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# Bioinformatics study of the TNFRSF1A mechanism involved in acute liver injury in sepsis through the mTOR signaling pathway

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**Summary.** Objectives. This study analyzed potential key genes involved in the mechanism of acute liver injury induced by sepsis through bioinformatics techniques, aiming to provide novel insights for the identification of early-stage sepsis-induced acute liver injury and its diagnosis.

Methods. Gene chip data sets containing samples from acute liver injury induced by sepsis and control groups (GSE22009 and GSE60088) were selected from the Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) with |log fold change| >1 and $p<0.05$ were screened with the GEO2R tool, which was also used for the selection of upregulated DEGs in the chips with $p<0.05$. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, Gene Ontology, and protein-protein interaction (PPI) analyses were then conducted. Results were visualized using R language packages, including volcano plots, Venn diagrams, and boxplots. The intersection of candidate genes with relevant genes in the Comparative Toxicogenomics Database (CTD) was performed, and the clinical significance of these genes was explored through a literature review. A rat model of acute liver injury was developed by inducing sepsis with the cecum ligation and puncture method. Real-time PCR was performed to determine the gene expression in rat liver tissues.

Results. A total of 646 upregulated DEGs were determined in GSE22009 and 146 in GSE60088. A Venn diagram was used to find the intersection of the upregulated DEGs between the two data sets, and 67 DEGs associated with sepsis-mediated acute liver damage were obtained. Enrichment analysis from the KEGG pathway showed that DEG upregulation was primarily associated with various pathways: TNF, NF-κB, IL-17, ferroptosis, mTOR, and JAK-STAT signaling pathways. DEGs resulted in three clusters and 15 candidate genes, as revealed by the PPI network and module analyses. Intersection with sepsis-induced acute liver injury-related genes in the CTD resulted in the identification of three significant differentially co-expressed genes: *CXCL1*, *ICAM1*, and *TNFRSF1A*. Sepsis-induced liver tissue indicated the overexpression of *CXCL1*, *ICAM1*, and *TNFRSF1A* mRNA, as compared with the control group ($p<0.05$).

Conclusion. The key genes identified and related signaling pathways provided insights into the molecular mechanisms of sepsis-induced acute liver injury. *In vivo* studies revealed the overexpression of *CXCL1*, *ICAM1*, and *TNFRSF1A* mRNA in sepsis-mediated injured liver tissues, providing a theoretical basis for early diagnosis and targeted treatment research.

**Key words:** Sepsis, Acute liver injury, Public chip database, Bioinformatics

## Introduction

Sepsis refers to a syndrome of organ dysfunction caused by infection. It is one of the dominant causes leading to mortality in ICU patients. Various factors, such as bacteria, fungi, and viruses, can induce infection (Font, 2020; Tang et al., 2023). Bacterial or viral invasion can activate inflammatory response-related cells in the body, inducing the release of numerous inflammatory mediators. An intensified inflammatory response can induce immune dysfunction, exacerbate systemic inflammation, promote pathological deterioration in various organs, induce organ failure, and even result in death (Grondman et al., 2020). The definition of sepsis was officially published in 2016, along with an evaluation of organ dysfunction. In this definition, organ dysfunction is identified as a sequential increase (≥2 points) in the Sequential Organ Failure

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Assessment score secondary to infection. Thus, infection as the primary driver leads to organ dysfunction when coupled with immune response dysregulation, posing a life-threatening risk. Globally, over 30 million people are diagnosed with sepsis, and five million deaths are recorded annually (Gauer et al., 2020). Sepsis-induced multiorgan dysfunction is a major cause of death in affected individuals, with mortality rates ranging from 23% to 70% (Song et al., 2023) in ICUs. Moreover, liver dysfunction is one of the severe complications associated with sepsis.

In sepsis pathology, the liver plays a crucial role in the progression of immune-inflammatory responses. It is responsible for clearing pathogenic microorganisms and endotoxins; undergoes inflammatory reactions, immune hyperactivity, and immune suppression; and can even cause secondary damage to other organs, particularly the liver. This damage leads to liver dysfunction and failure, emerging as a severe complication in patients with sepsis, and directly contributing to disease progression and death (Sun et al., 2020). Therefore, a compromised liver may be a key factor for the initiation and progression of multiorgan dysfunction. However, the lack of early and highly sensitive diagnostic tools presents challenges to the early detection of altered liver functions in sepsis (Srzić et al., 2022). The early identification and treatment of sepsis-related acute liver injury could halt disease progression to liver failure and improve patient prognosis. Thus, bioinformatics methods and gene chip technology are crucial for the analysis of novel biomarkers of early-stage diagnosis, treatment options, and prognosis of sepsis-associated acute liver injury.

This study is based on the Gene Expression Omnibus (GEO) public chip database, which explores differentially expressed genes (DEGs) in sepsis-mediated acute liver injury and their associated signaling pathways through bioinformatics analysis methods. The molecular level protein-protein interaction (PPI) network analysis highlights genes that may offer a fundamental role in the sepsis-associated pathogenesis of acute liver injury. Additionally, *in vivo* studies were then conducted to provide insights into uncovering molecular mechanisms associated with the acute liver injury induced by sepsis.

## Materials and methods

### *Data source*

The GEO database was used to download gene expression chip data sets containing sepsis-associated acute liver injury and control groups (GSE22009 and GSE60088). The following inclusion criteria were adopted for the data sets: (1) The selected data sets must be whole-genome mRNA expression chip data. (2) These data should include information on sepsis-related acute liver injury and control groups. (3) The original data sets must undergo standardized processing. (4) The selected data sets must include more than three samples. The GSE22009 data set contains six samples: three in the experimental group and three in the control group. The GSE60088 data set contains eight samples: five in the experimental group and three in the control group. The experimental group consisted of liver samples from mice euthanized 12h after sepsis induction. For the control group, mice were euthanized without any prior treatment. Finally, total RNA was isolated, and gene expression profiling was conducted.

### *Processing of data and DEG selection*

Principal component analysis (PCA) was performed using the R language on each sample of the two data sets according to chip type, and the distribution between groups was observed. DEGs in each data set were analyzed using the GEO2R online tool, and the filtering conditions were |log fold change| >1 and $p<0.05$. Common DEGs from the two chips included genes with varying expression levels (up- and downregulated). A Venn diagram presented all the common DEGs, and bioinformatics analysis was performed on the relevant pathways. To omit the effect of false-positive co-expressed genes and to select potential diagnostic and prognostic targets, we selected only overexpressed genes from the common DEGs for analysis. Heat maps and volcano plots were created for the DEGs obtained from each data set by using the R language. Upregulated DEGs selected from the two data sets were intersected with a Venn diagram, and a set of consistently upregulated genes related to sepsis-related acute liver injury was obtained.

### *Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, and gene set enrichment analysis*

To explore the biological functions of DEGs in sepsis-mediated acute liver injury or damage, we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway analyses. GO analysis was conducted to determine the DEGs associated with biological processes, cellular components, and molecular functions. Potential mechanistic pathways were explored with gene set enrichment analysis.

### *Protein-Protein Interaction (PPI) analysis associated with common DEGs*

PPI analysis of common DEGs was performed using the STRING online database. The visualization and correlation analysis were performed by importing results into Cytoscape software. The key protein expression molecules were filtered out by applying the Molecular Complex Detection (MCODE) plugin through protein

complex clustering.

### *Development of a sepsis-associated acute liver injury rat model*

Twenty healthy SD rats (male, 6 weeks old, SPF grade, body weight of 200-220 g) were selected. The entire experiment followed the requirements of the animal ethics committee. Before the experiment, the rats were acclimatized for one week and given full access to water and food. The animal living conditions were adjusted by maintaining a temperature of approximately 22-25°C and a humidity of approximately 40-45% under a 12h light/dark cycle. Then, the rats were fasted for 12h. The rats were categorized into control and experimental groups (n=10) and then injected with 4% chloral hydrate into the abdominal cavity. A rat sepsis model was developed using the cecum ligation and puncture method. Briefly, a midline abdominal incision was made and the cecum was exposed. The cecum was ligated with a 3/0 silk thread, and an 18-gauge needle was used to puncture it 12 times. The cecum was then slowly pressed until a small amount of feces was observed. The incision was sutured after cecum repositioning (Fang et al., 2021; Tian et al., 2020a,b).

### *Observation of liver pathological changes using H&E staining*

Liver tissues were collected and fixed in a 4% PFA solution after washing. The tissues were dehydrated and embedded in paraffin. Then, 3-μm-thick tissue sections were cut with a microtome. Standard H&E staining was performed, and the sections were sealed with neutral gum. Imaging was conducted under an optical microscope, and pathological changes in the tissues of the two rat groups were observed.

### *Quantification of DEG (CXCL1, ICAM1, and TNFRSF1A) mRNA expression in rat liver tissues using qRT-PCR*

Total RNA was extracted from rat liver tissues with Trizol reagent, and RNA integrity was assessed through electrophoresis. Then, cDNA was synthesized with a reverse transcription kit according to the instructions of the manufacturer. PCR analysis was performed using the specific primers for *CXCL1*, *ICAM1*, *TNFRSF1A*, and the reference gene (*GAPDH*). The reaction protocol followed was:

Initial denaturation: at 94°C for 10 min
40 cycles of
1. Denaturation: 94°C for 30 s
2. Annealing: 59°C for 30 s
3. Extension: 70°C for 1 min.

The fluorescent signals for *CXCL1*, *ICAM1*, and *TNFRSF1A* were collected, and data analysis was performed using the $2^{-\Delta\Delta Ct}$ method. The primer sequences were as follows:
*CXCR1*:
Forward: 5′-CTGTAAGAGGGTTCCAATG-3′
Reverse: 5′-AGGTTCAGCACGTAGACAT-3′
*ICAM1*:
Forward: 5′-CACCCCGCAGGTCCAATTC-3′
Reverse: 5′-TCCAGCCGAGGACCATACAG-3′
*TNFRSF1A*:
Forward: 5′-GCTGCGTGTAGTGTGTCTGC-3′
Reverse: 5′-ACTCGGCCTCTCTCACGAGT-3′

### *Statistical analysis*

Statistical analysis and graphical representation of the data were prepared using GraphPad Prism 7.0 software. Mean ± standard deviation was used to present all the data. DEG analysis was performed using a t-test, and *p*-values and adjusted *p*-values were determined. A *p*-value less than 0.05 was considered statistically significant.

## Results

### *Screening of DEGs in acute liver injury in sepsis*

Gene chip data sets (GSE22009 and GSE60088) containing the sepsis-induced acute liver injury and control groups were screened from the GEO database, and the volcano, PCA, and sample normalized box plots and heat map of DEGs were obtained as per the filtering conditions (Fig. 1A,B). The upregulated, differentially expressed genes were intersected to obtain Venn diagrams in two data sets. Finally, 67 upregulated DEGS were obtained (Fig. 1C). These specific genes are shown in Table 1.

### *Enrichment analysis of GO and KEGG pathways*

Common DEGs were screened, and enrichment analysis was performed using the DAVID online database. The biological processes of the upregulated DEGs mainly included cellular transition metal ion

**Table 1.** Upregulated DEGs common to the data sets GSE22009 and GSE60088.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Cxcl1* | *Lcn2* | *Psat1* | *Chac1* | *Socs3* | *Slc41a2* | *Rab30* | *Steap4* | *Gadd45a* | *Gnat1* |
| *Tacc2* | *St5* | *Thbd* | *2010003K11Rik* | *Slc41a1* | *Icam1* | *Orm2* | *Bcl3* | *Serpina7* | *Junb* |
| *Pdzrn3* | *Cebpd* | *Tmem87b* | *Prg4* | *Slc39a14* | *Nrg4* | *Nfkbiz* | *Snx10* | *Mt2* | *Snhg1* |
| *Nlrc5* | *Mt1* | *Lyve1* | *Fosl2* | *Gm7694* | *Plin2* | *Il13ra1* | *1810055G02Rik* | *4933426M11Rik* | *Arhgef26* |
| *Taf4b* | *Serpina3n* | *Pim3* | *Stat3* | *Lpgat1* | *Soat2* | *Lbp* | *Tnfrsf1a* | *Pla2g12a* | *Cebpb* |
| *Fgl1* | *Naf1* | *Lrg1* | *Ctbs* | *Clca1* | *Orm3* | *Hspb8* | *Baz1a* | *Ralgapa2* | *H6pd* |
| *Fnip1* | *Rbpms* | *Jam2* | *Slc3a2* | *Tsc22d2* | *Gjb2* | *Epc2* | | | |

homeostasis, regulation of IL-6 production, STAT receptor signaling pathways, reactive oxygen metabolism, α-α-β T-cell activation, regulation of the TNF superfamily, cytokine production, and neuronal apoptosis (Fig. 2A). Regarding cell composition, DEGs were primarily enriched in the RNA polymerase II transcription regulatory complex (Fig. 2B). As for molecular functions, the DEGs primarily bound to

**Table 2.** Top five results of KEGG and GO enrichment analysis.

| Ontology | ID | Description | Gene Ratio | Bg Ratio | *p*-value | p.adjust |
|---|---|---|---|---|---|---|
| BP | GO:0002526 | Acute inflammatory response | 6/64 | 112/28814 | 1.88e-07 | 1.88e-07 |
| BP | GO:0006953 | Acute-phase response | 4/64 | 41/28814 | 2.11e-06 | 2.11e-06 |
| BP | GO:0033212 | Iron import into the cell | 3/64 | 12/28814 | 2.27e-06 | 2.27e-06 |
| BP | GO:0046916 | Cellular transition Metal ion homeostasis | 5/64 | 111/28814 | 4.93e-06 | 4.93e-06 |
| BP | GO:0055076 | Transition metal ion homeostasis | 5/64 | 138/28814 | 1.42e-05 | 1.42e-05 |
| CC | GO:0090575 | RNA polymerase II transcription Regulator complex | 6/63 | 235/28739 | 1.29e-05 | 1.29e-05 |
| MF | GO:0035259 | Nuclear Glucocorticoid Receptor binding | 2/61 | 19/28275 | 0.0008 | 0.0008 |
| MF | GO:0046982 | Protein heterodimerization activity | 4/61 | 308/28275 | 0.0044 | 0.0044 |
| MF | GO:0016922 | Nuclear receptor binding | 3/61 | 155/28275 | 0.0046 | 0.0046 |
| MF | GO:0005126 | Cytokine receptor binding | 4/61 | 316/28275 | 0.0048 | 0.0048 |
| MF | GO:0008374 | O-Acyltransferase activity | 2/61 | 51/28275 | 0.0055 | 0.0055 |
| KEGG | mmu04668 | TNF signaling | 7/35 | 113/9000 | 2.05e-07 | 2.05e-07 |
| KEGG | mmu04064 | NF-kappa B pathway | 5/35 | 105/9000 | 4.83e-05 | 4.83e-05 |
| KEGG | mmu05417 | Lipid and atherosclerosis | 5/35 | 216/9000 | 0.0014 | 0.0014 |
| KEGG | mmu04380 | Osteoclast differentiation | 4/35 | 128/9000 | 0.0015 | 0.0015 |
| KEGG | mmu04920 | Adipocytokine signaling | 3/35 | 71/9000 | 0.0026 | 0.0026 |

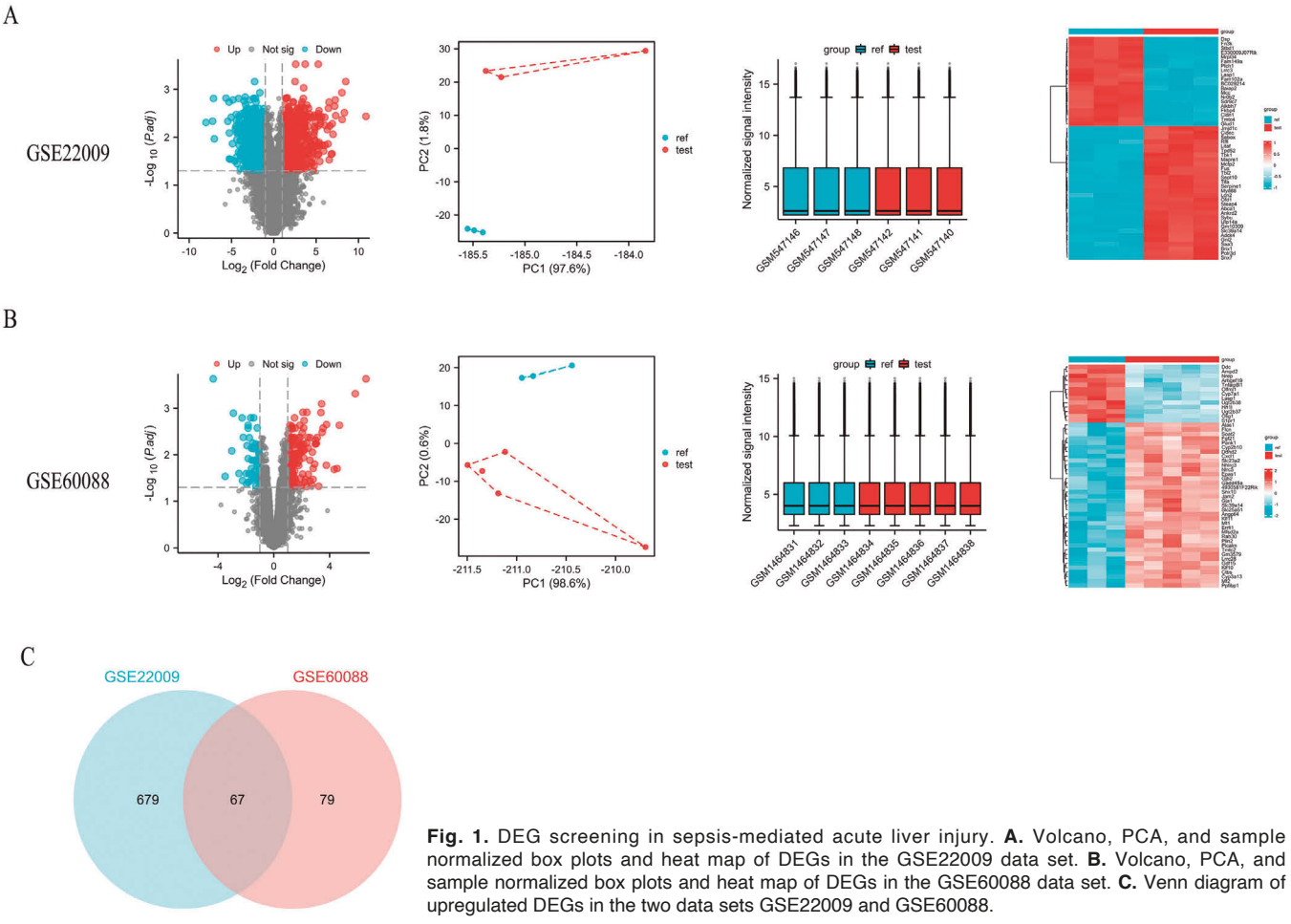


**Fig. 1.** DEG screening in sepsis-mediated acute liver injury. **A.** Volcano, PCA, and sample normalized box plots and heat map of DEGs in the GSE22009 data set. **B.** Volcano, PCA, and sample normalized box plots and heat map of DEGs in the GSE60088 data set. **C.** Venn diagram of upregulated DEGs in the two data sets GSE22009 and GSE60088.

glucocorticoid, cytokine, and chemokine receptors; telomerase RNA; and zinc ion transmembrane transporters, and were involved in carboxylate ester hydrolase activity (Fig. 2C). KEGG enrichment analysis showed that the upregulated DEGs were mainly associated with the TNF, NF-κB, IL-17, ferroptosis, mTOR, and JAK-STAT signaling pathways (Fig. 2D). The first five results are presented in Table 2.

### *Protein mutual assistance network construction and module analysis of common DEGs and core gene screening*

A PPI network for common DEGs was constructed using STRING. Visual analysis and screening of PPI networks were performed, and the obtained results were inputted into Cytoscape software. To obtain the degree value of each node, we used the Network Analyser tool in Cytoscape to perform nondirectional score calculations on each node in the PPI network. Node size was used to represent the degree values. The neighborhood connectivity of each node was indicated by node color (red to green; representing high to low values). The thickness of the edge represented the combined score values of the edge. An attribute circle layout was used in the arrangement of all protein nodes, and nodes with a degree ≥4 were surrounded in the inner layer (Fig. 3A). The protein molecules with the default parameters were clustered by the MCODE plugin (node score cutoff, 0.2; K-core, 2; and maximum depth, 100). Three high-score clusters were obtained, namely, Cluster1 (Fig. 3B), Cluster2 (Fig. 3C), and Cluster3 (Fig. 3D), through correlation analysis. Then, we queried the genes related to acute liver injury in sepsis from the CTD database (http://ctdbase.org/) and used Venn diagrams to intersect the 15 key protein molecules obtained by MCODE analysis. Finally, we identified three key genes: *CXCL1*, *ICAM1*, and *TNFRSF1A* (Fig. 3E).

### *Development of an acute liver injury model*

The rat sepsis model was established using the cecal ligation and puncture method. H&E staining showed that the liver cells of the rats in the normal control group were arranged in an orderly manner, exhibited a complete structure, clear nuclei and cytoplasm, and had no obvious hepatocyte necrosis or inflammatory cell infiltration. Hepatocyte necrosis and focal inflammation were observed in the rat liver after modeling treatment,

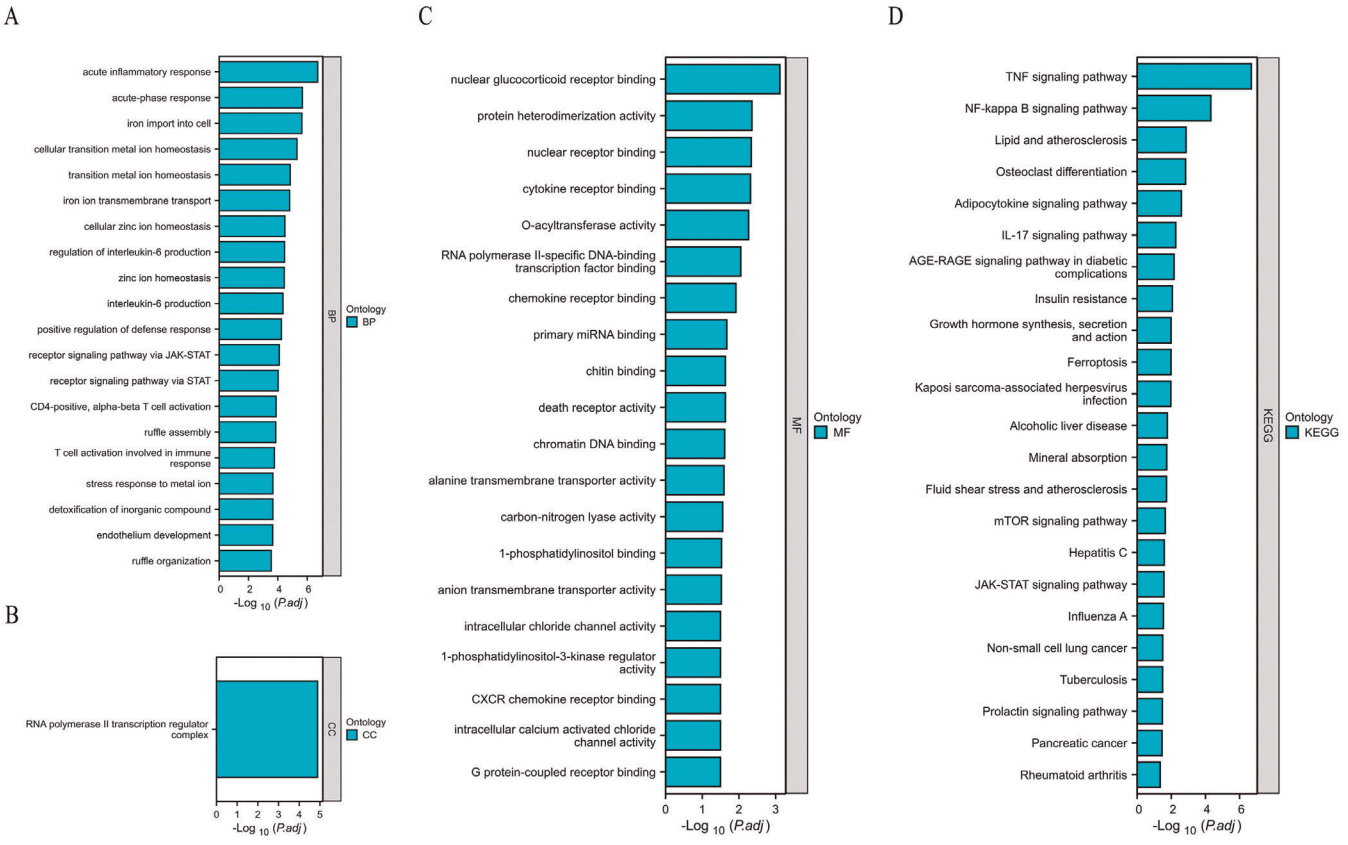


**Fig. 2.** KEGG and GO enrichment analysis of DEGs: enrichment analysis results of biological processes **(A)**, cellular components **(B)**, molecular functions **(C)**, and KEGG signal pathway **(D)**.

and inflammatory cells infiltrated the portal vein area and surrounding central veins. These results indicated that the modeling was successful (Fig. 4).

### *mRNA expression of CXCL1, ICAM1, and TNFRSF1A in liver tissue of rats with acute liver injury*

The sepsis-induced liver injury rat group showed significantly higher expression of *CXCL1*, *ICAM1*, and *TNFRSF1A* mRNA compared with the control group ($p<0.05$; Fig. 5).

## Discussion

The pathophysiological processes of septic acute liver injury are highly complex and can generally be explained by two main mechanisms: cholestasis and ischemia. The first is liver dysfunction (cholestatic), which is defined as impaired formation and excretion of bile and is typically caused by intrahepatic bile acids and bilirubin accumulation. Disturbances in bile metabolism, reduced bile secretion, and decreased flow of bile within the liver cells are consequences of altered signaling pathways at the cellular level. The transcriptional and posttranscriptional gene expression of bile acid transport proteins can be altered by endotoxins and pro-inflammatory cytokines, leading to their downregulation (Kluge and Tacke, 2019). Currently, no unified standard for defining cholestasis in critically ill patients has been established, but in most studies, a total bilirubin level greater than 2 mg/dL represents a practical threshold in clinical practice (Jenniskens et al., 2016). The second mechanism is ischemic hepatitis, which is also known as hypoxic liver injury. It often manifests as acute hepatic hypoxia induced by inflammatory immune responses or reduced (arterial) blood supply in critically ill patients. This condition leads to diffused hepatocellular necrosis. Various factors can cause the necrosis of central hepatocytes in hepatic lobules, and the "waterfall-like" inflammatory response and immune imbalance are considered central elements directly or indirectly causing liver damage. Stimulation by bacteria and endotoxins activates hepatocytes, sinusoidal endothelial cells, and Kupffer cells, leading to the release of pro-inflammatory factors, such as TNF-α, IL-6, and IL-1β (Wang et al., 2014). These factors not only chemotactically induce other immune cells to participate in the immune response, resisting foreign invasion, but also further promote the release of secondary inflammatory mediators and acute-phase proteins. These substances actively participate in the systemic immune response, playing a crucial role in clearing bacteria and endotoxins, causing inflammation and immune responses in the body, once again damaging the liver,

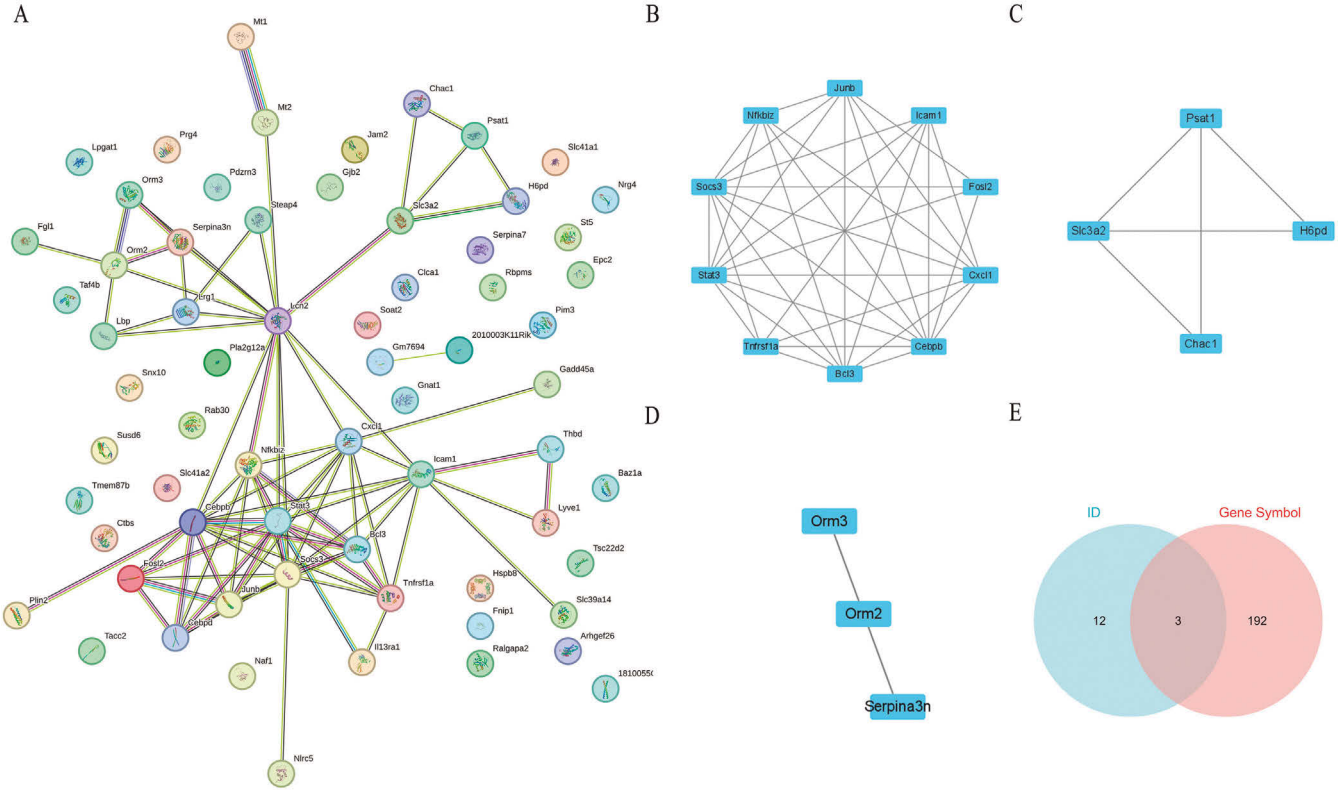


**Fig. 3.** Venn diagram of protein interaction networks and candidate genes. **A.** Protein interaction network diagram. **B.** Cluster1. **C.** Cluster2. **D.** Cluster3. **E.** Venn diagram of candidate genes.

and potentially affecting other organs. Additionally, the involvement of low perfusion is associated with decreased blood flow and increased oxygen demand in the liver during septic shock. Hepatic ischemia may also result from reoxygenation during the ischemia-reperfusion process. Ischemic hepatitis often leads to disseminated intravascular coagulation and bleeding, indicating abnormal coagulation function, which is

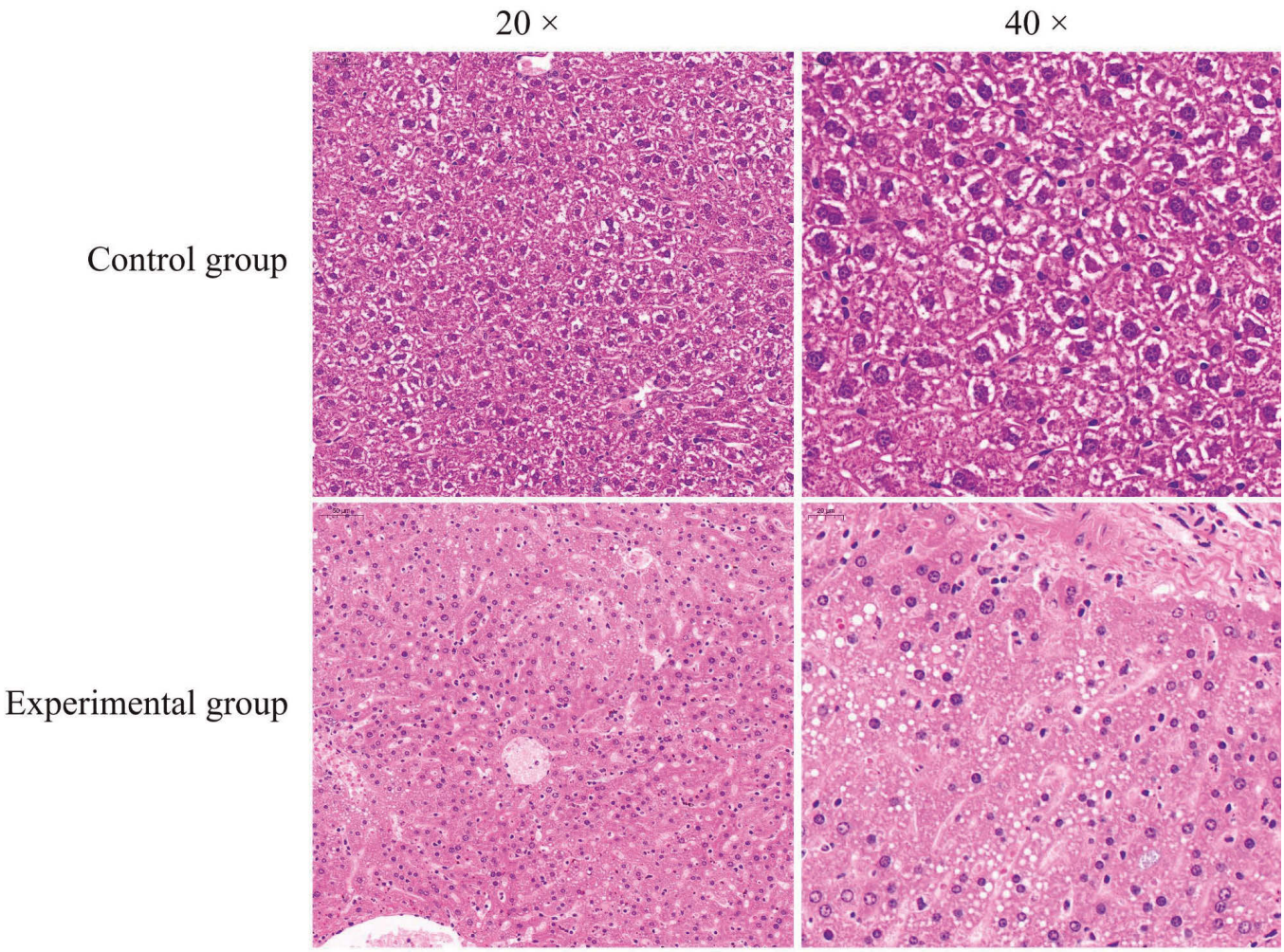


**Fig. 4.** Pathological structure of rat liver tissue (H&E staining).

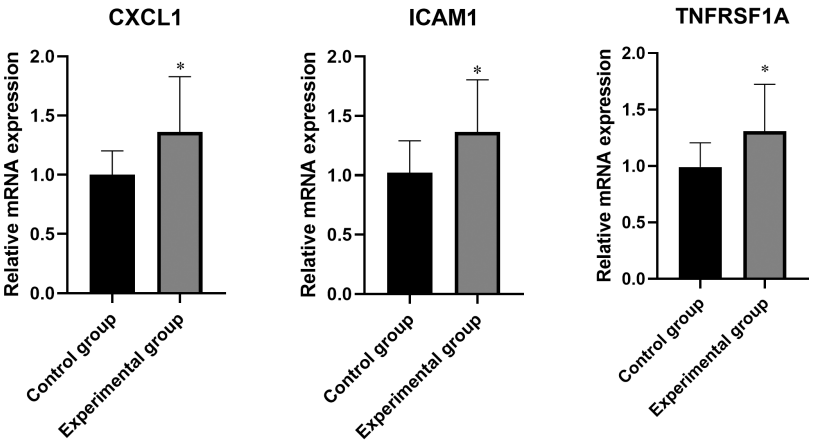


**Fig. 5.** *CXCL1*, *ICAM1*, and *TNFRSF1A* mRNA expression in rats with acute liver injury. *$p<0.05$.

typically manifested by prolonged prothrombin time, decreased prothrombin activity, and increased international normalized ratio (INR) values (Liu et al., 2022; Saini et al., 2022). Therefore, the liver not only plays a crucial role in defense mechanisms, clearance of bacteria, and production of inflammatory mediators but also serves as a potential dysregulated target organ in sepsis pathology.

Recently, gene chips have been extensively applied to the study of acute liver injury (Chang et al., 2023). In this study, by searching the GEO database for data sets related to septic acute liver injury, we identified 67 upregulated DEGs through bioinformatics analysis. We utilized the DAVID online software to determine the DEG functions for GO and KEGG enrichment analysis. KEGG enrichment pathways were mainly associated with TNF, NF-κB, IL-17, ferroptosis, mTOR, and JAK-STAT signaling. These mechanisms have been reported in the context of septic acute liver injury, such as GOLPH3-promoting liver and kidney damage induced by endotoxemia through Golgi stress-mediated apoptosis and inflammatory responses (Dusabimana et al., 2023). Additionally, KLF15 reduces sepsis-induced apoptosis in mouse liver cells by inhibiting the p38MAPK/ERK1/2 signaling pathway (Tian et al., 2020b). The knockdown of proteins interacting with Cα kinase exacerbates septic acute liver injury through the regulation of the TLR4/NF-κB pathway (Wang et al., 2023). Dexmedetomidine-mediated protection against liver injury, as a result of sepsis dependent on the downregulation of the NF-κB/TLR4/MyD88 pathway, is partly induced by cholinergic anti-inflammatory mechanisms (Zi et al., 2019). Paclitaxel alleviated inflammation and reduced septic mouse liver injury through the microRNA-27a/TAB3/NF-κB signaling pathway (Yang et al., 2018). Chuanxiong dipisu alleviated sepsis-related acute liver injury by preventing ferroptosis through modulation of the gut microbiota (Huang et al., 2023). Albiflorin mitigated acute liver injury induced by sepsis through the mTOR/p70S6K pathway (Liu et al., 2024). These findings indirectly reflect the accuracy and research value of our study. In the PPI network and module analysis of common DEGs, we identified three clusters and 15 candidate genes. Intersection with the CTD database yielded three crucial genes: *CXCL1*, *ICAM1*, and *TNFRSF1A*. These genes have been studied in the context of septic acute liver injury. For instance, Korbecki et al. (2022) found that CXCL1 acts as a chemotactic factor, attracting neutrophils to high-expression sites under adverse conditions related to inflammation and neutrophil accumulation. Kaur et al. (2021) proposed that elevated plasma ICAM1 can serve as an independent predictor of 28-day mortality in sepsis-related deaths among cirrhotic patients. Zhou et al. (2022) confirmed the specific reduction of *TNFRSF1A* in septic mouse liver tissue and suggested it is a potential biomarker of septic liver injury. Finally, we hypothesized that *TNFRSF1A* is involved in septic acute liver injury through the mTOR signaling pathway.

This study utilized cecal ligation and puncture surgery to simulate liver damage induced by sepsis. The effectiveness of the septic liver injury model was confirmed through H&E staining. We analyzed the mRNA expression of *CXCL1*, *ICAM1*, and *TNFRSF1A* in the control group and septic liver injury rats by using qRT-PCR. The results showed the overexpression of these genes in rats with septic liver injury, and the elevated expression of these genes may reflect an enhanced inflammatory response in the liver under septic conditions. CXCL1 is a chemotactic factor involved in guiding white blood cells, especially neutrophils, thereby mitigating inflammation. ICAM1 is a cell surface molecule, and its upregulation indicates increased levels of white blood cell-endothelial cell adhesion, which is a crucial step in an inflammatory response. TNFRSF1A is a TNF receptor, and its upregulation may be associated with enhanced apoptosis and inflammation signal transduction. Therefore, the upregulation of these genes could be molecular markers of exacerbated inflammation in septic liver injury.

However, our study has limitations. First, the sample size in each experimental group was relatively small, which may have limited the statistical power and generalizability of our conclusions. Additionally, our study focused solely on changes at the mRNA level without further exploring how these gene expression changes affect protein levels and the specific cellular and molecular mechanisms involved.

## *Conclusion*

The occurrence of septic acute liver injury may involve the regulation of multiple factors, and the results obtained by exploring the key genes and their related signaling pathways provided valuable insights into the associated molecular mechanisms. Additionally, the animal experiments provided a theoretical basis for early clinical diagnosis and targeted treatment studies. However, the results must be further validated through relevant *in vivo* experiments and clinical data. Future research should consider conducting further multicenter, large-sample, and prospective studies to validate and explore these results.

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*Ethics approval and consent to participate.* Not applicable.

*Competing interests.* No conflict of interest exists in this manuscript.

*Consent for publication.* The manuscript is approved by all authors for publication.

*Availability of data and materials.* The data and materials of this experiment are available.

*Author contributions.* Zhidong Chen was responsible for conception and design. Kankai Tang wrote the manuscript. Hui Zhang and Zhidong Chen collected and organized the data. Hui Zhang and Kankai Tang analyzed and interpreted the data. All authors were responsible for manuscript writing. All authors were responsible for the final approval of the manuscript.

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