

Assessing the Global Impact on the Mouse Kidney After Traumatic Brain Injury: A Transcriptomic Study

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Purpose: In this study, we use animal models combined with bioinformatics strategies to investigate the potential changes in overall renal transcriptional expression after traumatic brain injury.

Methods: Microarray analysis was performed after kidney acquisition using unilateral controlled cortical impact as the primary mouse TBI model. Multi-oriented gene set enrichment analysis was performed for differentially expressed genes.

Results: The results showed that TBI affected the gene set associated with mitochondria function in kidney cells, and a negative enrichment of gene sets associated with immune cell migration and epidermal development was also observed. Analysis of the disease phenotype gene set revealed that differential expression of mitochondria-related genes was associated with lactate metabolism. Alternatively, activation and adhesion of immune cells associated with the complement system may promote autoinflammation in kidney tissue. The simulated immune cell infiltration analysis showed an increase in the proportion of activated memory CD4 T cells and a decrease in the proportion of resting memory CD4 T cells, suggesting that activated memory CD4 T cell infiltration may be involved in the inflammation of renal tissue and cause damage to renal cells, such as principal cells, mesangial cells and loops of Henle cells.

Conclusion: This study is the first to reveal the effects of brain trauma on the kidney. TBI may affect the expression of mitochondria function-related gene sets in renal cells by increasing lactate. It may also affect renal mesangial cells by inducing increased infiltration of immune cells through mechanisms related to complement system activation or autoimmune antibodies.

Keywords: traumatic brain injury, nephropathy, complement system, lactate metabolism, immune cell infiltration

Introduction

Traumatic brain injury (TBI) is an acquired brain injury caused by direct damage to the brain from external mechanical forces and is one of the most common causes of death and disability in healthy adults, resulting in significant medical financial costs to individuals and society.^{1,2} TBI not only has acute and chronic neurological consequences but also leads to non-neurological complications.³ Traditionally, the study of extracranial organ dysfunction after severe TBI has been based on individual organ systems. However, many studies have shown that multisystem organ dysfunction after TBI is common and may have similar mechanisms and pathophysiological features to single organ system dysfunction.

In particular, acute kidney injury (AKI) is thought to be potentially associated with TBI, and the concomitant occurrence of AKI is thought to be associated with causing unfavorable outcomes in TBI patients.^{3–7} Studies have previously found that the incidence of AKI after TBI is approximately 10–20%.^{5–8} Although elevated serum creatinine and decreased urine output are criteria for the diagnosis of AKI in TBI, patients who do not meet these criteria may be at risk of developing subclinical AKI due to acute tubular injury or other possible mechanisms.³ In addition to using RIFLE, AKIN and KDIGO criteria for AKI diagnosis, biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL), cystatin C, and serum lactate have been evaluated as screening tools for predicting AKI in critically ill patients including those with TBI.^{9–11} Different mRNA expression profiles may also facilitate to identify potential biomarkers associated with AKI with specific causes.^{12–14} However, the mechanism of how TBI is associated with AKI still needs to be elucidated.

Trauma-related excess blood loss may result in glomerular hypoperfusion and subsequent kidney injury. However, in profound hypoperfusion situation such as out of hospital cardiac arrest (OHCA), the incidence of AKI is only about 8.7%.¹⁵ Besides, most the TBI patients are younger and have normal pre-existing renal function compared with OHCA patients.¹⁶ This implied that hypoperfusion is not the only risk factor of AKI after TBI. Several medications for TBI, including mannitol for intracranial pressure (ICP) control,¹⁷ non-steroidal for analgesia, and radiocontrast agent for diagnosis, may also contribute to AKI.

Intracranial inflammatory responses in the injured brain may be responsible for neurological sequelae and lead to systemic inflammatory responses that may result in multiple organ dysfunction syndrome (MODS) and death.^{18,19} Exploring the linkage of immune responses and elucidating cellular and molecular interactions and biochemical pathways is important for both potential diagnostic biomarkers and therapeutic strategies for AKI. In this study, we used an animal model combined with a bioinformatics analysis strategy to elucidate the potential changes in the kidney after suffering TBI.

Materials and Methods

Traumatic Brain Injury Animal Model

We used unilateral controlled cortical impact as the primary TBI model. The heads of adult C57BL/6 male mice (7 weeks old) were fixed in a stereotaxic frame. Mice were anesthetized with isoflurane (4% induction and 2% maintenance),²⁰ topical anesthetic was applied to the surgical site to minimize discomfort. Mice were placed on a warming pad at 37°C to maintain temperature during surgery and monitored with a rectal probe. Toe pinch method was used to confirm the depth of anesthesia. After retraction of the scalp, a burr drill was used to perform a 4-mm diameter circular craniotomy on the right parietal temporal cortex.²⁰ A 3.0 mm diameter impactor tip with a speed of 4 m/s, a dwell time of 100 ms, and a deformation depth of 1 mm were used at 2.5 mm posterior to fontanelle and 2.0 mm right of midline (TBI group, n = 5). The sham group (n = 4) underwent the same craniotomy but without cortical impact. Warm saline (0.1 mL/10 g) was administered after the TBI operation to rehydrate the animals and the warming pad was used to maintain their body temperature. Animals were weighed and evaluated for behavioral testing 24 hours after the surgery. Modified neurologic severity scoring (mNSS) was performed based on the study of Li et al²¹ and behavioral change was assessed by the contralateral swing method.^{22,23} All mice were sacrificed using euthanasia methods. The mice were anesthetized with 4% isoflurane in an induction chamber. After the confirmation of unconsciousness and respiratory cessation, exsanguination of the mice via cardiac puncture and perfused with PBS were performed. Urine sample was collected by bladder puncture simultaneously. The kidneys were initially minced and placed in 2-mL tissue homogenizing mix tubes containing 3 mm Zirconium Oxide beads and TRIzol reagent (ThermoFisher Scientific Inc., MA, USA). The samples were stored at –80°C in an ultra-low temperature freezer before further procedure. The tissue homogenizer (Lawson, Ningbo, China) was set at 55 Hz for 30 seconds and 2 cycles were performed to homogenize the kidney tissue fragments. All procedures were approved by the Laboratory Animal Center of National Defense Medical Center, where they have been granted a full accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Urine, blood, and kidney tissue collection were performed under approved protocols. Urine and blood cytokine concentrations (pg/mL) were quantified by the Cytometric Bead Array Flex Set system (BD Biosciences, San Jose, CA, USA).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA of the mouse kidney was isolated using the TRIzol reagent. The OneStep RT-PCR Kit (QIAGEN, CA, USA) was used for RT-PCR and reactions were run on a GeneAmp PCR system 9700 (Applied Biosystems, NJ, USA). The following primers were used for RT-PCR as shown in Table 1.

mRNA Expression Profiling

Mouse kidney tissues were pre-treated and total RNA was extracted with TRIzol RNA isolation reagent according to the manufacturer's instructions. Total RNA from various mouse kidney tissues were sent to Phalanx Biotech Group (Hsinchu, Taiwan) for gene expression profiling services by HOA OneArray method and HmiOA v5. The amount and purity of RNA was assessed by NanoDrop ND-1000 (ThermoFisher Scientific Inc., MA, USA). The passing criteria for absorbance ratio were determined as $A_{260}/A_{280} \geq 1.8$ and $A_{260}/A_{230} \geq 1.5$, indicating acceptable RNA purity. RNA integrity number (RIN) values were determined using the Agilent RNA 6000 Nano assay (Agilent Technologies, Waldbronn, Germany) to determine the integrity of the RNA. The passing standard for RIN values was determined to

Table 1 Primers Used for RT-PCR

| Gene Name | Primer Sequence (5'→3') |
|------------------------|---|
| GAPDH | Forward: 5'-CTTCATTGACCTCAACTAC-3' Reverse: 5'-GCCATCCACAGTCTTCTG-3' |
| Myl7 | Forward: 5'-ATGGCCAGTAGGAAGGCTGG-3' Reverse: 5'-GTCTGCCTGGGTCATGAGAAG-3' |
| DKK3 ²⁴ | Forward: 5'-GGCAATGGGACCATCTGTGA-3' Reverse: 5'-TCTCCGCCTAGTGACTCCAC-3' |
| Adcy1 ²⁵ | Forward: 5'-CTCCAACGTGATGACCTGCGA-3' Reverse: 5'-CACCACACAGCCTTGAGCTAC-3' |
| Cacna1d ²⁶ | Forward: 5'-CCTCATCGTAATCGGCAGCAT-3' Reverse: 5'-TGGCAAAGTTGCTCCCACAAG-3' |
| Cacna1b ²⁶ | Forward: 5'-CTGGTGGCATTGCGTTCTCG-3' Reverse: 5'-GCATCCACAGAGTGTTCAGC-3' |
| Cacna2d2 ²⁶ | Forward: 5'-CCCAGCAGCTTTGAGTCTGAA-3' Reverse: 5'-CGCACTTCTGAGGTTGGTCC-3' |
| Cxcl13 ²⁷ | Forward: 5'-ATGAGGCTCAGCACAGCAACG-3' Reverse: 5'-TCAGGCAGCTCTTCTCTTACTC-3' |
| Tfec ²⁸ | Forward: 5'-GGAGCTTGGCACTCTTATTCC-3' Reverse: 5'-AGAGAGTAGTGGGTCCGTCC-3' |
| Sfrp5 ²⁹ | Forward: 5'-CAAGCGCTGCCACTCAGACA-3' Reverse: 5'-GTTCTTCATATGCAGGACCAGC-3' |
| Dmkn ^{30,31} | Forward: 5'-GATGCATCTACCTTGCCCTTC-3' Reverse: 5'-CTTCAGCCACTTCAGCAGGC-3' |
| Mlana ³² | Forward: 5'-ATGCCCCAAGAAGACATTAC-3' Reverse: 5'-TCAGGGTGAATAAGGTGGCGG-3' |
| Opn5 ³³ | Forward: 5'-GCCTGATTACCATGACTGCTG-3' Reverse: 5'-CTGACCACACAGAGACGACC-3' |
| Vdr ³⁴ | Forward: 5'-ATGGAGGCAATGGCAGCCAG-3' Reverse: 5'-GTGGGGTCGTAGGTCTTGTG-3' |

be ≥ 6 , indicating acceptable RNA integrity. gDNA contamination was assessed using gel electrophoresis. Data analysis was processed by the Rosetta Resolver[®] system (Rosetta Biosoftware). The majority of various databases are built on human gene symbol-based gene-set only. In order to increase the interpretability of mouse samples for analysis and to evaluate the association with human diseases, we pre-converted mouse genes to homologous human gene symbols using the Collapse Dataset tool provided by GSEA software with “Mouse Gene Symbol Remapping Human Orthologs MSigDB.v7.5.chip”.³⁵ Subsequent analyses were carried out using the homologous human gene symbol for comparison and analysis in each package in R (version 4.1.3).

GSE131179 Gene Expression Profiling

The GSE131179 database is provided by Verma et al in the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) Database and has published literatures discussing acute T-cell mediated rejection (ACR) of human kidney allografts.^{36,37} There are 34 samples in the GSE131179 database, including 16 ACR kidney samples and 18 Normal/Non-specific kidney samples. The raw data were downloaded and processed using GEOquery,³⁸ and grouped with the authors’ annotations for subsequent analysis.

Differential Expression Analysis and Enrichment Analysis

Analyses were performed using RStudio version 2022.02.0 Build 443 and R version 4.1.3. Differentially expressed genes (DEGs) between each group were analyzed using the EdgeR (empirical analysis of digital gene expression in R) package.³⁹ Please refer to [Supplementary Table 1](#) for the detailed raw data comparing TBI kidney to sham group. We used the Benjamin-Hochberg procedure to adjust all enriched p-values to assess statistical significance. Genes were considered to be differentially expressed when they had a $|\text{fold change}| \geq 2$ and a false discovery rate (FDR) < 0.001 . To fully represent the biological processes of renal tissue most affected by TBI, we performed multi-facet differential gene expression analyses and gene-set enrichment analyses.³⁵ For the MAplot, we used the ggmaplot function of the ggpubr package for plotting. Genes were sorted by FDR and fold change values, and we labeled the top 50 genes with the smallest FDR on the plot. For g:profiler analysis, the list of up-regulated and down-regulated genes was entered into the g:profiler web page, and the datasets were checked for Gene Ontology Biological Processing (GOBP), Gene Ontology Molecular Function (GOMF), Gene Ontology Cellular Component (GOCC), Reactome pathway (REAC) and Human phenotype ontology (HP), and analyzed with the default options.⁴⁰ The top 10 gene-sets in each dataset were filtered by FDR for up- and down-regulated DEGs, respectively. For over-representation analysis (ORA), DEG screening was performed with $|\text{fold change}| \geq 2$ and false discovery rate (FDR) < 0.001 , then using the clusterProfiler R package cnetplot and heatplot functions to produce plots.⁴¹ For the Enrichment map, we used the Enrichment map app (version 3.3.4) from Cytoscape 3.9.1 for the plotting.^{42,43} The “c5.go.bp.v7.5.1.symbols.gmt” file was downloaded from MSigDB for GOBP enrichment analysis.³⁵ Filter was set to “FDR cutoff < 0.05 ”, “Edge cutoff > 0.375 ”. We use AutoAnnotate 1.3.5 to annotate the clusters and delete the clusters with Node number less than 2. For SigCom LINCS analysis, we follow the instructions of the web tool to enter the list of up- and down-regulated genes and select the signature that provides 431 Datasets for scoring.⁴⁴ The top 30 results were ranked by the sum of Z scores [Z score (up) + Z score (down)]. For enriched gene-set comparison, the compareCluster function in clusterProfiler was used and the enrichKEGG, enrichWP and enrichDO functions were employed to analyze the enrichment scores and p -values for each gene set.⁴¹ For GSEA and Ridge plot, we searched for kidney-specific single-cell biomarkers in PanglaoDB and made a gmt file (detailed gene list please referred to [Supplementary Table 2](#)),⁴⁵ and used the gseaplot2 and ridgeplot functions in enrichplot package (v1.16.1) to plot the graphs, respectively.⁴⁶ CIBERSORT-X was used to evaluate cell abundance and cell type-specific gene expression patterns from a large number of tissue transcriptomic profiles.⁴⁷ We followed the web instructions to input microarray data for immune cell infiltration analysis.

Results

Execution and Verification of TBI Animal Models

In this study, we established an animal model of TBI with unilateral controlled cortical impact and performed behavioral pattern analysis 24 hours after the surgery, following which the animals were sacrificed and the kidneys were exteriorized

for total RNA extraction. The differences in renal whole-gene transcription expression between the TBI and sham groups of mice were analyzed by microarray method, and the corresponding procedure is shown in [Figure 1A](#). Twenty-four hours after the surgery, the mice in the TBI group showed significant weight loss ([Figure 1B](#)) and a substantial increase in mNSS ([Figure 1C](#)), as well as a significant increase in the percentage of contralateral swing ([Figure 1D](#)). When blood and urine were analyzed for inflammation-related cytokine concentrations, no significant differences were observed in blood. In urine, an increase in IFN- γ and TNF- α was observed ([Figure 1E](#)), although it was not significant due to the limitation of the amount of samples collected from animals. In addition, we extracted renal total RNA for RT-PCR and evaluated it against several genes identified in the literature as being associated with renal injury or function ([Figure 1F](#)). The results showed significant changes in the mRNA expression of DKK3, Tfec, and Vdr, three biomarkers associated with kidney injury.^{28,34,48} With these results, we confirmed that the execution of TBI caused significant physiological effects in mice kidney and resulted in a slight increase in inflammatory cytokines in the urine.

Over-Representation Analysis Reveals That TBI Causes Abnormalities in the Acid-Base Homeostasis and Mitochondria Function in the Kidney of Mice

The microarray results were analyzed by EdgeR and the distribution of differentially expressed genes was presented by MA plot. Among them, 1104 genes were significantly up-regulated, and 1190 were significantly down-regulated with the following screening criteria: $|\text{Fold change}| > 2$, $\text{FDR} < 0.001$ ([Figure 2A](#)). In addition, the distribution of differentially expressed genes was presented in the heatmap with hierarchical clustering ([Figure 2B](#)). Up- and down-regulated differentially expressed genes were entered into g:profiler. Gene Ontology Biological processing (GOBP), Gene Ontology Molecular function (GOMF), Gene Ontology Cellular Component (GOCC), Reactome pathway (REAC), and Human Phenotype Ontology (HP) were used for Over-representation analysis ([Figure 3](#)). Among the down-regulated differentially expressed genes, gene sets in GOBP and GOMF were significantly enriched mainly in relation to cell migration ability. Similarly, GOCC showed that differentially expressed genes were predominantly located at the pericellular membrane, suggesting a slowed migration or infiltration of specific cells in the kidney. In REAC, the Interleukin-4 and Interleukin-13 signaling gene sets were significantly enriched, suggesting a potential change in the regulation of immune-related responses. Among the up-regulated differentially expressed genes, gene sets in GOBP and GOMF were enriched considerably in oxidative phosphorylation and mitochondria-related functions. GOCC showed that differentially expressed genes were mainly located in mitochondria. REAC also suggested an increased enrichment of respiratory electron transport-related gene sets. HP showed significant enrichment of several gene sets associated with kidney-related diseases, such as acidosis, abnormality of acid-base homeostasis, lactic acidosis and increased serum lactate, as well as gene sets associated with mitochondria abnormalities.

We further performed the Complex Network Analysis (CNET plot) with the ClusterProfiler 4.0 to present the differentially expressed genes in HP gene sets associated with nephropathy. [Figure 4A](#) shows the top five HPO gene sets with the lowest P-value, namely, mitochondrial disease, creatine phosphokinase serum increased, increased CSF lactate, Acidosis, and Increased serum lactate. [Figure 4B](#) presents the expression of the genes contained in each gene set, showing an up-regulation trend for most of the genes (red). [Figure 4C](#) shows the overall distribution of gene sets and accessory genes. In addition, Heatplot showed the expression of five HPO gene sets and their accessory genes ([Figure 4D](#)), which showed a significant upregulation of gene families related to mitochondria function, including COQ family, NDUF family, and SLC25A family.^{49–51}

Enrichment Map Shows Negative Enrichment of Immune Cell Migration-Related Genes and Epithelial Morphogenesis Development

We used GSEA software combined with GOBP database to comprehensively analyze TBI kidney or Sham group enrichment gene sets and visualized the association and clustering of GOBP gene sets with Enrichment map in Cytoscape ($\text{FDR} < 0.05$) ([Figure 5](#)). The results showed negative enrichment with the largest cluster (274 nodes) with immune cell migration, followed by epithelial morphogenesis development (61 nodes). Potassium ion action (48 nodes) and mitochondrial respiratory chain (35 nodes) were both positively enriched, which is consistent with the results of ORA analysis.

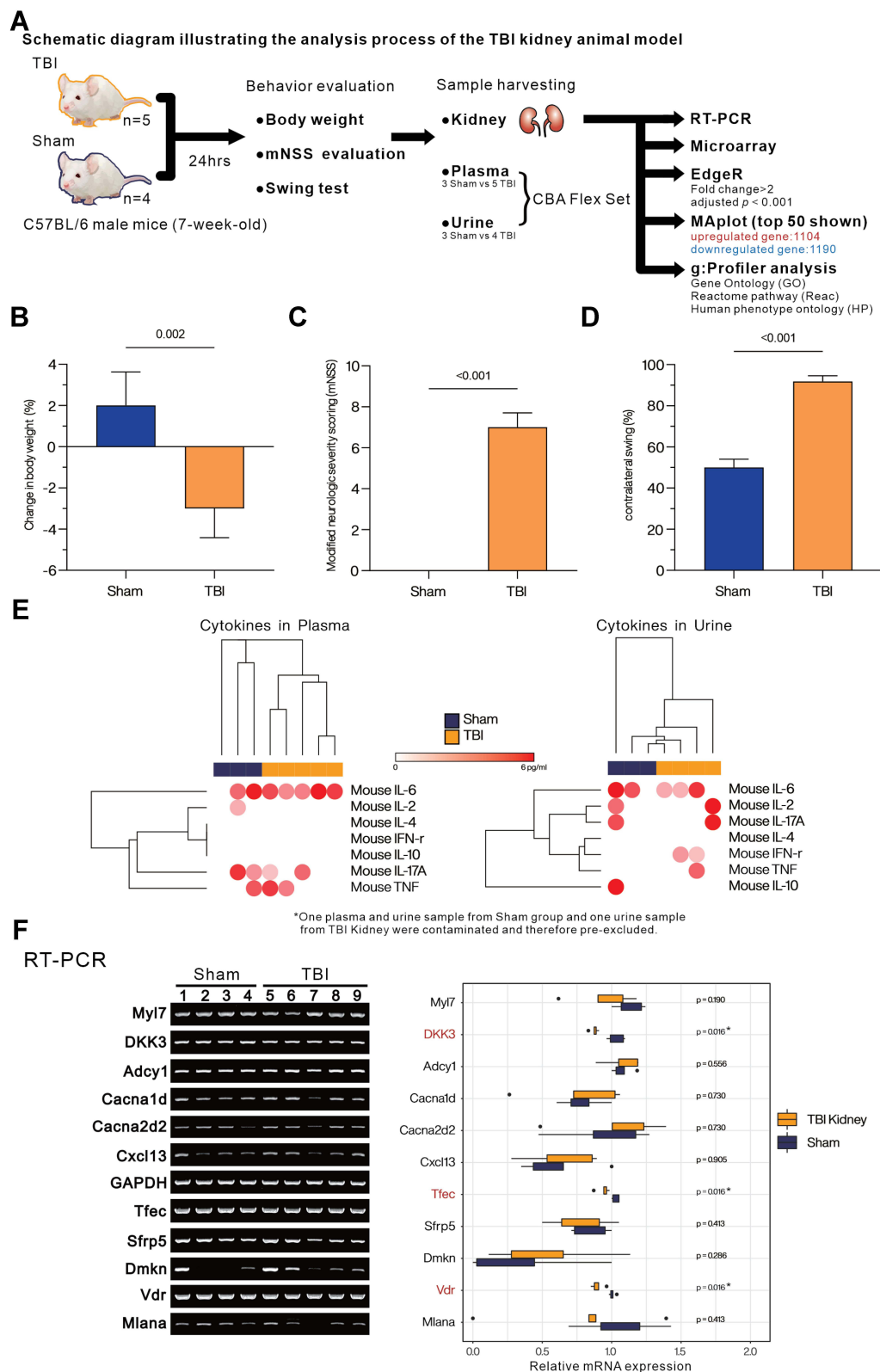


Figure 1 Assessment of the overall effect of TBI on mice. **(A)** The overall experimental design and analysis process. **(B)** Assessment of the degree of weight change (%). **(C)** Evaluation of modified neurological severity scoring (mNSS). **(D)** Comparison of contralateral swing (%). **(E)** The heatmap with hierarchical cluster analysis (Euclidean distance) presents the concentration (pg/mL) of IFN- γ , TNF- α , IL-2, IL-4, IL-6, IL-10, and IL-17A in plasma and urine from Sham or TBI group mice by cytometric bead array (CBA). **(F)** Evaluating the expression of biomarkers related to kidney function and injury by RT-PCR. Boxplot represents the quantified mRNA expression level (by ImageJ v1.53s), of kidney related biomarkers. Student's *t*-test was used to analyze the statistical significance of differences between groups, and $p < 0.05$ was considered a significant difference. * $p < 0.05$.

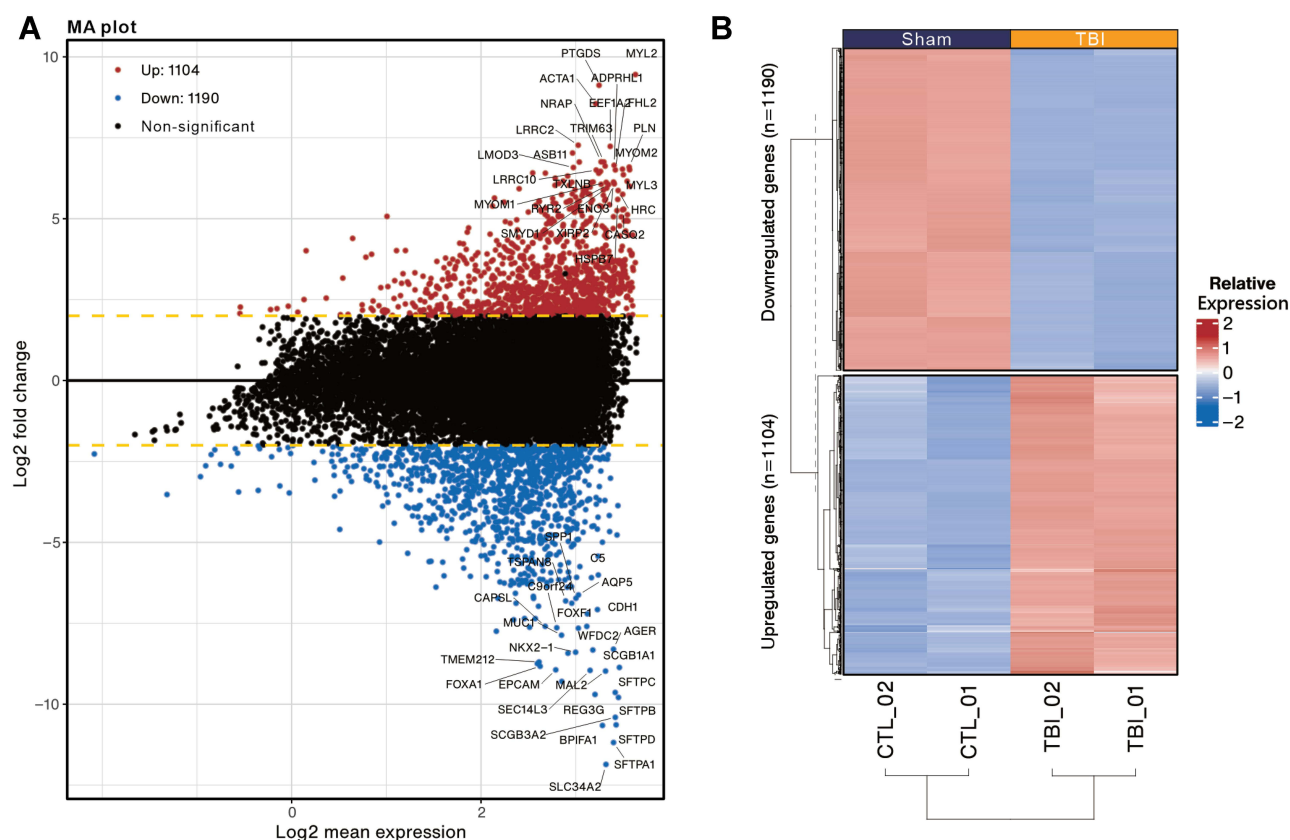


Figure 2 MA plot and Heatmap present the distribution of differentially expressed genes identified in the kidneys of TBI and Sham mice. **(A)** MA plot represents single gene responses, plotted as \log_2 mean expression (x-axis) and \log_2 fold change (y-axis), with the filtering criteria of fold change > 2 ($FDR < 0.001$), negative fold change representing down-regulated genes in TBI (Blue) and positive fold change representing up-regulated genes (Red). The top 50 genes with the smallest FDR are marked on the MAplot. **(B)** Differentially expressed genes are presented in the heatmap with hierarchical clustering (determined by Euclidean distance). Gene expression values are converted to Z-scores for visualization.

TBI Causes Differential Expression of Genes in the Mouse Kidney Similar to the Acute T-Cell Mediated Rejection Response in the Human Transplanted Kidney

To understand the potential impact of TBI on the kidneys, we used SigComLINCS to compare the correlation of differentially expressed genes with other disease datasets (Figure 6). Among the top 30 significantly related databases, the GSE131179 database was the most relevant for kidney disease, presenting a set of differentially expressed genes for acute T-cell mediated rejection (ACR) in human transplanted kidneys, suggesting that TBI may be associated with damage caused by T-cell accumulation and may attack on kidney tissue.

To evaluate the similarities and differences between the two databases, we used the “compareCluster” function of ClusterProfiler to evaluate the commonly enriched gene sets in KEGG, WIKIPATH and Disease Ontology (Figure 7). Analysis of the KEGG database revealed the presence of significant Hematopoietic cell lineage in ACR kidneys, significant activation of inflammation-associated cytokine associated pathways, and enrichment of multiple immune rejection-associated gene sets. The predominant enrichment of multiple metabolic pathways in TBI kidneys, particularly mitochondria-related metabolism, is consistent with previous observations. Interestingly, the common denominator is the enrichment of hematopoietic cell lineage and cell adhesion molecules, suggesting the infiltration and adhesion of immune cells in the kidney tissue. The results of the WIKIPATH analysis showed that the ACR kidney was enriched for the activation of numerous immune cell-related signaling pathways, while the TBI kidney was still enriched for metabolic pathways. The commonality between the two immune-related gene sets is the complement system and genes associated with the development of rheumatoid arthritis, suggesting that the complement system may be involved in the infiltration of immune cells in the kidney tissue causing autoinflammation. Disease ontology analysis showed that the common

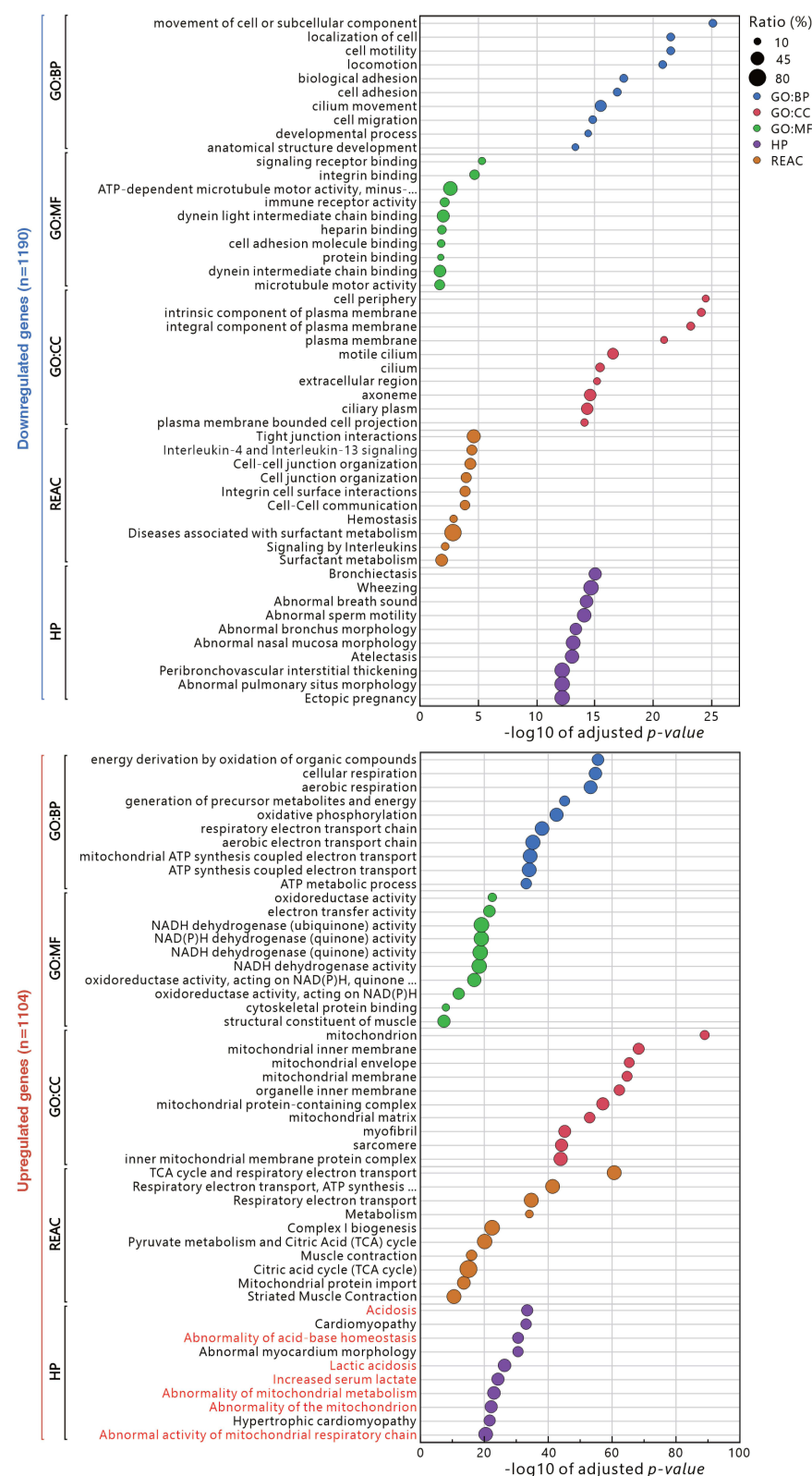
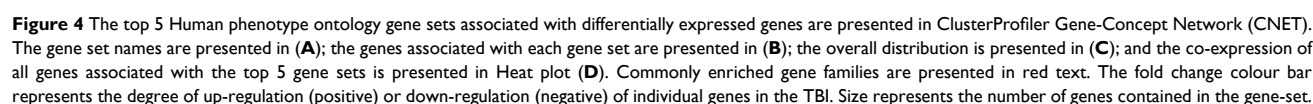


Figure 3 Functional enrichment analysis of up- and down-regulated differentially expressed genes between the kidney and Sham groups in TBI mice using g:Profiler. The analysis was performed in g:Profiler with the selection of GO:BP, GO:MF, GO:CC, Reactome (REAC) and Human Phenotype ontology (HP) databases and grouped by the color of the source database used, with the x-axis showing the negative decimal logarithmic scale values of adjusted *p*-value. Highlighted dots indicate the ratio of enrichment containing 10 to 80%. Highlighted dots indicate the percentage (%) of differentially expressed genes contained in individual gene sets. Gene-sets associated with kidney diseases in HP database were red-colored.



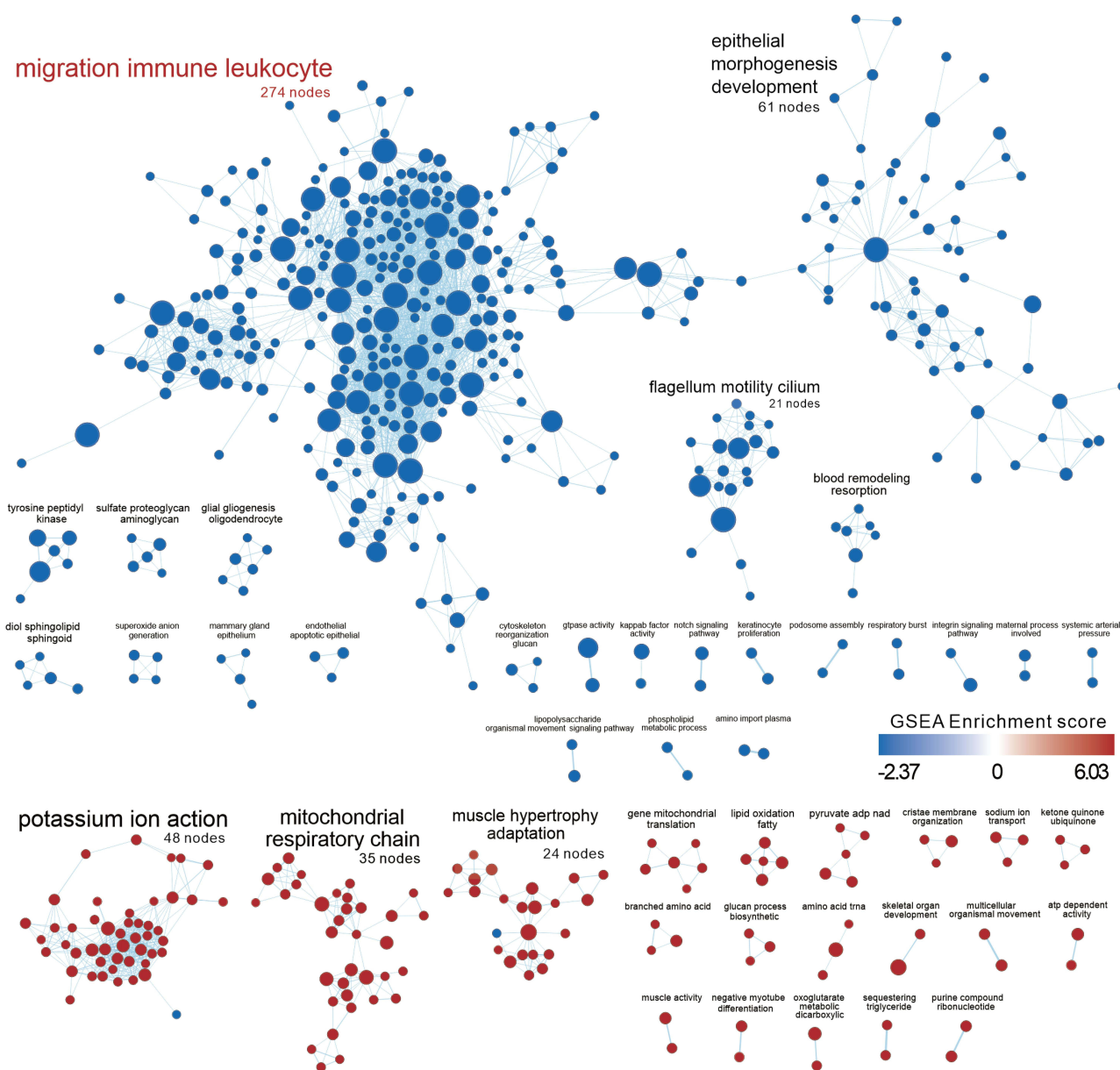


Figure 5 Enrichment map presents the distribution and enrichment of GOBP gene sets in TBI Kidney compared to Sham group. Each circle represents a gene set, and the size of the circle represents the number of genes contained in the gene-set. The color of the circle represents the GSEA score, red means the gene-set is positively enriched, blue means negative enrichment. The edges between nodes represent the similarity between gene-sets. Pre-screened clusters with more than 2 nodes are labeled with AutoAnnotate app v1.3 in Cytoscap 3.9.1, and the font size reflects the number of nodes contained in each cluster. Clusters are arranged according to the number of clustered nodes. The largest node in the figure were red-colored.

denominator in a set of genes related to the urological system is the autoimmune disease of the urogenital tract, suggesting that TBI may cause the immune system to attack the autologous kidney cells and damage the kidney tissue.

Simulated Immune Cell Infiltration Analysis Showed That the Proportion of Activated CD4 Memory T Cells Was Significantly Increased in TBI Kidney or ACR

To compare the similarity of immune cell population infiltration in TBI Kidney and ACR Kidney, we analyzed the distribution of 22 immune cells in TBI Kidney or ACR Kidney using the CIBERSORT-x method (Figure 8). In ACR Kidney, the proportion of T cell CD4 memory resting was significantly decreased and the proportion of T cell CD4 memory activated was significantly increased compared to the control group. Similarly, in TBI Kidney, the proportion of

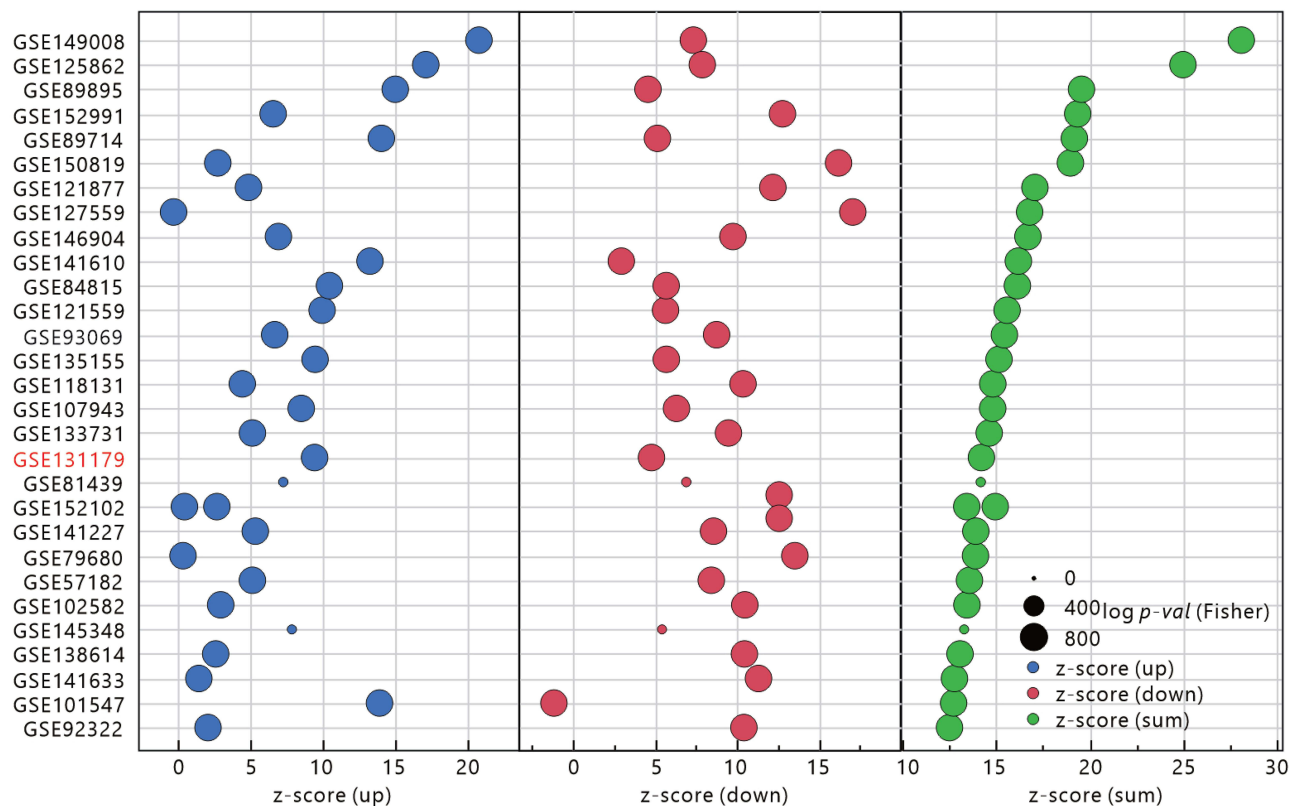


Figure 6 Similarity of comparing the up- and down-regulated gene sets in the TBI Kidney to other GEO databases as assessed by SigCom LINCS. The size of the points represents the negative decimal logarithmic scale values of adjusted *p*-value. Data sets are sorted by z-score (sum). Database associated with kidney diseases was marked as red text.

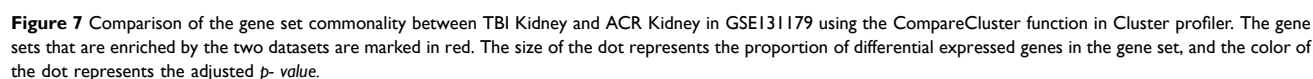
T cell CD4 memory resting decreased significantly compared to mock, while the proportion of T cell CD4 memory activated increased significantly. The results of this analysis suggest that the increased infiltration of activated CD4 memory T cells in TBI kidneys may be related to the renal effects of TBI.

GSEA Analysis Based on Multiple Renal Cell Gene Sets Showed That ACR Kidney and TBI Kidney Have Different Populations of Damaged Cells

Finally, to understand whether TBI affects specific cells in the kidney tissue, we performed simultaneous analysis using a set of nine kidney cell biomarker genes obtained from the PangLao single-cell database (Figure 9). The results showed that in TBI kidney, principal cells ($FDR < 0.001$) were most significantly affected, followed by Mesangial cells ($FDR = 0.0362$) and Loops of Henle cells ($FDR = 0.0386$). In ACR kidney, proximal tubule cells were most significantly affected ($FDR < 0.001$). The related GSEA score distribution is presented by Ridge plot.

Discussion

In this study, we evaluated the potential impact of TBI on mouse kidneys from a transcriptional perspective, basing on the classical TBI model. The results showed that TBI affected the gene set associated with mitochondria function in kidney cells, and a negative enrichment of gene sets associated with immune cell migration and epidermal development was also observed. Analysis of the disease phenotype gene set revealed that differential expression of mitochondria-related genes was associated with lactate metabolism. Alternatively, activation and adhesion of immune cells associated with the complement system may promote autoinflammation in kidney tissue. The simulated immune cell infiltration analysis showed an increase in the proportion of activated memory CD4 T cells and a decrease in the proportion of resting memory CD4 T cells, suggesting that activated memory CD4 T cell infiltration may be involved in the



Increased intracranial pressure (ICP) is common after TBI and may result in autonomic dysregulation, such as sympathetic hyperactivity.^{52,53} The increase in visceral sympathetic nervous system activity impairs glomerular perfusion

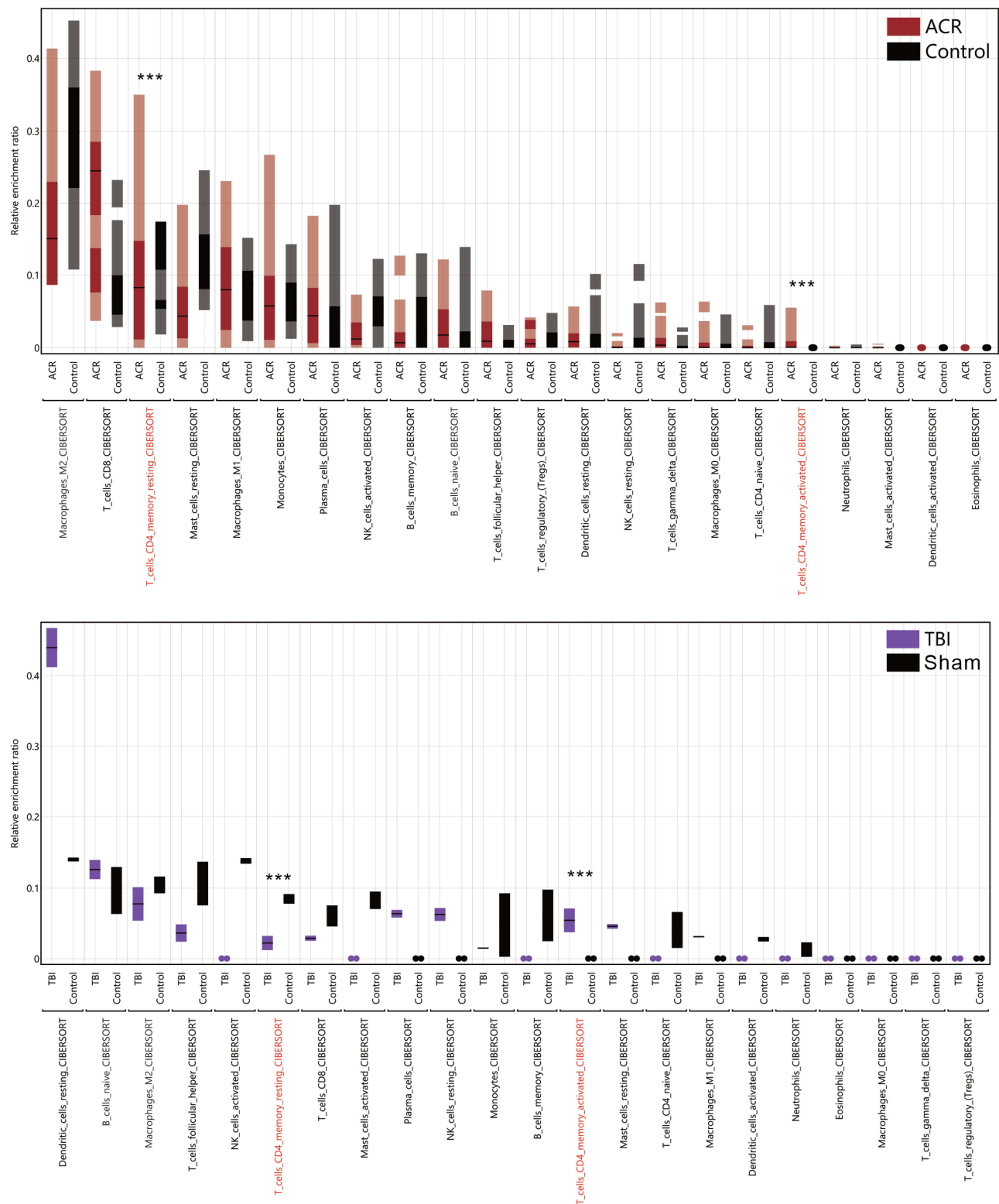


Figure 8 Simulated infiltration ratio of 22 immune cells evaluated by CIBERSORT-X. The different immune cell types were ranked in descending order according to the infiltration ratio of ACR or TBI. The Highest Density Region (HDR) boxplot was used to present the main areas of infiltration distribution. T cell CD4 memory resting or activated infiltration is marked as red text. Student's t-test was used to analyze the significance of the differences between ACR/Control or TBI/Sham. *** $P < 0.001$.

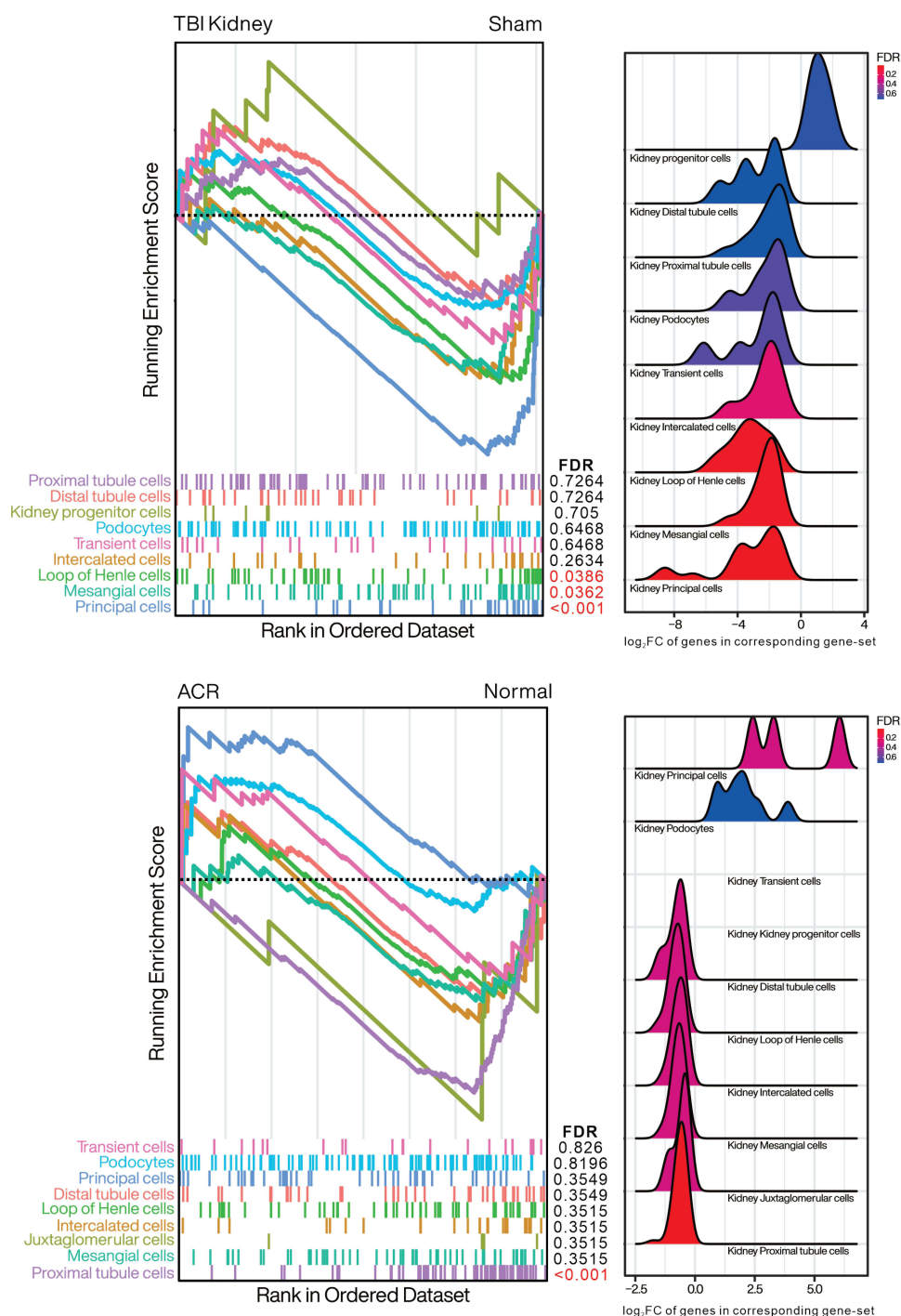


Figure 9 Distribution of 10 kidney cell biomarker gene sets in TBI/Sham or ACR/Normal presented by GSEA and Ridgeplot. GSEA presents the arrangement of each kidney-specific cell biomarker in TBI Kidney/Sham or ACR kidney/Control. False discovery rate (FDR, or q-value) less than 0.05 is marked in red. Ridge plots depict the overall distribution of genes in corresponding gene-set, with negative value representing decreased gene expression within TBI or ACR.

and the sustained hypertension also leads to red cell fragmentation and thrombi formation in the glomeruli,¹⁶ which contribute to AKI. Disrupted hemodynamics after TBI impaired oxygen delivery and led to hypoxic renal tubules.^{53,54} It contributed to mitochondrial dysfunction and altered kidney metabolism with decreased fatty acid oxidation and increased lactic acid generation.⁵⁴

Elevated extracellular lactate concentrations and lactate/pyruvate ratios are well recognized in patients with TBI.⁵⁵ Current research suggests that the increase in lactate contributes to the astrocyte-neuron lactate shuttle hypothesis.^{56–58}

High extracellular levels of lactic acid accumulate when the neurons are severely damaged and unable to use the lactic acid produced by astrocytes from glucose, which means a decoupling of neuronal and glial metabolism.^{58,59} Under normal physiological conditions, excess lactic acid is removed by the liver and a small portion is metabolized and reabsorbed by the kidneys. The increase in lactate caused by TBI may contribute to the mechanism of increased renal metabolism of lactate, which may account for the significant increase in mitochondria function-related genes we observed in renal tissues of TBI animal models.⁶⁰

On the other hand, TBI promotes microglia activation and expression of various complement receptors, including C1q and C3 lysis products.⁶¹ As an important participant in secondary injuries, the complementary system may have an impact on other parts of the body.⁶² There is also growing experimental evidence that complement activation contributes to the pathogenesis of AKI.^{63–65} Complementary suppressive therapy may be an effective strategy to mitigate or prevent AKI and its complications.⁶⁵ Activation of the complementary system will impact the renal filtration barrier, affecting the glomerular, tubular interstitial and/or vascular systems and causing renal injury.^{63,66} In particular, mesangial cell damage induced by activation of the complement system has been reported for many years.^{67,68} Mesangial cells are specialized kidney stromal cells that play an important role for the response to kidney injury and maintaining glomerular homeostasis.⁶⁹ Mesangial cells and neighboring stromal cells also participate in developmental, immunity, inflammation,⁷⁰ and regeneration processes. Previous studies showed that genetic defects in mesangial cells resulted in small kidneys as a consequence of impaired glomeruli formation.^{71,72} Mesangial cells might have phagocytic function to clear proteins that get lodged in the glomerular filter.⁷³ Mesangial cells could function as sentinel cells, reacting quickly to innate immune stimuli or to tissue injury by modulating extracellular matrix (ECM) components and by producing chemokines to direct immune cell recruitment.⁶⁹ The impairment of crosstalk between mesangial cells and the immune system may result in dysregulated immune response and glomerular diseases.⁷⁴ Previous study showed that combined mesangial deposition of IgA and IgG aggravates renal inflammation in Wistar rats⁷⁵ and is associated with more severe clinical features (more proteinuria and capillary wall IgA deposits) in patients with IgA nephropathy, which is the most common glomerulonephritis and is the principal cause of chronic kidney disease (CKD).⁷⁶ Similarly, TBI may induce an increase in serum IgG,⁷⁷ which filters and accumulates in the glomerulus,⁷⁸ further causing an immune response of TH1 cells to glomerular tissue.^{79–82} It suggested that TBI-related kidney injury may share some common immune-related pathological pathways with IgA nephropathy, such as complement activation.⁷⁵ Besides, immune complexes deposited in the kidney can alter the structure and function of resident renal cells and promote immune cell adhesion and increased infiltration.⁸³ Among these, T cells are thought to play an important role in kidney damage.^{84,85} Mesangial cells may also be involved in the activation of CD4 TH1 cells to promote inflammation in kidney tissues.⁸⁶ Correspondingly, the RT-PCR analysis of several immune-related genes in the kidney between the TBI and sham groups showed significant differences in Dickkopf-related protein 3 (DKK3), Tfec, and Vitamin D receptor (Vdr) (Figure 1F). DKK3 is a secreted glycoprotein which is associated with renal tubular atrophy⁴⁸ and AKI.²⁴ However, DKK3 may prevent idiopathic membranous nephropathy by affecting T cell polarization and decreasing IFN γ expression.⁴⁸ Tfec is the mouse transcription factor EC, which can be induced upon stimulation with the Th2 cytokines, such as IL-4 and IL-13.⁸⁷ IL-4 participates in an alternative pathway of macrophage activation that tunes inflammatory responses, scavenges debris and promotes tissue repair.⁸⁸ The activation of Vdr is associated with the suppression of RAS activation, restoring mitochondrial function,⁸⁹ the suppression of autoimmunity⁹⁰ which is involved in the protection against renal injury in kidney diseases.³⁴

Clinically, early elevated serum lactate levels in TBI patients are associated with AKI. Lactate is valuable for clinicians to assess the likelihood of AKI in patients with TBI.⁶ However, whether the lactate-related metabolic gene set was enriched directly due to TBI or due to damage to renal cells by TBI through complement system activation or IgG-mediated autoimmune response could not be determined in this study. It is certain that the significant enrichment of inflammation-related gene sets in the transcriptional expression profile of renal tissues after TBI caused by the autoimmune response may be related to the increased accumulation of antibodies or complements in glomerular filtration that promotes the attack of immune cells including TH1 cells. This phenomenon may explain the association of acute renal tubular injury with severe traumatic brain injury.⁹¹ Compared to human ACR kidneys, activated CD4 memory T cell infiltration was also observed in the kidneys after TBI.⁹² Nevertheless, in the analysis of kidney-specific cells, we

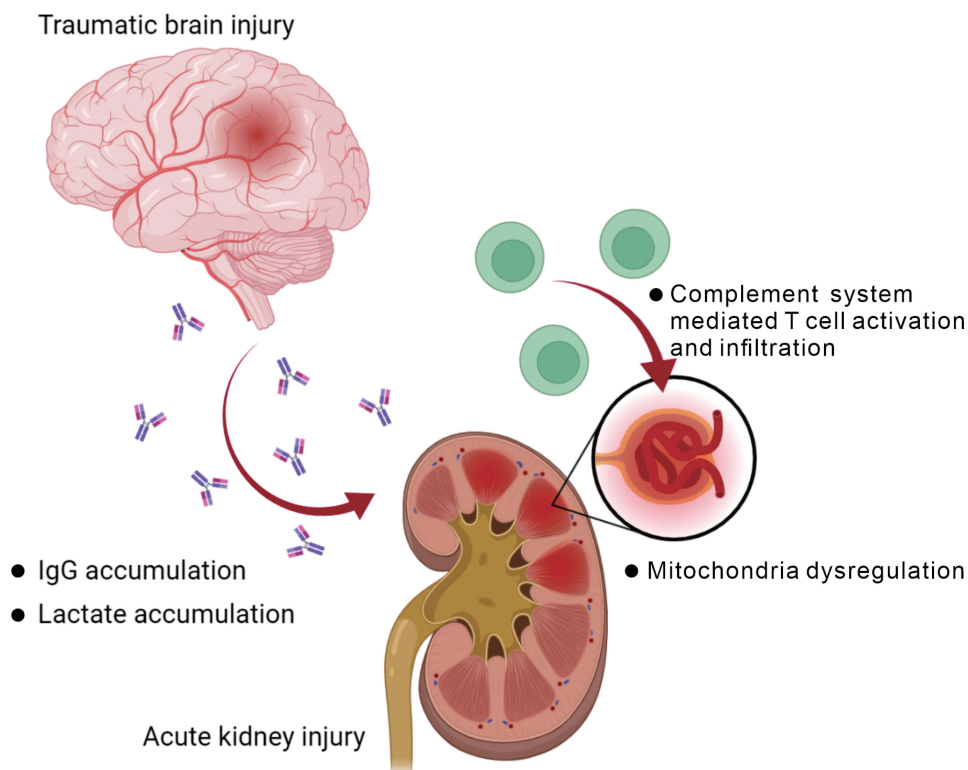


Figure 10 Summary of the postulated impact on kidney after traumatic brain injury. The graph was created with BioRender.com.

observed that proximal tube cells were the most severely affected in ACR kidneys, echoing the histological observations.⁹³ This suggests that although activated CD4 memory T cells are involved in renal injury, active rejection or passive deposition of antibodies and complement may be the cause of renal cell injury at different sites.⁹⁴

Conclusion

In summary, this study reveals for the first time that brain trauma has direct impacts on the kidney, mainly through lactate metabolism-related mechanisms that impact the expression of mitochondria-associated gene sets in renal cells, and possibly through complement system activation or autoimmune antibody-related mechanisms that induce increased infiltration of immune cells into renal mesangial cells (Figure 10).

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Disclosure

The authors report no conflicts of interest in this work.

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