepsy (GSE271985) Datasets. (A) Volcano plot of DEGs in traumatic brain injury. (B) Volcano plot of DEGs in post-traumatic epilepsy.

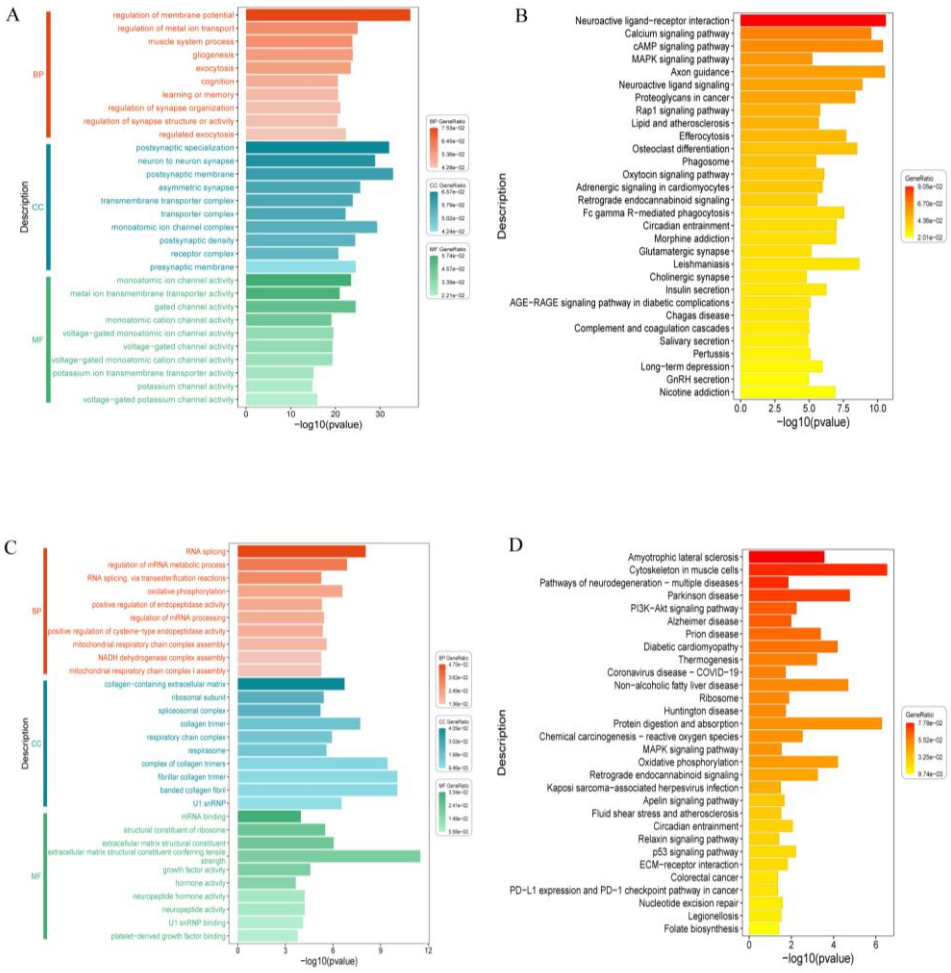


Figure3 (A-D) GO and KEGG enrichment pathway maps for traumatic brain injury and post-traumatic epilepsy, respectively.

Identification and Functional Profiling of Shared Differentially Expressed Genes in Traumatic Brain Injury and Post-Traumatic Epilepsy

Venn analysis was performed to identify overlapping differentially expressed genes (DEGs) between the traumatic brain injury dataset (GSE242025) and the epilepsy dataset (GSE143272), applying the previously established filtering criteria. This analysis revealed 48 common DEGs (Figure 4A). Following the exclusion of genes exhibiting opposite expression trends between the two conditions, a final set of 19 genes—comprising 11 upregulated and 8 downregulated—was retained (Figure 4B, 4C). Functional enrichment analysis demonstrated that these shared DEGs are predominantly involved in biological processes related to learning, memory, cognition,

and synaptic plasticity. Moreover, they were significantly associated with key signaling pathways, including the IL-17 and Wnt cascades (Figure 4D, 4E)

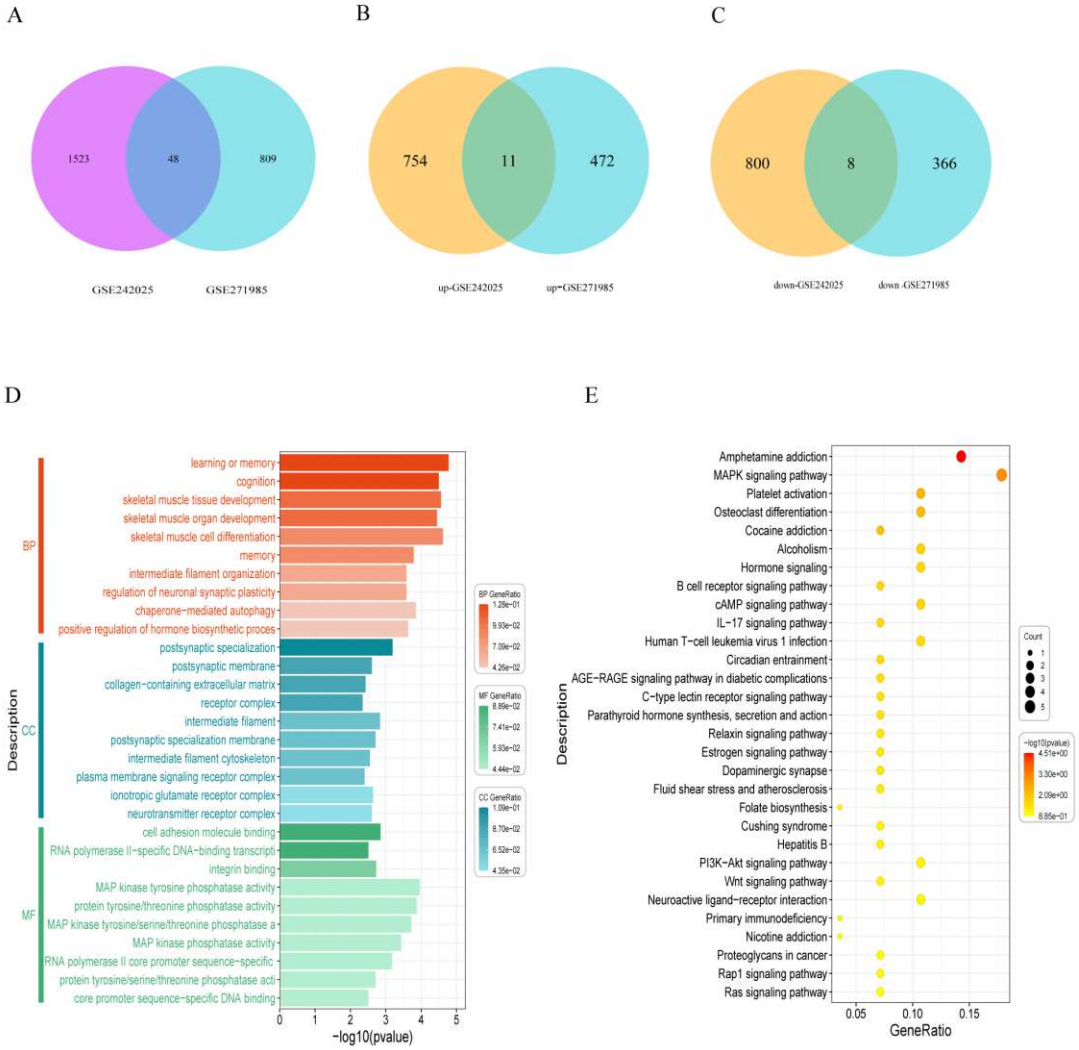


Figure4: Identification and Enrichment Analysis of Common Differentially Expressed Genes (DEGs) in Traumatic Brain Injury and Post-Traumatic Epilepsy Datasets: (A) Venn diagram showing the 48 common DEGs. (B) Eleven upregulated genes. (C) Eight downregulated genes. (D) GO enrichment pathway map for common DEGs. (E) KEGG enrichment pathway map for common DEGs.

Construction of Protein-Protein Interaction (PPI) Network, Modular Analysis, and Identification of Hub Genes

The PPI network for differentially expressed genes (DEGs) was constructed using String and Cytoscape. The top eight central genes identified through this approach were visualized (Figure 5A). In the network, nodes represent DEGs, while edges indicate interactions between genes. The size of the nodes corresponds to the gene's centrality or expression level, with darker colors indicating higher significance. Venn diagram analysis (Figure 5B) shows the overlapping genes identified by different algorithms, including Arc, DUSP1, DUSP6, EGR1, Fos, Foab, and Nr4a1. To investigate the expression levels of these hub genes, we analyzed their expression in both TBI and PTE. Figures 4B-H illustrate the expression of these seven genes, showing that MEST treatment significantly suppressed their expression. These genes play key roles in biological processes such as synaptic plasticity, learning and memory, and neuroinflammation (Figures 6A-G).

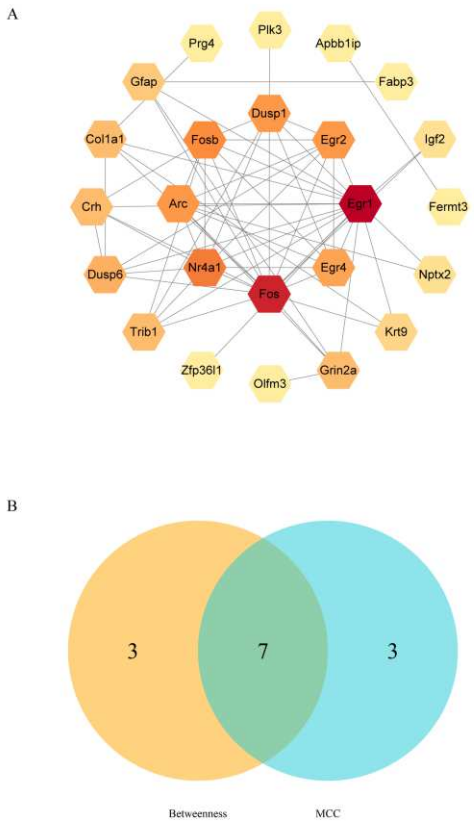


Figure 5: Identification and Expression Levels of Hub Genes. (A) PPI network of

common differentially expressed genes (DEGs) in TBI and PTE. (B) Venn diagram of overlapping genes identified by three algorithms.

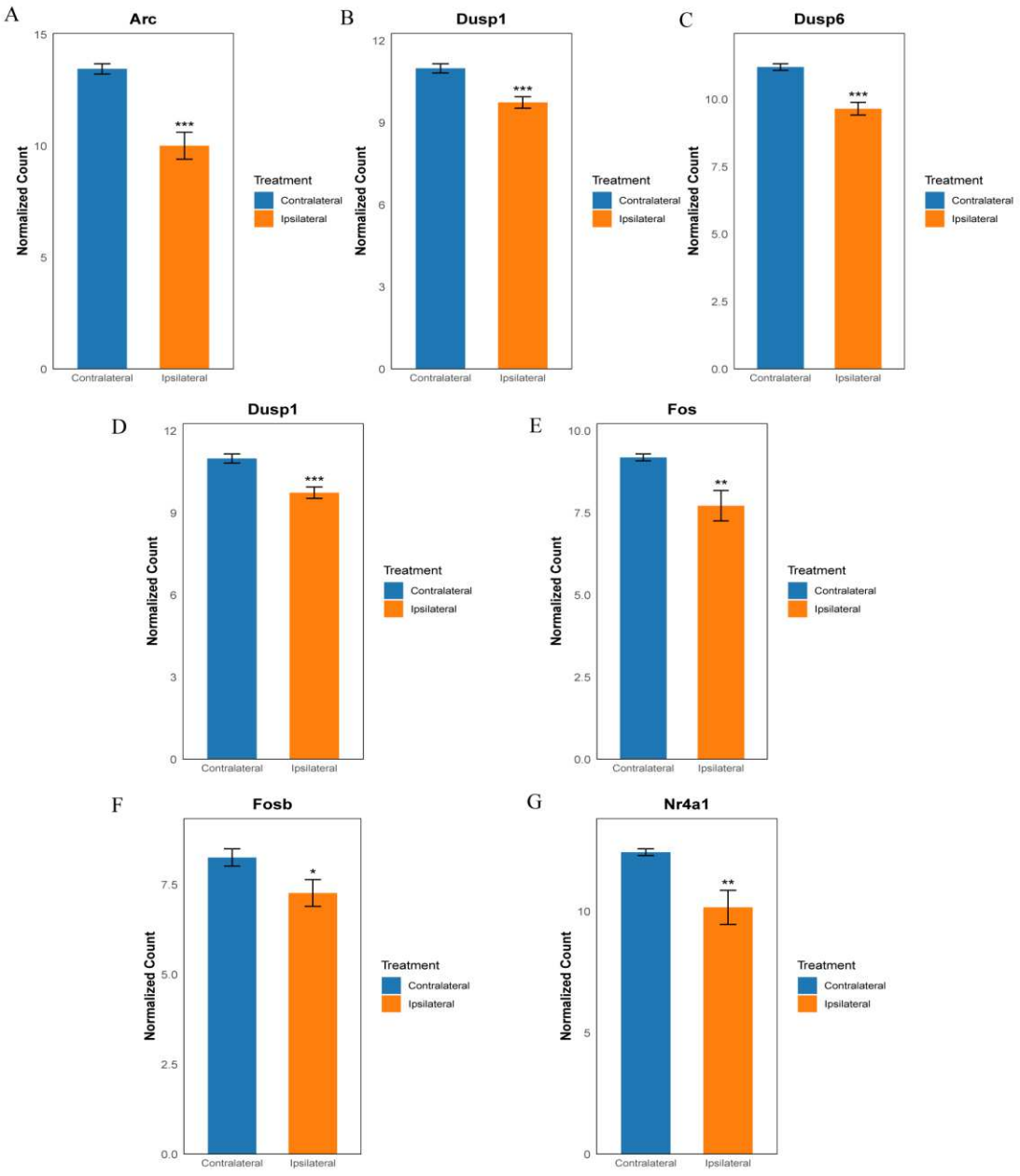


Figure 6(A–G) Expression levels of hub genes from the GSE40490 database. Data comparisons between the two groups were performed using a t-test for means, **p < 0.01, ***p < 0.001.

Validation of Hub Genes through Database Analysis

Correlation analysis of the hub genes using the CTD database further confirmed that DUSP1 and EGR1 are key molecular nodes linking brain injury and the onset of epilepsy. The analysis revealed that these two genes play central roles in the pathological processes of traumatic brain injury (TBI) and post-traumatic epilepsy (PTE) (Figure 7A), suggesting their involvement in shared pathways of disease progression and highlighting them as potential therapeutic targets.

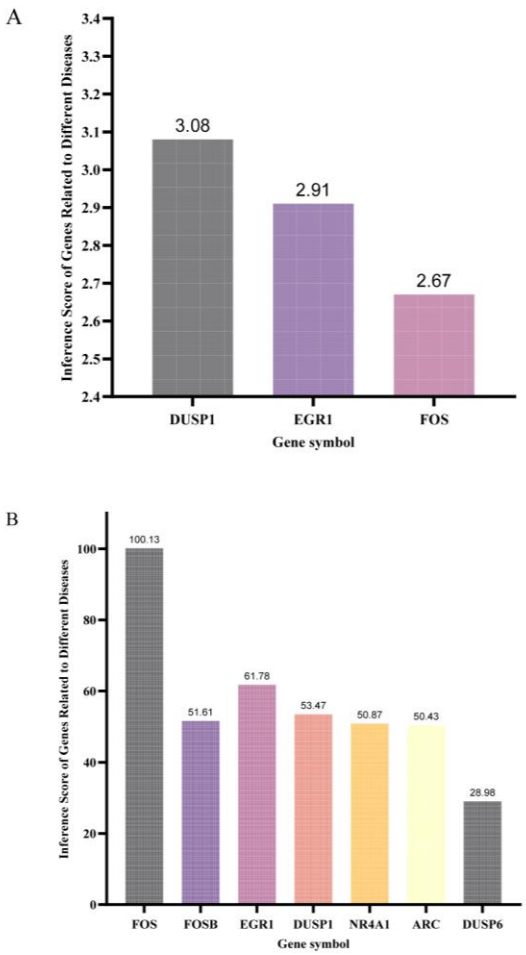


Figure 7: (A) Analysis of the relationship between hub genes and traumatic brain injury (TBI) based on the Comparative Toxicogenomics Database (CTD). (B) Analysis of the relationship between hub genes and post-traumatic epilepsy (PTE) based on the CTD.

Single-Cell Expression Profiling and Immunofluorescence Validation of DUSP1 and EGR1

To further investigate the cellular distribution of key hub genes, We obtained single-cell datasets for TBI and PTE from GSE290150 and GSE274303, and performed single-cell analysis using the Seurat package with t-SNE for visualization. Cell populations were annotated with the SingleR package and manually curated based on established marker genes, resulting in the identification of astrocytes, dendritic cells, endothelial cells, microglia, and other subtypes (Figures 8A, 8B).Expression patterns of DUSP1 and EGR1 across these cell types are displayed in (Figure 8C,8D), where expression intensity is projected onto the t-SNE map, with darker hues indicating higher transcript levels.We subsequently established a mouse model of traumatic brain injury (TBI). Figures (E) and (F) show the morphology of normal and TBI-affected brain tissue in mice, respectively; Figures (G) and (H) present haematoxylin and eosin (HE) stained images of mouse brain tissue; and Figures (I)and (J) present the results of behavioural tests using the rotarod and balance beam. The HE staining and behavioural data described above collectively confirm the successful establishment of the mouse brain injury model. immunofluorescence staining was conducted on cortical tissues from sham-operated and TBI (day 7) mice to validate protein expression and localization. Quantitative analysis of fluorescence intensity demonstrated distinct upregulation of both DUSP1 and EGR1 following TBI (Figure K,8L). Notably, merged images revealed that DUSP1 and EGR1 signals predominantly co-localized with IBA1-positive microglia in the injured cortex, suggesting their specific induction within this cell population after TBI.Quantitative analysis of the relative fluorescence intensity of DUSP1 and EGR-1 in brain sections from the control group and traumatic brain injury (TBI) group (Figure 8M,8N, respectively; n=6). Data are presented as mean ± standard error of the mean (SEM). *, $p<0.05$; **, $p<0.01$.

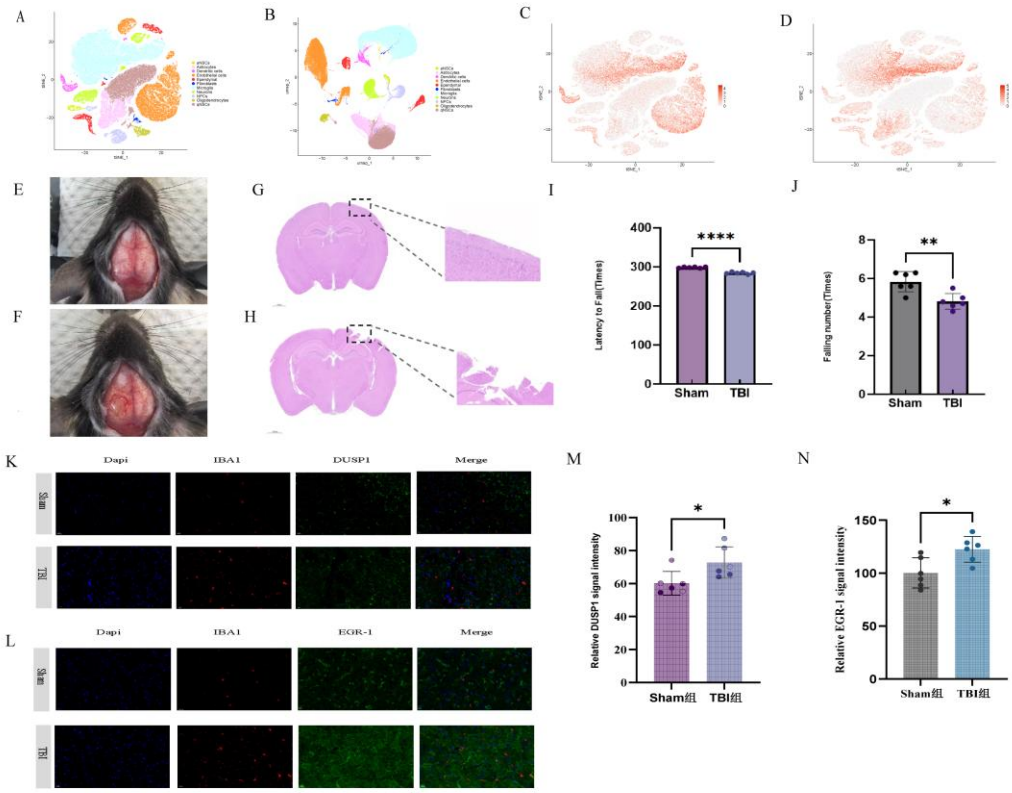


Figure 8: (A) and (B) show the cell type identification results from single-cell RNA sequencing analysis in TBI (traumatic brain injury) and PTE (post-traumatic epilepsy), including astrocytes, dendritic cells, endothelial cells, microglia, and others. (C) and (D) display the expression levels of the DUSP1 and EGR-1 genes, respectively, visualized on t-SNE dimensionality reduction plots, with color intensity representing the level of gene expression, (E)and (F) show the morphology of normal mouse brain tissue and TBI brain tissue,(G) and (H) show HE-stained images of mouse brain tissue; (I) and (J)present the results of the rotarod tests and the balance beam test.(K) presents immunofluorescence staining results: DAPI (blue) labels cell nuclei, IBA1 (green) marks microglia, and DUSP1 (red) indicates the distribution of DUSP1 protein. (L) similarly shows immunofluorescence staining, depicting DAPI (blue)-labeled nuclei, IBA1 (green)-labeled microglia, and the distribution of EGR-1 protein in red. (M) and (N) are quantitative statistical graphs of DUSP1 and EGR-1 immunofluorescence signals, respectively.

Discussion

The dysregulated expression of DUSP1 and EGR1 in both traumatic brain injury (TBI) and post-traumatic epilepsy (PTE) suggests their potential involvement in the shared pathological continuum linking these two conditions [21]. Increasing evidence indicates that TBI is not merely an acute neurological insult but often progresses into a chronic disease state that contributes to epileptogenesis and long-term neurological dysfunction [22,23]. The concurrent dysregulation of DUSP1 and EGR1 in both TBI and PTE may therefore provide important insights into the molecular mechanisms underlying this pathological transition. Notably, single-cell analysis of the CTD dataset revealed that both genes were predominantly expressed in microglia. This observation was further validated by immunofluorescence staining in a murine TBI model, which demonstrated significant cortical upregulation of DUSP1 and EGR1 accompanied by marked co-localization with IBA1-positive microglia.

DUSP1 as a Regulator of Neuroinflammation and Neural Repair

DUSP1 is a dual-specificity phosphatase that negatively regulates the mitogen-activated protein kinase (MAPK) signaling pathway through dephosphorylation, thereby preventing excessive pathway activation. This regulatory function contributes to the attenuation of neuroinflammation, reduction of cellular injury, and promotion of tissue repair following brain damage. Previous studies have shown that trauma-induced upregulation of DUSP1 suppresses the production of pro-inflammatory cytokines and inhibits the activation of microglia and astrocytes, thereby mitigating neuronal damage and facilitating neurorepair [24]. Given the critical role of the MAPK signaling cascade in cellular proliferation, differentiation, and stress responses [25], DUSP1 serves as an essential negative regulator that helps maintain neural homeostasis and limits secondary injury.Following TBI, DUSP1 expression is significantly elevated, further highlighting its neuroprotective role. Hyperactivation of the MAPK pathway after injury is known to exacerbate neuroinflammatory responses and neuronal loss. However, DUSP1-mediated dephosphorylation effectively restrains aberrant MAPK signaling, thereby suppressing downstream inflammatory cascades [26,27]. Specifically, increased DUSP1

expression modulates trauma-induced neuroinflammation, reduces the release of inflammatory mediators, and attenuates glial activation. Collectively, these effects slow neuronal degeneration and promote neurological recovery. Consequently, DUSP1 is widely recognized as a key neuroprotective factor that preserves neuronal integrity and supports functional restoration following TBI.

EGR1 in Neuronal Plasticity and Epileptogenesis

EGR1 (early growth response 1) is an immediate-early gene and transcription factor that plays a pivotal role in neuronal development, injury responses, and synaptic plasticity. As a rapidly inducible transcriptional regulator, EGR1 responds to both physiological and pathological stimuli by controlling the expression of genes involved in cell survival, differentiation, and tissue repair [28]. In addition to its role in neuronal regeneration, EGR1 contributes to the maintenance of neural homeostasis through the modulation of neuroinflammatory processes.Following TBI, EGR1 expression is markedly increased, suggesting a close association with repair and regenerative mechanisms. In response to neuronal injury and cell death, EGR1 promotes the expression of neurotrophic factors, including brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), both of which are essential for neuronal repair and neurogenesis [29]. These neurotrophic factors support neuronal survival, synapse formation, and circuit reconstruction, thereby facilitating adaptive responses to traumatic injury.Moreover, EGR1 has been implicated in neural network remodeling during post-injury recovery [30]. By regulating neuronal excitability and promoting adaptive reorganization of neural circuits, EGR1 contributes to the restoration of functional connectivity within the injured brain [31]. This remodeling capacity is particularly important during the recovery phase, as it helps re-establish the balance between excitatory and inhibitory signaling required for normal neural network function. However, persistent or excessive EGR1 activation may also contribute to maladaptive synaptic reorganization and neuronal hyperexcitability, processes that have been closely associated with epileptogenesis. Therefore, EGR1 may play a dual role in the post-traumatic brain, supporting neural repair while simultaneously participating in mechanisms that increase susceptibility to PTE.

Dual-Edged Role of EGR1 in Epileptogenesis

Despite its established role in neuronal repair and plasticity, EGR1 may also contribute to epileptogenesis when its expression is sustained or excessively elevated. By modulating neuronal excitability and neural network reorganization, aberrant EGR1 activation can reduce seizure thresholds and promote the development of hyperexcitable neuronal circuits. Furthermore, excessive EGR1 expression has been associated with neuronal apoptosis and functional deterioration, potentially aggravating epileptic pathology and contributing to adverse neurological outcomes. These findings suggest that EGR1 exerts a dual role following brain injury, functioning as both a mediator of neural repair and a potential driver of epileptogenic processes under pathological conditions.

Integrated Co-Expression in Microglia and Pathophysiological Implications

Both single-cell transcriptomics and immunofluorescence analyses confirmed that DUSP1 and EGR1 are co-expressed predominantly in microglia following TBI. This coordinated expression pattern implies that the two genes may function synergistically within microglia to regulate neuroinflammatory and repair processes relevant to both TBI and PTE. Although existing studies have partially elucidated their roles, the precise molecular interplay between DUSP1 and EGR1—and their net effect on disease progression—remains to be fully defined. Future investigations should focus on their interaction mechanisms, their combined influence on neuronal repair and network remodeling, and their potential as dual-target therapeutic strategies. Collectively, the dynamic expression profiles of DUSP1 and EGR1 offer valuable biomarker insights and mechanistic clues linking acute brain injury to chronic epileptogenesis. A limitation of this study is the lack of functional validation due to constraints in both sample size and experimental resources. Specifically, budgetary and technical limitations precluded in vivo loss-of-function experiments (e.g., gene knockout or knockdown of DUSP1 and EGR1) to assess their roles in the progression from traumatic brain injury (TBI) to post-traumatic epilepsy (PTE). Consequently, the

conclusiveness and generalizability of our findings are somewhat constrained. Future studies addressing these functional aspects are warranted to further elucidate the mechanistic roles of these two genes.

Conclusion

We analyzed transcriptomic data from TBI and PTE patients, identifying common DEGs and hub genes shared by both conditions, and performed a series of downstream analyses. Our findings suggest that DUSP1 and EGR1 may play critical regulatory roles in this process, potentially acting in synergy to modulate neuroinflammation, tissue repair, and neural plasticity, thereby conferring neuroprotection against TBI-induced injury and reducing the risk of PTE development. These insights offer a novel perspective that may inform the prevention and treatment of TBI-related PTE in the future.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

Data related to this study are available from the GEO database, including GSE242025 (https://www.ncbi.nlm.nih.gov/geo/). Single-cell RNA sequencing data from TBI (GSE290150) and PTE (GSE274303).The data generated in the present study may be requested from the corresponding author.

Authors' contributions

The conceptualization and design of the study were conducted by Limei Liu, Jiamei Jia, and Yu Pan. Data curation and analysis were performed by Limei Liu and Jiamei Jia. Limei Liu additionally carried out the experiments and drafted the initial manuscript. Manuscript review and editing were undertaken by Jiamei Jia and Yu Pan. All authors have reviewed and approved the final submitted version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

[1] Maas AIR, Menon DK, Manley GT, et al. Traumatic brain injury: progress and challenges in prevention, clinical care, and research. *Lancet Neurol*. 2022;21(11):1004-1060.

[2] Tang J, Kang Y, Zhou Y, et al. TIMP2 ameliorates blood-brain barrier disruption in traumatic brain injury by inhibiting Src-dependent VE-cadherin internalization. J Clin Invest. 2023;134(3):e164199. Published 2023 Nov 28.

[3] Munoz-Ballester C, Mahmutovic D, Rafiqzad Y, Korot A, Robel S. Mild Traumatic Brain Injury-Induced Disruption of the Blood-Brain Barrier Triggers an Atypical Neuronal Response. Front Cell Neurosci. 2022;16:821885. Published 2022 Feb 18.

[4] Sulimai N, Brown J, Lominadze D. Caffeic Acid Phenethyl Ester Protects Neurons Against Oxidative Stress and Neurodegeneration During Traumatic Brain Injury. Biomolecules. 2025;15(1):80. Published 2025 Jan 8.

[5] Sulhan S, Lyon KA, Shapiro LA, Huang JH. Neuroinflammation and blood-brain barrier disruption following traumatic brain injury: Pathophysiology and potential therapeutic targets. J Neurosci Res. 2020;98(1):19-28.

[6] Pitkänen A, Immonen R, Kharatishvili M, et al. Modeling Post-Traumatic Epilepsy for Therapy Development [M]//Handbook of Clinical Neurology. Elsevier, 2017: 217-246.

[7] Han Z, Zhao Z, Wang L, et al. Targeted Pyroptosis with Resveratrol Nanoparticles to Reduce Secondary Brain Injury and Post-Traumatic Epilepsy. ACS Appl Mater Interfaces. 2024;16(48):65850-65862.

[8] Javalgekar M, Jupp B, Vivash L, et al. Inflammasomes at the crossroads of traumatic brain injury and post-traumatic epilepsy. J Neuroinflammation. 2024;21(1):172. Published 2024 Jul 16.

[9] Shi Z, Mao L, Chen S, et al. Reversing Persistent PTEN Activation after Traumatic Brain Injury Fuels Long-Term Axonal Regeneration via Akt/mTORC1 Signaling Cascade. Adv Sci (Weinh). 2025;12(6):e2410136.

[10] Shandra O, Mahmutovic D, Maharathi B, et al. Fast Ripple-Delta Coupling as Early Biomarker for Post-Traumatic Epileptogenesis in Repetitive Brain Injury. Preprint. bioRxiv. 2025;2025.09.16.676387. Published 2025 Oct 2.

[11] Slobodyanyuk M, Bahcheli AT, Klein ZP, Bayati M, Strug LJ, Reimand J. Directional integration and pathway enrichment analysis for multi-omics data. Nat Commun. 2024;15(1):5690. Published 2024 Jul 7.

[12] Kernie SG, Parent JM. Forebrain neurogenesis after focal Ischemic and traumatic brain injury. Neurobiol Dis. 2010;37(2):267-274.

[13] Taylor DM, Moser R, Régulier E, et al. MAP kinase phosphatase 1 (MKP-1/DUSP1) is neuroprotective in Huntington's disease via additive effects of JNK and p38 inhibition. J Neurosci. 2013;33(6):2313-2325.

[14] Zhao H, Lv J, Meng L, Lv J, Li Z. Dual-specificity phosphatase 26-dificient neurons are susceptible to oxygen-glucose deprivation/reoxygenation-evoked apoptosis and proinflammatory response by affecting the TAK1-medaited JNK/P38 MAPK pathway. Int Immunopharmacol. 2023;117:109980.

[15] Kalinina A, Krekhno Z, Yee J, Lehmann H, Fournier NM. Effect of repeated seizures on spatial exploration and immediate early gene expression in the hippocampus and dentate gyrus. IBRO Neurosci Rep. 2021;12:73-80. Published 2021 Dec 31.

[16] Chen T, Zhu J, Wang YH, Hang CH. Arc silence aggravates traumatic neuronal injury via mGluR1-mediated ER stress and necroptosis. Cell Death Dis. 2020;11(1):4. Published 2020 Jan 2.

[17] Veremeyko T, Jiang R, He M, Ponomarev ED. Complement C4-deficient mice have a high mortality rate during PTZ-induced epileptic seizures, which correlates with cognitive problems and the deficiency in the expression of Egr1 and other immediate early genes. Front Cell Neurosci. 2023;17:1170031.

[18] López-López D, Gómez-Nieto R, Herrero-Turrión MJ, et al. Overexpression of the immediate-early genes Egr1, Egr2, and Egr3 in two strains of rodents susceptible to audiogenic seizures. Epilepsy Behav. 2017;71(Pt B):226-237.

[19] Siddiqui N, Oshima K, Hippensteel JA. Proteoglycans and glycosaminoglycans in central nervous system injury. *Am J Physiol Cell Physiol* 2022 Jul 1;323(1):C46-C55.

[20] Lefton M, Flores CC, Owada Y, et al. Astrocyte Fabp7 modulates nocturnal seizure threshold and activity-dependent gene expression in mouse brain. PNAS Nexus. 2025;4(5):pgaf146. Published 2025 May 7.

[21] Jiao W, Jiang L, Zhang Y. SNHG1 alleviates the oxidative stress and inflammatory response in traumatic brain injury through regulating miR-377-3p/DUSP1 axis. Neuroreport. 2023;34(1):17-29.

[22] Li N, Lu W, Tang L, et al. Microglia in Post-Traumatic Brain Injury (TBI) Cognitive Impairment: From Pathological Changes to Therapeutic Approaches. CNS Neurosci Ther. 2025;31(8):e70568.

[23] Ruchika F, Shah S, Neupane D, Vijay R, Mehkri Y, Lucke-Wold B. Understanding the Molecular Progression of Chronic Traumatic Encephalopathy in Traumatic Brain Injury, Aging and Neurodegenerative Disease. Int J Mol Sci. 2023;24(3):1847. Published 2023 Jan 17.

[24] Hu Y, Zhan F, Wang Y, et al. The Ninj1/Dusp1 Axis Contributes to Liver Ischemia Reperfusion Injury by Regulating Macrophage Activation and Neutrophil Infiltration. Cell Mol Gastroenterol Hepatol. 2023;15(5):1071-1084.

[25] He X, Deng B, Zhang C, et al. HSPA1A inhibits pyroptosis and neuroinflammation after spinal cord injury via DUSP1 inhibition of the MAPK signaling pathway. Mol Med. 2025;31(1):53. Published 2025 Feb 9.

[26] Pignatelli M, Luna-Medina R, Pérez-Rendón A, Santos A, Perez-Castillo A. The transcription factor early growth response factor-1 (EGR-1) promotes apoptosis of neuroblastoma cells. Biochem J. 2003;373(Pt 3):739-746.

[27] Jiao W, Jiang L, Zhang Y. SNHG1 alleviates the oxidative stress and inflammatory response in traumatic brain injury through regulating miR-377-3p/DUSP1 axis. Neuroreport. 2023;34(1):17-29.

[28] Rahmi U, Goenawan H, Sylviana N, et al. Exercise induction at expression immediate early gene (c-Fos, ARC, EGR-1) in the hippocampus: a systematic review. Dement Neuropsychol. 2024;18:e20230015. Published 2024 Apr 15.

[29] Yin F, Liu Z, Zhang D, Shen Z, Niu Z, Guo L. Identification of key genes involved in neural regeneration and the repairing effect of BDNF-overexpressed BMSCs on spinal cord ischemia-reperfusion injury in rats. Biomed Pharmacother. 2023;160:114293.

[30] Förstner P, Knöll B. Interference of neuronal activity-mediated gene expression through serum response factor deletion enhances mortality and hyperactivity after traumatic brain injury. FASEB J. 2020;34(3):3855-3873.

[31] Sampaio ASB, Real CC, Gutierrez RMS, et al. Neuroplasticity induced by the retention period of a complex motor skill learning in rats. Behav Brain Res. 2021;414:113480.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- figureabstract.doc