BMC Infectious Diseases

RESEARCH

Open Access

CTSO and HLA-DQA1 as biomarkers in sepsis-associated ARDS: insights from RNA sequencing and immune infiltration analysis

Yu Zhou Shen[1†], Yan Dong Yao[1†], Hai Li Li[1†], Yang Li[1†] and Ying Chun Hu[1*]

Abstract

The onset of sepsis frequently coincides with *acute respiratory distress syndrome* (ARDS), which constitutes a significant contributor to severe acid–base disturbances in septic patients. In the pathogenesis of sepsis, it conducts a crucial role. lysosomal metabolic disorders and immune imbalance conduct a pivotal role. Despite extensive research into the alterations in immune status during sepsis, few studies have been reported to thoroughly examine the association between lysosomes and sepsis. As a result, this study is predominantly Intended to delve into the link between lysosome-related genes and alterations in the lysosome in the immune microenvironment from the standpoint of bioinformatics in sepsis. The Registration Number was ChiCTR1900021261. Registration Date is 2019/02/04.

Method Sepsis data source: Sepsis data was collected from previous clinical data and sequencing results (Originated from BGI Shenzhen Co., Ltd.) and the GO database was utilized for data collection of lysosome-related genes. Differential expression genes (DEGs) were screened on clinical sequencing data by employing IDEP 0.93 software subsequent to quality control. Afterwards, enrichment analysis was conducted by adopting Gene Set Enrichment Analysis (GSEA) and Weighted Gene Co expression Network Analysis (WGCNA), followed by cross referencing of lysosomal genes to identify DEGs associated with lysosomes. GO and KEGG pathway analysis wereperformed subsequently. The genes obtained from PLSGs and WGCNA by Creating a PPI network entails the following steps: the points were intersected at first. Afterwards, CytoHubba and MCODE analysis were performed by utilizing cytoscape software. Next, the intersection was taken to confirm Hub gene sequences, and subsequently the central DEGs tightly associated with existing CTD scores. Notwithstanding the fact that the causes of sepsis are multifaceted, ARDS can often trigger the development of sepsis in numerous cases. Simultaneously, with an aim to predict transcription factor levels in the central nervous system, Cytoscape software was adopted DEGs and to find relevant target miRNAs in the miRWalk database, and a correlated regulatory network was established accordingly. The SEPSIS immune infiltration model was constructed by employing ImmuCellAI software. Afterwards, the association between DEGs and immune microenvironment abundance was constructed by adopting Spearman's method. Last but not least, it is worth noting that single-cell sequencing has been validated as a method to analyze hub gene expression in immune cells of sepsis patients, enabling the selection of key genes that are closely associated with predictive outcomes.

†Yu Zhou Shen, Yan Dong Yao, Hai Li Li, Yang Li contributed equally to this work.

*Correspondence:
Ying Chun Hu
huyingchun913@swmu.edu.cn

Result When acute respiratory distress syndrome (ARDS) is present, the differentially expressed genes (DEGs) are implicated in lysosomal metabolism and the regulation of the immune microenvironment. Six hub DEGs were bound up with sepsis or was attributable to the examinations. On top of that, it was determined that the patients had acute respiratory distress syndrome. The associated immune analysis illustrated a remarkable augment in T cell infiltration in the immune microenvironment of sepsis, while the infiltration relative to DC was reduced at certain level. Positive correlations were found between the two by employing Spearman analysis between hub DEGs and the regulatory role of immune cells. Moreover, it was universally acknowledged that anti-inflammatory immune cells were responsible for the negative correlation. On the basis of single-cell sequencing, it has been determined that CTSO and HLA-DQA1 were expressed in immune cells in sepsis. Aside from that, the survival-death curve direction suggested that they could be utilized as core genes for predicting sepsis-related prognosis analysis.

Conclusion An analysis of this study demonstrates the interaction between sepsis lysosome-related metabolism and changes by understanding the pathogenesis of immune cells in the microenvironment. On this basis, we can develop new clinical diagnostics and therapeutic approaches of sepsis and identifying drug targets. Nonetheless, ARDS and sepsis can differ simply by the difference in site of infection; as the etiology of numerous ARDS cases is quite complex, progression to sepsis can occur if infection exacerbates or other complications arise, meeting the diagnostic criteria of sepsis 3.0.

Keywords Sepsis, Acute respiratory distress syndrome, Immune microenvironment, Immune infiltration

Background

Septicemia, a life-threatening condition, stands as a prominent cause of mortality, primarily resulting from severe impairment of the host's anti-infective defenses [1–3]. As already demonstrated by several studies, anaerobic glycolysis of immune cells can give rise to septicemia [4–7]. Currently, there is an absence of an effective therapeutic intervention for ARDS, a rapidly progressing disease characterized by a high mortality rate in clinical settings [8]. Multiple studies have shown that ARDS often results from diseases that cause an inflammatory storm, among which septicemia and pneumonia are two typical examples. It is particularly noteworthy that septicemia is the leading cause [9, 10]. As a consequence, lessening the likelihood of cellular and molecular events that trigger inflammatory responses and modulating the equilibrium between host immunity and inflammation can be beneficial in the treatment of ARDS.

In general, iysosomes are considered to be integral to the degradation of components that are associated with cells. They are a structural unit of intracellular digestive enzymes. It has been demonstrated that they are not merely involved in the metabolism of intracellular substances but also play crucial roles in the synthesis of extracellular materials, the regulation of amino acid level fluctuations, and the modulation of autophagy processes [11, 12]. On top of that, associated studies reveal that transcription conducts a pivotal role in factor family participates in the biological [13]. There are reports that the occurrence of lysosomal stress response is due to the accumulation of internal substances in lysosomes [14]. Apart from that, there is a possibility that it may result in respiratory distress disorder [15].

In a multitude of cellular events, including potassium efflux, calcium influx and an excessive amount of Reactive Oxygen Species (ROS) is produced. Moreover, the immune system becomes overly activated ascribable to the activation of the NLRP3 inflammasomes. This is an important mechanism that may cause tissue damage and inflammatory response [16–20]. Factors that cause inflammation, such as tumor necrosis factor (TNF-a) and interleukin1 (IL-1) serve as inflammatory mediators, which conduct a pivotal role in the acute inflammatory response induced by sepsis. On top of that, these factors influence the development of anti-inflammatory factors and the recovery of immune function [21–23]; As previously reported, programmed cell iron death plays a crucial role in the impact of sepsis and acute respiratory distress syndrome [24]. Sepsis inflammation induced by NLRP3 inflammasomes occurs as follows. First and foremost, IL-1β and IL-18 conduct certain role in mediating inflammation. In addition, metabolic regulation are responsible for the formation of biologically active forms [25].

Nonetheless, exploring the correlation is crucial between lysosomal metabolism and immune infiltration so as to better understand what makes these diseases occur and develop inflammation by changing the immune microenvironment. As experimental findings suggest, NLRP3 inflammasomes are activated by inflammatory factors such as caspase −1 and IL-1β [26]. Observations conducted on animals reveal the following insights: When septic mice are subjected to inflammatory factors, the expression exhibits a regulatory nature, potentially contributing to the amelioration of

their intestinal dysfunction [27]. In accordance with relevant research, changes in lysosomal pH not only affect the function of macrophages, but also give rise to corresponding changes in their polarity, thereby affecting their function [28–30]. Nevertheless, although lysosomal metabolic function and sepsis or immune inflammation influence the direction of acute respiratory distress syndrome. there are few reports on their interaction in sepsis. For this reason, it is essential to conduct further research in this area. By integrating and sequencing the clinical data collected at an early stage, we have demonstrated the differentially expressed genes between septic patients and healthy individuals, and subsequently visualized these findings using existing bioinformatics methods. Hence, by adopting this method, after organizing and summarizing the clinical data, we can delve into the roles of lysosome-associated genes in modulating inflammation and immune cells in both ARDS and severe sepsis. Most importantly, we can probe deep into how immune infiltration manifests in sepsis, hoping to help us further understand the connection between disease prognosis and immune metabolism.

Method

Clinical data

Blood samples of twenty-one patients with sepsis and ten healthy individuals were collected between January 2019 and December 2020 (Admission to the ICU of Southwest Medical University Emergency Department). The inclusion criteria for the studied patients are as follows: (1) They must be diagnosed with sepsis and admitted to the EICU; (2) They must meet the diagnostic criteria of sepsis 3.0 version [31] released by the Society of Critical Care Medicine (SCCM) and the European Society of Intensive Care Medicine (ESICM) in 2016; (3) Their ages ranges from 16 to 65 years old; (4) The clinical participants involved in this study have all signed written informed consent forms. (5) The study was conducted in strict accordance with the rules of the Declaration of Helsinki. The study protocol has been approved by the ethics committee of the Affiliated Hospital of Southwest Medical University (Ethical Approval No. ky2018029). The Registration Number was ChiCTR1900021261. Patients who have (1) previous organ failure; (2) previous immune system disease; (3) previous blood system disease; (4) refuse to participate in the study must be excluded. This study protocol complies with all principles specified in the Helsinki Declaration. ARDS is the term applied to a spectrum of conditions with dissimilar etiologies which share common clinical-pathological characteristics including: (1) augmented permeability of the alveolo-capillary membrane, giving rise to infammatory edema; (2) heightened non-aerated lung tissue resulting in higher lung elastance (lower compliance); and (3) elevated venous admixture and dead space, which trigger hypoxemia and hypercapnia [32].

RNA sequencing

The mRNA sequencing was commissioned to BGI (Shenzhen, China). RNA-seq upstream processing ①: Quality Control: Assess the quality of raw sequencing data by examining sequencing quality indicators such as sequence length distribution and sequencing error rates, thereby ensuring the accuracy and reliability of the data. ②: Data pre-processing involves removing adapter sequences, eliminating low-quality sequences, and eliminating sequences containing unknown bases to purify the original sequencing data. ③: Alignment: Compare the pre-processed sequencing data with a reference genome or transcriptome to determine the expression levels of genes or transcripts. ④: Expression Matrix: Calculated by comparing results, an expression matrix of genes or transcripts is obtained, denoting their expression levels. Total RNA was extracted from peripheral blood cells by adopting TRIZOL (Invitrogen, Carlsbad, CA, USA). Subsequently, first-strand cDNA was synthesized from 800–1000 ng of total RNA by utilizing the Clontech SMARTer PCR cDNA Synthesis Kit. The CDS primer IIA was first annealed to the polyA + tail of the transcript, followed by first-strand synthesis by employing the SMARTScribe™ reverse transcriptase. Afterwards, large-scale PCR was performed by adopting the PRIMESTAR GXL DNA Polymerase and the 5′PCR Primer IIA (5′- AAGCAG TGGTATCAACGCAGAGTAC-3′) to obtain a sufficient amount of double-stranded cDNA. At last, a cDNA library was constructed correspondingly. The library was subsequently assessed and quantified by utilizing the Agilent 2100 Bioanalyzer (Thermo Fisher Scientific, MA, USA) and real-time quantitative PCR (TaqMan Probe). MRNA quality control achieved a 28S/18S ratio of bbb1. Sequencing of eligible libraries was performed on the DNBSEQ platform (BGI-Shenzhen, China). Sequencing data were subsequently collated and non-compliant quality data were excluded.

The list of lysosome-related genes was collected gene set enrichment analysis was performed conducted by employing the GO database (http://149.165.154.220/go/) and the GSEA gene set enrichment database (http://www.gsea-msigdb.org/gsea/index.jsp) [33, 34]. IDEP 0.93 software was employed for data processing and data was obtained on DEGs (http://bioinformatics.sdstate.edu/idep93/). The identified DEGs had to meet the criteria of adj $P < 0.01$ and $|\log2(\text{foldchange})| \geq 1$. The DEGs obtained under these conditions were utilized to construct volcano plots and heat maps.

An analysis of functional enrichment rooted in GSEA

GSEA analyzes whether all genes within a gene set are enriched at the top or bottom of a ranked list. If they exhibit enrichment at the upper end, it can be asserted that the gene set generally displays an upward regulatory trend. On the contrary, if they are enriched at the bottom, it indicates a downregulation trend. The GSEA module of the omicshare platform (https://www.omicshare.com/tools/home/) was employed to perform enrichment analysis on existing datasets, with a minimum parameter of 5, maximum parameter of 500, and Signal2Noise method of noise analysis.

GO and KEGG are gene ontologies and gene databases

Shiny GO software was employed to conduct centralized analysis of DEGs, ultimately selecting adj $p < 0.05$. Subsequently, the results were displayed by adopting a circle graph. lysosome-related genes was output from the GO database, and subsequently the resulting lists were downloaded and saved. A total of 875 lysosome localization genes were obtained. A Venn diagram was utilized to intersect clinical sequencing data DEGs with 875 mitochondrial-related genes to obtain lyso-related DEGs. WGCNA was employed to intersect related genes with lyso-related DEGs for hub gene screening.

Analysis of PPI interactions and identification of hub genes

By utilizing the PPI database (https://string-db.org/) [35], mutual interaction analysis was performed on overlapping DEGs. Cytoscape 3.10.0 [36] was employed to generate a visual network correlation diagram. The CytoHubba and MCODE modules were adopted to select hub DEGs, so as to probe into potential disease-related mechanisms of DEGs rooted in the CTD database, environmental exposure, and drugs (http://ctdbase.org/) [37].

This database contains data on the interactions between chemical substances, gene products, functional phenotypes, and diseases. The CTD database displays the mutual correlation between hub DEGs and the risk of developing sepsis or ARDS.

Predicting hub DEGs-Transcription Factors (TF)-miRNAs Network

To search for upstream regulatory factors of hub DEGs, transcriptional factors expressed by hub DEGs was predicted by employing the I regulon analysis module of one of the plugins of Cytoscape 3.10.0 [38]; we adopted the mirwalk database (http://mirwalk.umm.uni-heidelberg.de/) [39]. Then, hubDEG-miRNA prediction was carried out. The core DEGs, TFs, and miRNAs were visualized as a network diagram by employing Cytoscape 3.10.0.

WGCNA

WGCNA is a systems biology method employed to describe the correlation patterns of genes among dissimilar samples. It can identify gene sets that exhibit high co-variation. Grounded in the connectivity within these gene sets and their association with phenotypes, it can identify candidate biomarker genes or therapeutic targets. We utilized the OEBIOTECH platform (https://cloud.oebiotech.com/task/) for this computation. The following stages are involved. The WGCNA R package was adopted to construct and modularize at different stages of development. There are different gene networks. Samples were organized into clusters to identify any potential significant outliers. Subsequently, an automated network system was utilized to establish a co-expression network. Module construction adopted hierarchical clustering and dynamic tree cutting function detection methods. Subsequent to the above steps, MM and GS were estimated to determine the association between module membership (MM) and gene significance (GS). The module with the highest Pearson MM correlation and an absolute adj $P < 0.05$ is selected as the hub module. The MM is above 0.8, and the GS is above 0.2, respectively, high connectivity and clinical relevance of the module.

Immunoassay

Our goal was to understand the correlation between clinical data and the abundance of 24 immune cells by uploading the normalized gene expression list for Immune Cell AI.

Afterwards, the Wilcoxon rank-sum test was utilized to compare intergroup differences (http://bioinfo.life.hust.edu.cn/web/ImmuCellAI) [40]. By employing Spearman correlation analysis, it was determined explore the association between lysosome-related DEGs and immune cells.

Meta-analysis

Meta-analysis is a statistical method employed to synthesize results from multiple independent studies, commonly in medical and scientific research fields. It provides a stronger evidence base by aggregating and quantitatively analyzing the data from these studies, which can arrive at more trustworthy conclusions. This approach can handle heterogeneous data by utilizing random-effects or fixed-effects models and is considered a high-level form of evidence in evidence-based medicine, particularly when rooted in high-quality randomized controlled trials. Meta-analysis is advantageous for researchers to resolve inconsistencies in study results,

enhance statistical power, identify new research directions, and ultimately provide robust support for clinical decision-making. In this study, differential expression will be examined to determine hub DEGs at the transcriptional level. Afterwards, we selected 5 human peripheral blood sepsis samples (GSE28750 [41], GSE69528 [42]) from the public database GEO (https://www.ncbi.nlm.nih.gov/geo/) [1] website and homogenized them (log2). After that, the participants were divided into two groups: one with sepsis and the other with health. The R software package multi meta was adopted to perform meta-analysis on individual genes in the same group from different datasets, which was utilized to delve into the trend expression of core genes in sepsis.

Cell experiment

① Cell Culture:

(1) Retrieve the RAW264.7 cell cryovial from liquid nitrogen, rapidly thaw the cells, and dilute them in complete medium supplemented with 10% fetal bovine serum (FBS);
(2) Transfer the cell suspension to a petri dish or flask and incubate at 37 °C in a 5% CO2 incubator;
(3) Replace the medium every other day to sustain cell growth.

② Cell Preparation:

After 3 days, when the cells cover 60%−70% of the surface in the petri dish or flask, proceed with the experiment. Discard the supernatant of the culture medium and gently wash the cells three times with PBS buffer to remove residual serum and metabolic products.

③ Cell Treatment:

(1) In a 6-well plate, add 2 ml of medium containing 10% FBS without penicillin–streptomycin to each well. Continue incubating the cells in the incubator for 24 h;
(2) After 24 h, add medium containing 500 ng/ml LPS to each well of the experimental group to induce a sepsis model. The control group is replaced with the same volume of regular medium. Incubate the cells for an additional 6 h in a 37 °C, 5% CO2 incubator to allow for LPS-induced changes in gene expression.

④ RNA Extraction:

(1) After incubation, discard the medium and wash the cells once with PBS.

Extract total RNA from the cells following the instructions provided with the ORISCIENCE RNA extraction kit (China).
(2) Determine the concentration and purity of the RNA by utilizing a spectrophotometer, ensuring an A260/A280 ratio between 1.8 and 2.0cDNA Synthesis:
(3) The extracted RNA was reverse transcribed into cDNA by employing the Seq-Hunt RT Kit (China, CA01), following the kit's instructions.

⑤ RT-qPCR:

(1) Real-time quantitative PCR (RT-qPCR) was performed by adopting the Seq-Hunt SYBR Green Kit (China, AF01-5).
(2) The PCR reaction mixture was prepared, comprising cDNA template, specific primers (CTSO: forward AGTCAGCTCGTGCAATCCAA, reverse TGTCTCATTTAGCCAGCGCA; HLA-DQA1: forward GACCTCCCAGAGACCAGGAT, reverse GGAACACAGTCGCTTGAGGA), and SYBR Green reaction mixture. Operate the reaction on a real-time PCR instrument and generate a melting curve to verify amplification specificity.

⑥ Data Analysis:

(1) Investigate the RT-qPCR results by utilizing the 2^-ΔΔCt method to determine the relative expression levels of the CTSO and HLA-DQA1 genes in experimental and control group cells.
(2) Calculate the Ct values for each gene and compare them to the Ct values of a housekeeping gene (such as GAPDH) to normalize expression differences between samples.

Through these steps, the expression of target genes CTSO and HLA-DQA1 in RAW264.7 cells during sepsis can be identified.

The statistical analysis of data

An analysis of the data was conducted by adopting the following statistical methods: GraphPad Prism 9.0

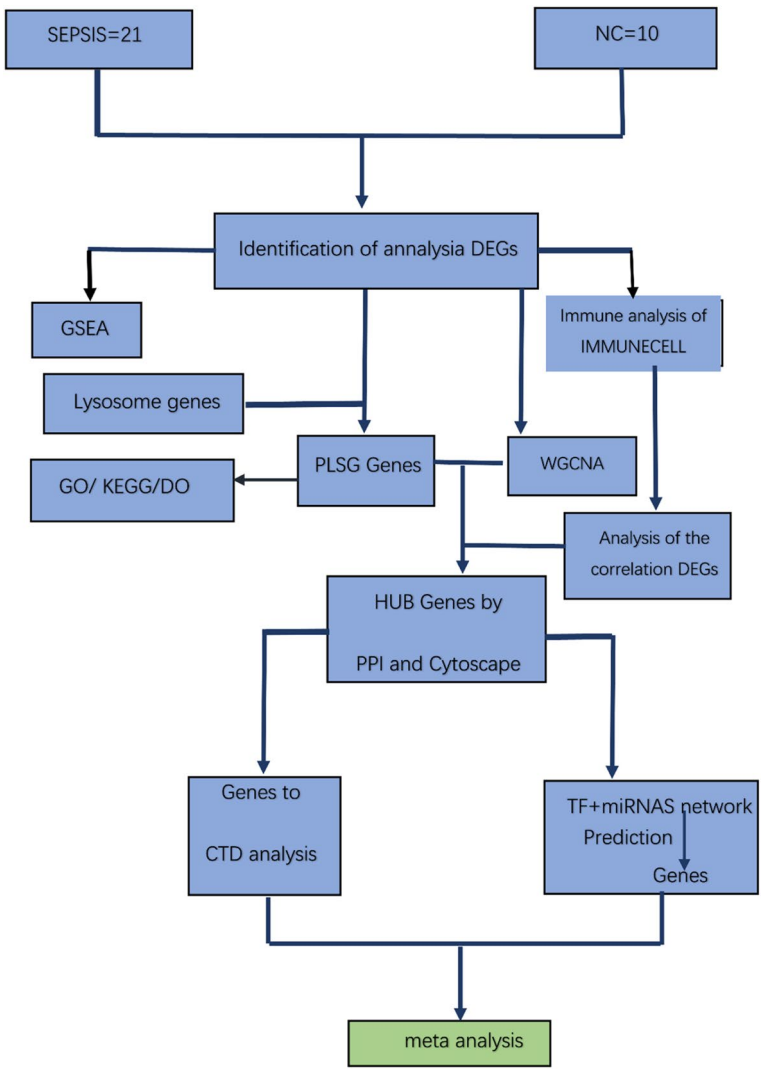

Fig. 1 Flowchart of gene selection strategy in bioinformatics analysis

software to analyze the data obtained from enzyme-linked immunosorbent assay and clinical data of patients. As a continuous variable, the mean was rounded to standard deviation or the median was rounded to the interquartile range (IQR). Differences between continuous variables. Afterwards, the data was analyzed by utilizing non-parametric Mann–Whitney U tests and unpaired Student's t tests. Group comparison was completed by employing Fisher's exact test the number of cases between two groups. It was set at a cutoff value of $P<0.05$.

Result

Analyzing clinical data for DEGs and related functional enrichment

The overall data screening strategy flowchart is demonstrated in the Fig. 1.

A visual representation of clinical data sequencing analysis. The number of DEGs was 3112, including 1625 upregulated genes and 1487 downregulated genes in comparison with normal samples in sepsis samples, which were visualized as volcano plots, PCA and heat maps in Fig. 2A-C. GSEA was performed on sepsis patients and healthy populations in Fig. 2D to explore their biological significance signaling pathways. The top five terms determined by HALLMARK analysis. Light response signal, aldehyde dehydrogenase family, hormone stress response, beta catenin signal. Afterwards, transplant rejection signal was significantly enriched in sepsis patients (adj $P<0.05$).

Identification of co-expressed gene modules in sepsis

A multitude of genes were identified by employing WGCNA modules that are co-expressed in sepsis

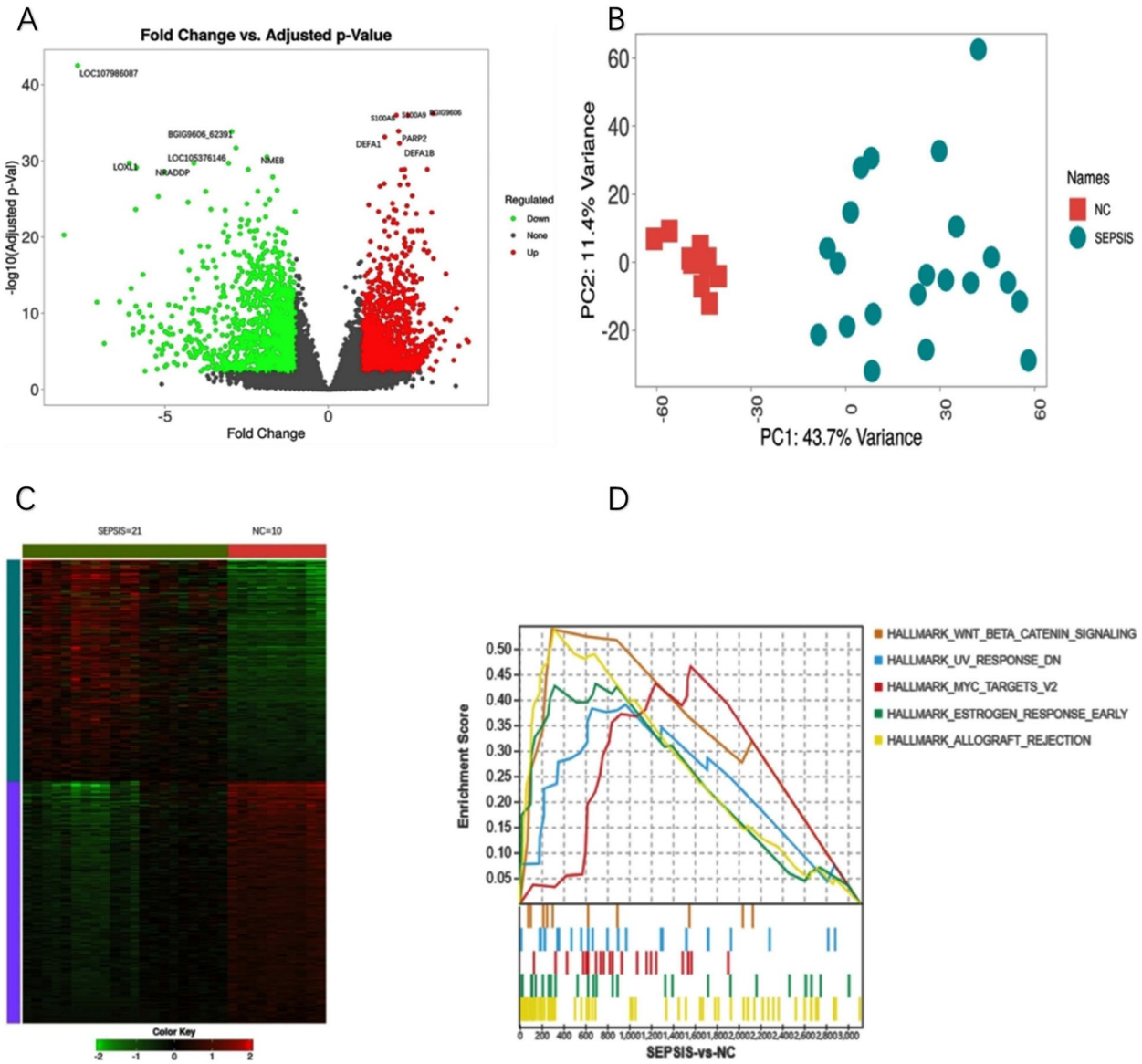

Fig. 2 Analysis results of DEGs in clinical sequencing data. **A** Volcano plot in the dataset; **B** PCA of DEGs. **C** Cluster heatmap of DEGs. **D** GSEA analysis

sequencing datasets. In the first step, the dataset samples were categorized into two distinct groups: one comprising individuals diagnosed with sepsis and the other consisting of individuals without sepsis. Next, no abnormal expression values were observed in Fig. 3A. In the second step, with the independence set to > 0.8, a soft threshold power of 13 was selected β, so as to ensure that the network was biologically significant in Fig. 3B. Afterwards, hierarchical clustering analysis was utilized, and the joint gene tree and the dynamic branch cutting method were adopted to divide the genes into 12 modules in Fig. 3C-E. The light cyan, blue, and the number of light green

(See figure on next page.)

Fig. 3 Gene co-expression networks connected with sepsis were analyzed on the basis of a weighted gene co-expression network analysis dataset. **A** clustering dendrogram was generated from 31 samples; **B** an analysis of network topology by employing various soft threshold powers; **C** An analysis of gene clustering dendrograms; **D** Gene tree derived from average linkage hierarchical clustering; **E** Module-feature correlations; **F** Scatter plot of GS and MM recurrence in the purple module; **G** Recursive scatter plot of GS and MM in the light gray module; **H** Recursive scatter plot of GS and MM in the light blue module. GS defines gene significance, and MM denotes module membership

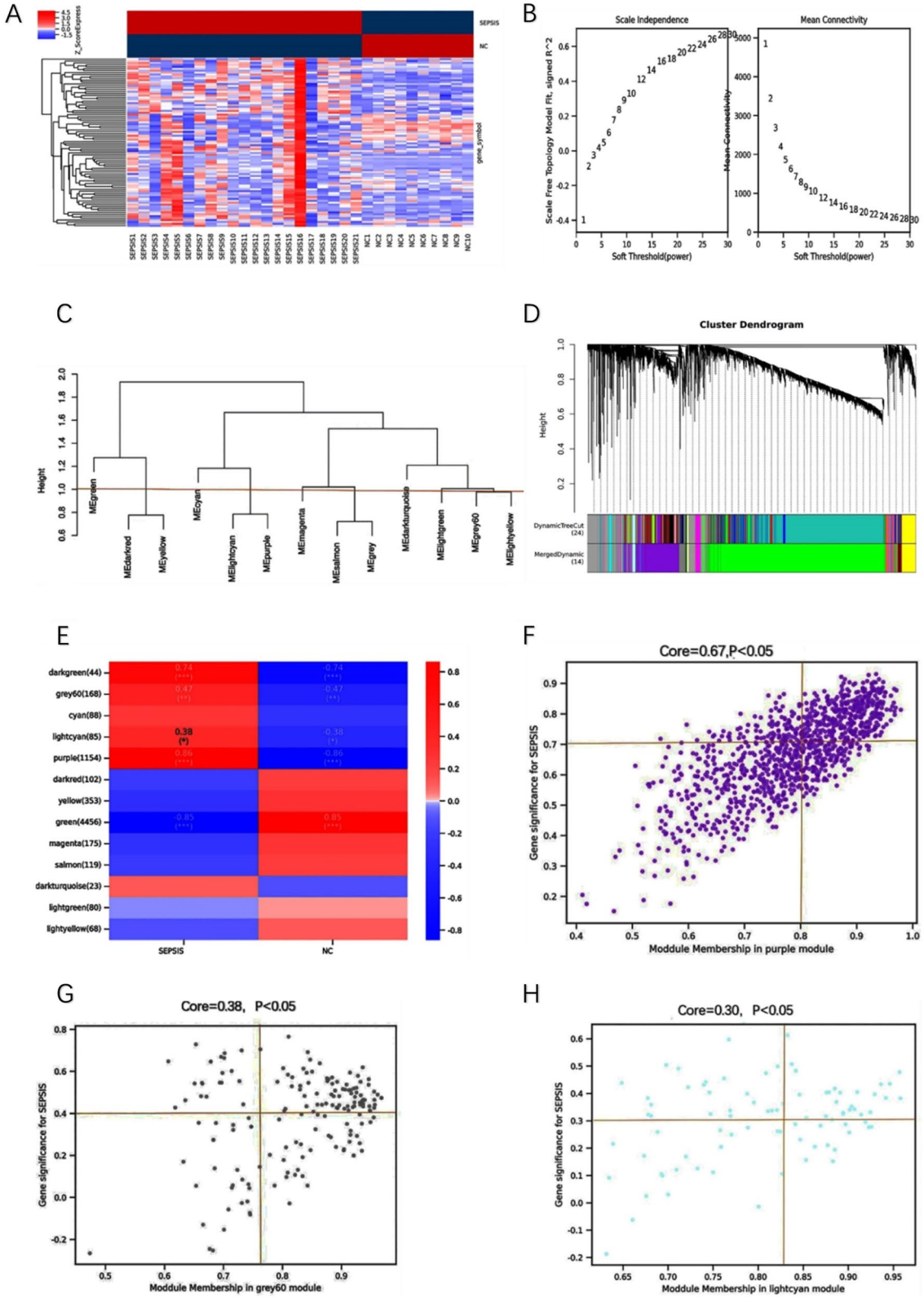

Fig. 3 (See legend on previous page.)

modules significantly with the number of patients with sepsis increased and were selected for further analysis in Fig. 3F-H, adj $P < 0.05$), with a total of the gene significance of sepsis and module membership was associated with 6,952 genes.

DEGs' functional enrichment analyzed

LYSG DEGs were identified by retrieving lysosome-related genes from the GO database in SEPSIS lysosome-related genes and overlapping them with the previously obtained DEGs. A total of 182 DEGs were obtained in Fig. 4A. A GO analysis demonstrated that DEGs conduct a paramount role in immune regulation, actively regulating cytokine production, mediating leukocyte differentiation, activating T cells, as well as other immune-related processes and myeloid cell activation by activating immune responses in Fig. 4B-D. In accordance with the KEGG analysis, the differentiation of Th1 and Th2 cells, as well as activating the MTOR pathway in Fig. 4E are indisputably linked with hematopoietic cell lineage. These DEGs have been associated with immune system diseases, multiple sclerosis, demyelinating diseases, hypersensitivity diseases, diseases associated with the respiratory system and digestive system diseases in line with DO analysis results in Fig. 4F.

The DEGs are differentially expressed genes, while the GSEAs are gene set enrichment analyses. A disease ontology is called a disease ontology, while a gene ontology is called a gene ontology, The PPI is referred to as a protein–protein interaction and a genome ontology is called a genome ontology.

From the WGCNA-generated related genes, genes connected with Lyso-degs were selected as Wgcna-Lyso-degs. A total of 155 DEGs were screened from the DEGs data in Fig. 5A. In an effort to analyze the PPI networks and the central DEGs of the 155 DEGs, the STRING database was transformed into a visual network in Fig. 5B. The MCODE plugin implemented by Cytoscape. With an aim to identify pivotal modules, this method was adopted (gene clusters) on the basis of the following screening criteria: degree cutoff = 2; Score cutoff = 0.2 for nodes; k-core = 2; and maximum depth = 100. A module consisting of 17 nodes and 36 edges was identified as important, and A number of HLA variants were also tested, including HLA-DPA1, HLA-DPB1, HLA-DOB, HLA-DOA, HLA-DMB, HLA-DMA, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DRA, HLA-DRB1, HLA-DRB5, B2M, and CD74, CTSC, CTSF, and CTSO in Fig. 5C. The MCC algorithm of the CytoHubba plugin in Fig. 5D, 10 candidate Hub genes have been identified from the PPI network, this includes HLA-DQA2, HLA-DPB1, HLA-DQA1, HLA-DPB2, HLA-DPB1 and HLA-DRA, HLA-DRB5 and HLA-DRB1. After removing the intersection of the two, a final set of 8 HUB DEGs was obtained, including HLA-DQB1, HLA-DOB, CTSO, HLA-DMA, HLA-DPB1, HLA-DQA1, HLA-DOA, and HLA-DMB in Fig. 5E.

Employing the CTD database to predict the association between wheel hub DEGs and SEPSIS/MODS.

Figure 6A Analysis revealed 6 DEGs that intersected with clinical data and SEPSIS/MODS-related genes, including CTSO, HLA-DMA, HLA-DMB, HLA-DPB1, HLA-DQA1, and HLA-DQB1. Hub DEGs was regulated by TFs and miRNAs, so the upstream regulation of Hub DEGs can be investigated by predicting the relevant TFs and miRNAs. By utilizing the Cytoscape plugin iRegulon, 21 TFs (GCLC, DMD, CTGF, CENPF, CDKN1A, CD1D, BNIP3L, BCL2, AQP9, ANG, AKT3, AKT1, TTN, TGFB2, TGFB1, SRC, RAF1, MDM2, MAPK3, IL-6, IL-12B, and PGK1) were predicted to regulate the hub DEGs-TFs network in Fig. 6B. By adopting miRWalk 3.0 to predict the mirna of hub DEGs, a hub DEGs miRNAs regulatory network involving 780 nodes and 2500 edges was generated in Fig. 6C.

During our study, we analyzed 24 types of immune cell infiltration by adopting the ImmuCellAI algorithm. There were 19 immune cell types with statistical differences (adj $P < 0.05$) between the SEPSIS group and the NC group. Specifically, the SEPSIS group had an abundance of NK cells, CD4-T cells, CD8-T cells, Tgd cells, CD4-navie cells, Tr1, nTreg, iTreg, Th2 cells, Tfh, CD8-navie cells, Tc, Tex, MAIT, and Tcm, while macrophages, neutrophils, and Th17 cells were more abundant in the NC group in Fig. 7A and B. In line with the correlation matrix plot in Fig. 7C, there were multiple correlations between immune cells infiltrating SEPSIS. The degree of correlation was expressed as a fraction. Cells in the Tfh lineage illustrated the greatest synergistic effect (0.99), followed by the nTreg lineage (098), the nTreg lineage and the iTreg lineage (098), and nTreg cells with ITreg cells (0.98) and CD8T cells and MAIT cells (0.98). In comparison, A competition effect of −0.9−8.9 between iTreg cells and macrophages.

Figure 8A and B illustrate the positive/negative correlation between DEGs (20 upregulated and 20 downregulated) and immune cells. Among the six hub DEGs, their negative correlation with macrophages, neutrophils, TH17 cells, and lymphocytes was well documented, monocytes, B cells, DC cells, and NKT cells, while they were positively correlated with Monocytes, NK, CD4_T, CD8_T, Gamma delta, CD4_naïve, Tr1, nTreg, iTreg, Th1, Th2, Tfh, CD8_naïve, Cytotoxic, Exhausted, MAIT, Central memory, Effector memo, and monocytes. They were negatively associated with

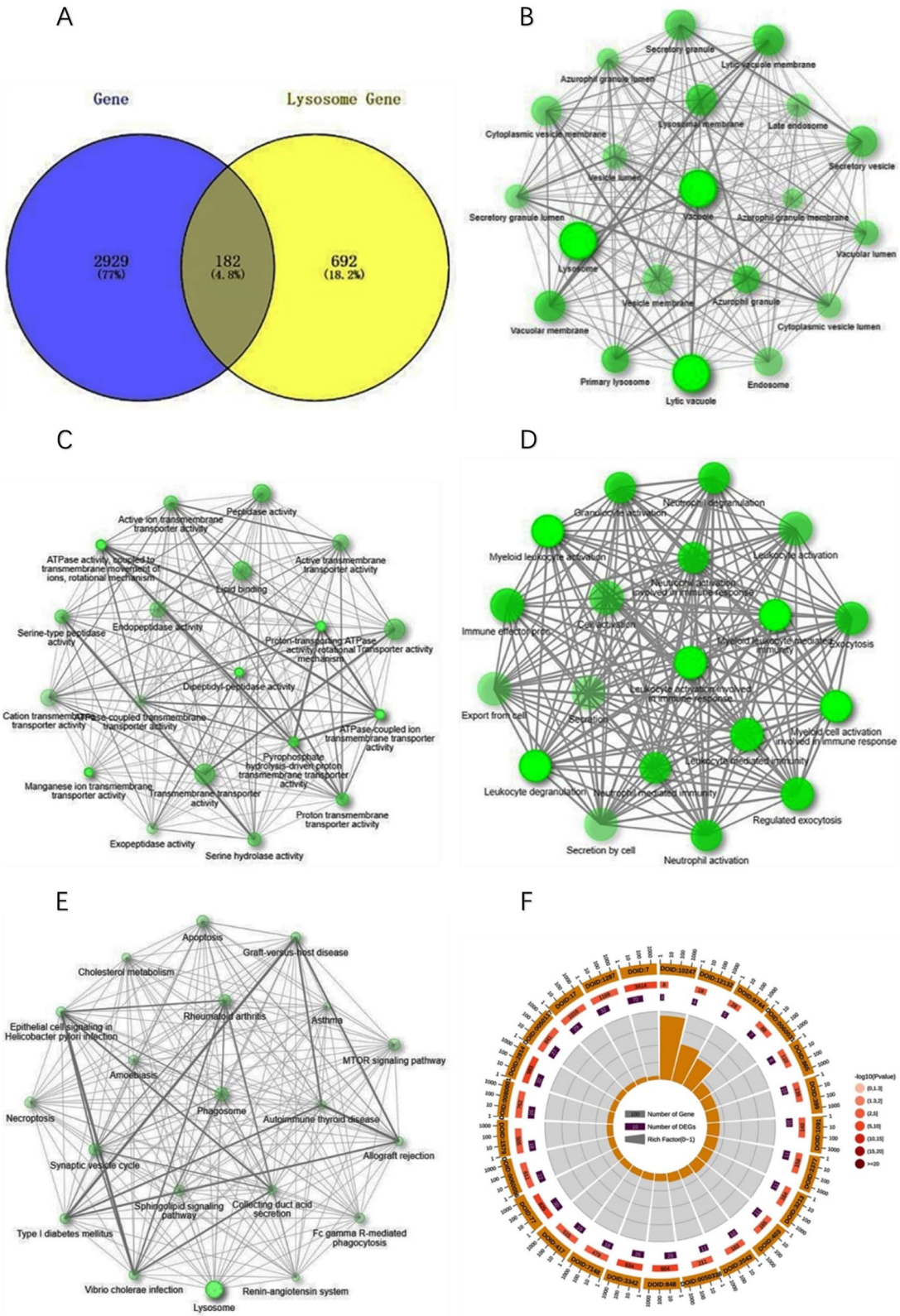

Fig. 4 An analysis of the functional characteristics of the LYSG GENE system is presented in the diagram below. **A** the deg of LYSG-GENEs; (**B**-**D**) graphene oxide enrichment analysis; There are two types of GO pathway enrichment analyses: (**E**) KEGG pathway enrichment analysis and (**F**) DO analysis

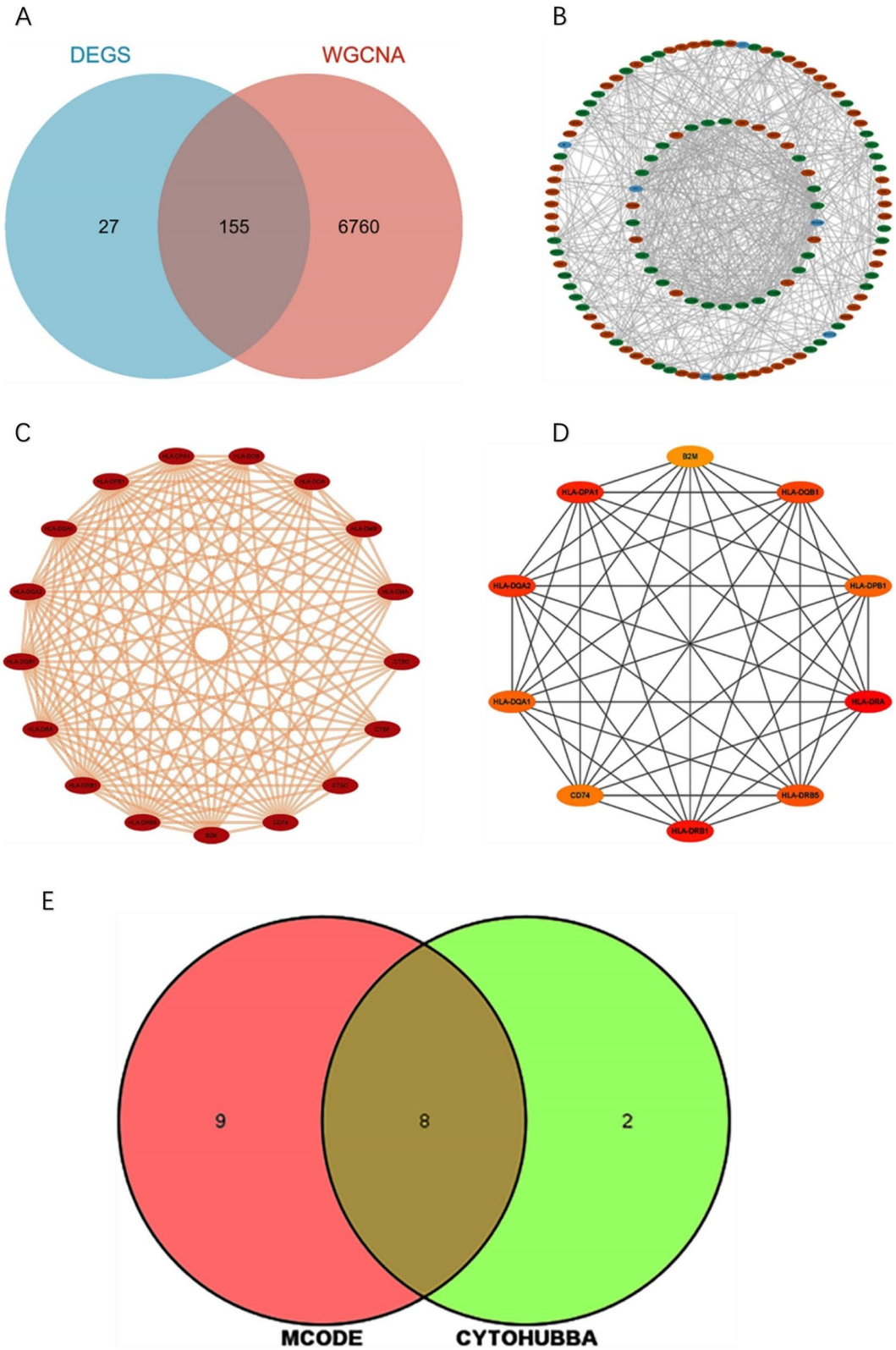

Fig. 5 WGCNA-LYSO-DEGs in SEPSIS; PPI network analysis and hub DEGs identification. An illustration of the number of people in a venn diagram genes shared between WGCNA and LYSO-DEGs; **B** PPI network of WGCNA-LYSO-DEGs; **C** Further selection of a key cluster containing 17 genes by employing MCODE as hub genes; **D** Top 10 hub genes discovered by CytoHubba; **E** MCODE-CytoHubba DEGs

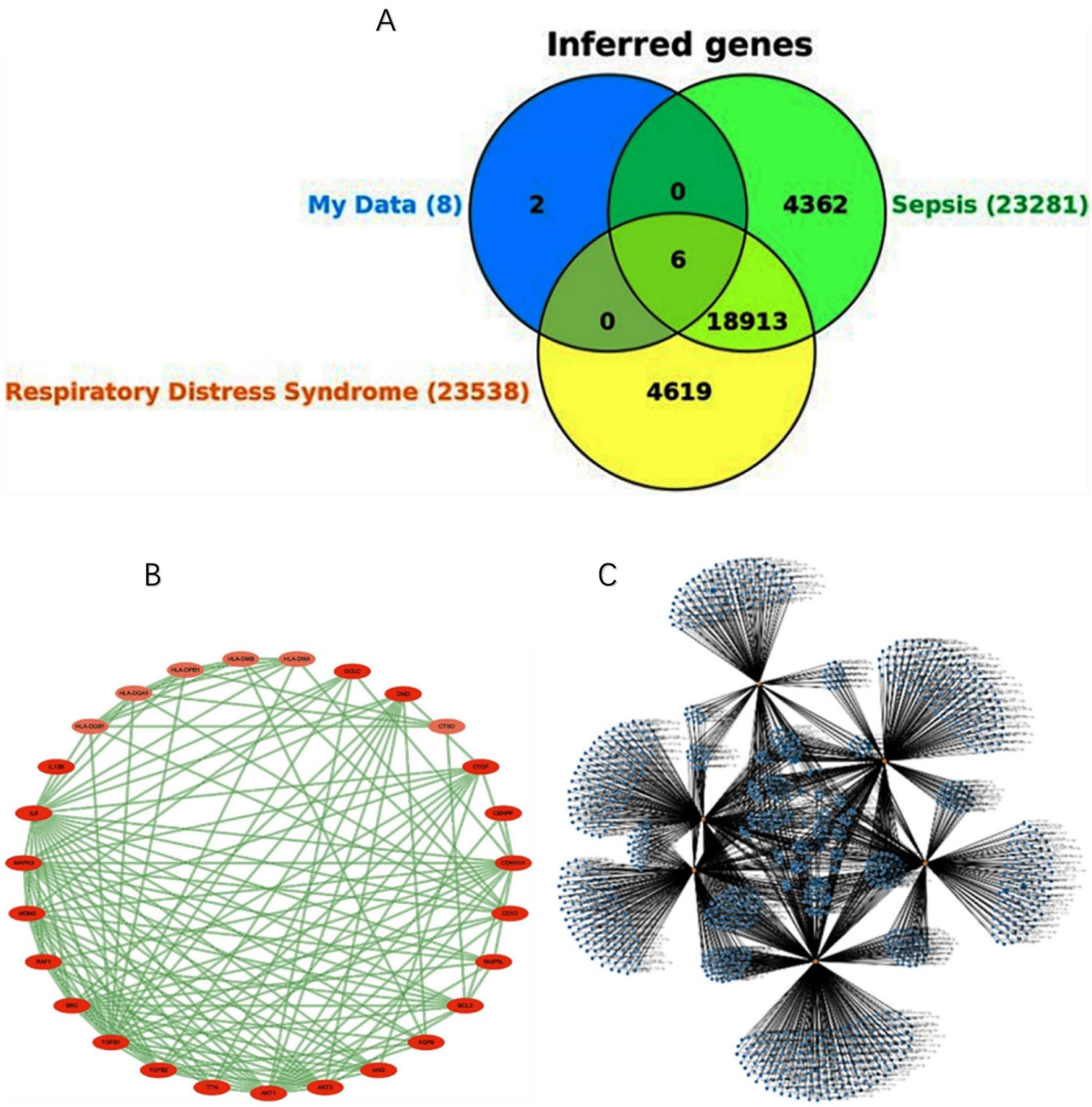

Fig. 6 Correlation between Hub DEGs and SEPSIS/MODS; Regulatory network of Hub DEGs-TFs-miRNAs. **A**, **B** Hub degs associated with SEPSIS and MODS grounded in the CTD database; TF-hub DEGs regulatory network: pink circles define hub DEGs, red dots refer to transcription factors; **C** miRNA-hub DEGs regulatory network: light pink squares denote hub DEGs, there are blue circles representing miRNAs

macrophages, neutrophils, Th17 cells, B cells, DC cells, and NKT cells in Fig. 8C.

Meta-analysis

Utilizing sepsis datasets GSE28750 and GSE69528 sourced from the publicly accessible GEO database, a meta-analysis was conducted at the transcriptional level concentrating on the aforementioned differentially expressed genes (DEGs). The results, presented in Fig. 9A, B, revealed that within the sepsis group, there was a statistically significant upregulation of CTSO and HLA-DQA1 genes in comparison with the control group comprising healthy individuals (adjusted P-value < 0.05).

Discussion

Bloodstream infections, posing a significant risk of life-threatening complications, represent the most ubiquitous cause of sepsis. Among numerous potential

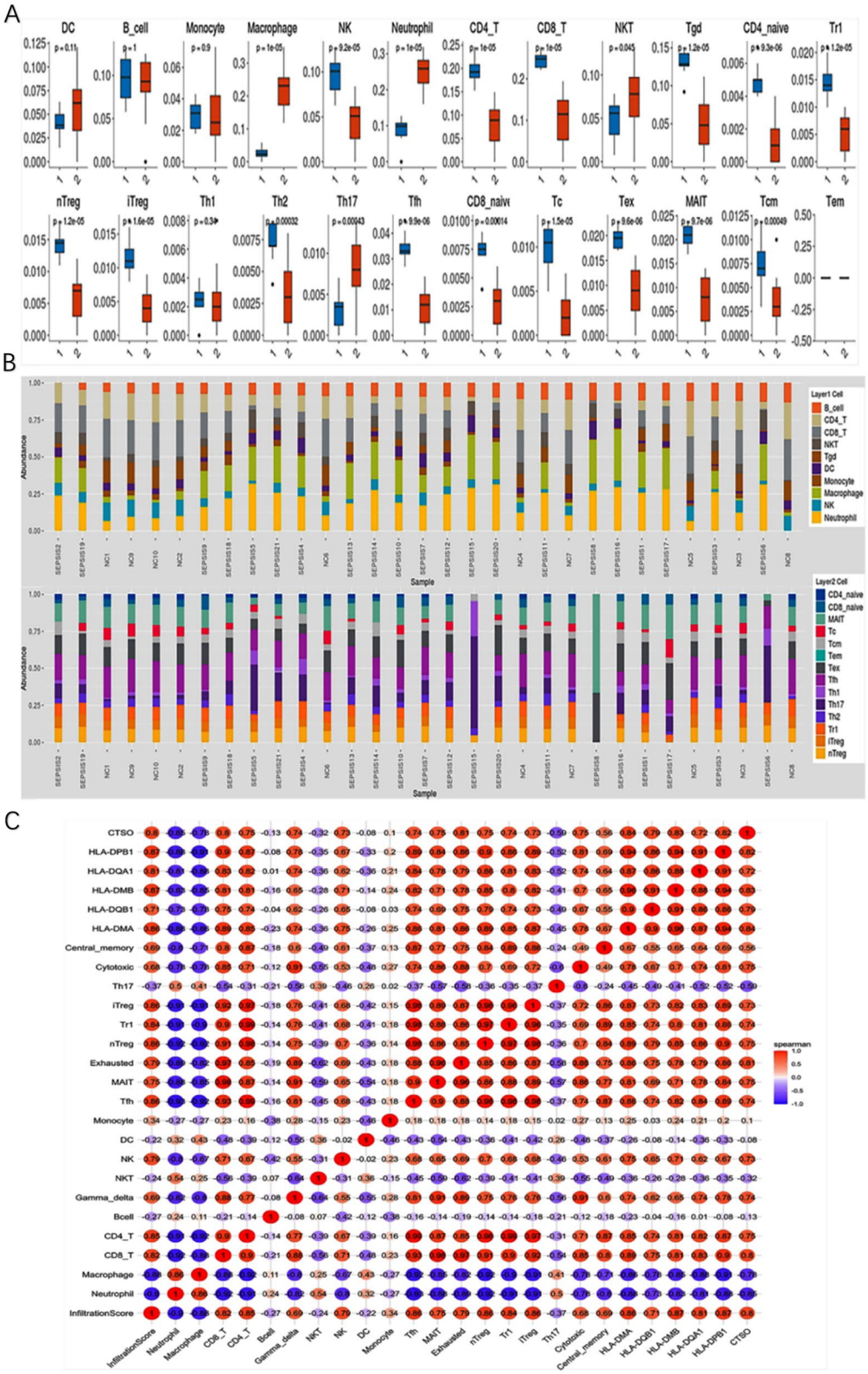

Fig. 7 Analogy between SEPSIS and NC immune cell infiltration types. **A** violin plot reveals the proportions of immune cells; **B** this chart demonstrates the proportion of immune cells in a stacked bar chart; **C** a correlation matrix suggests the proportions of immune cells

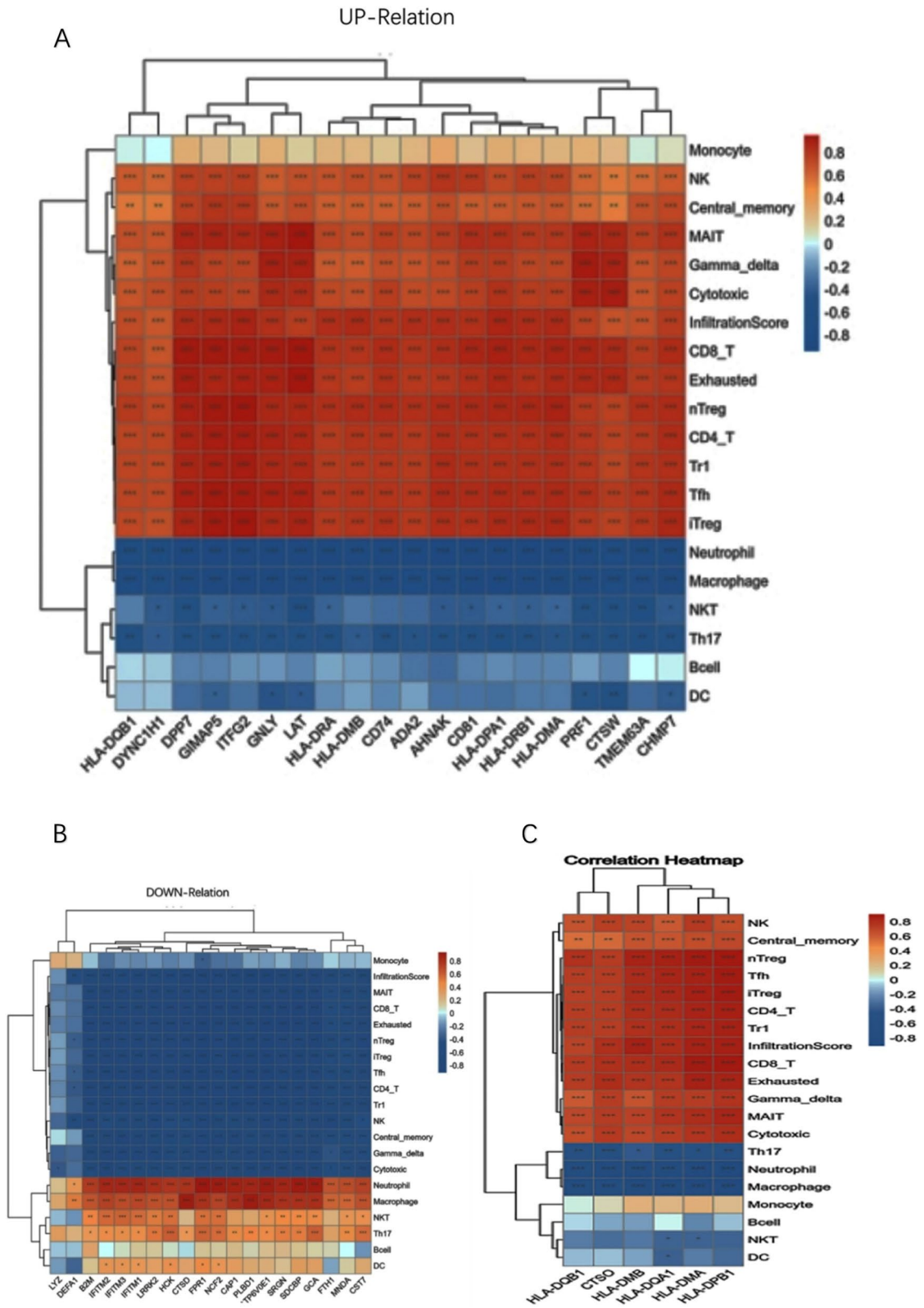

Fig. 8 Applies the Spearman method to explore the potential association between DEGs/hub DEGs and immune cells (**A**-**C**)

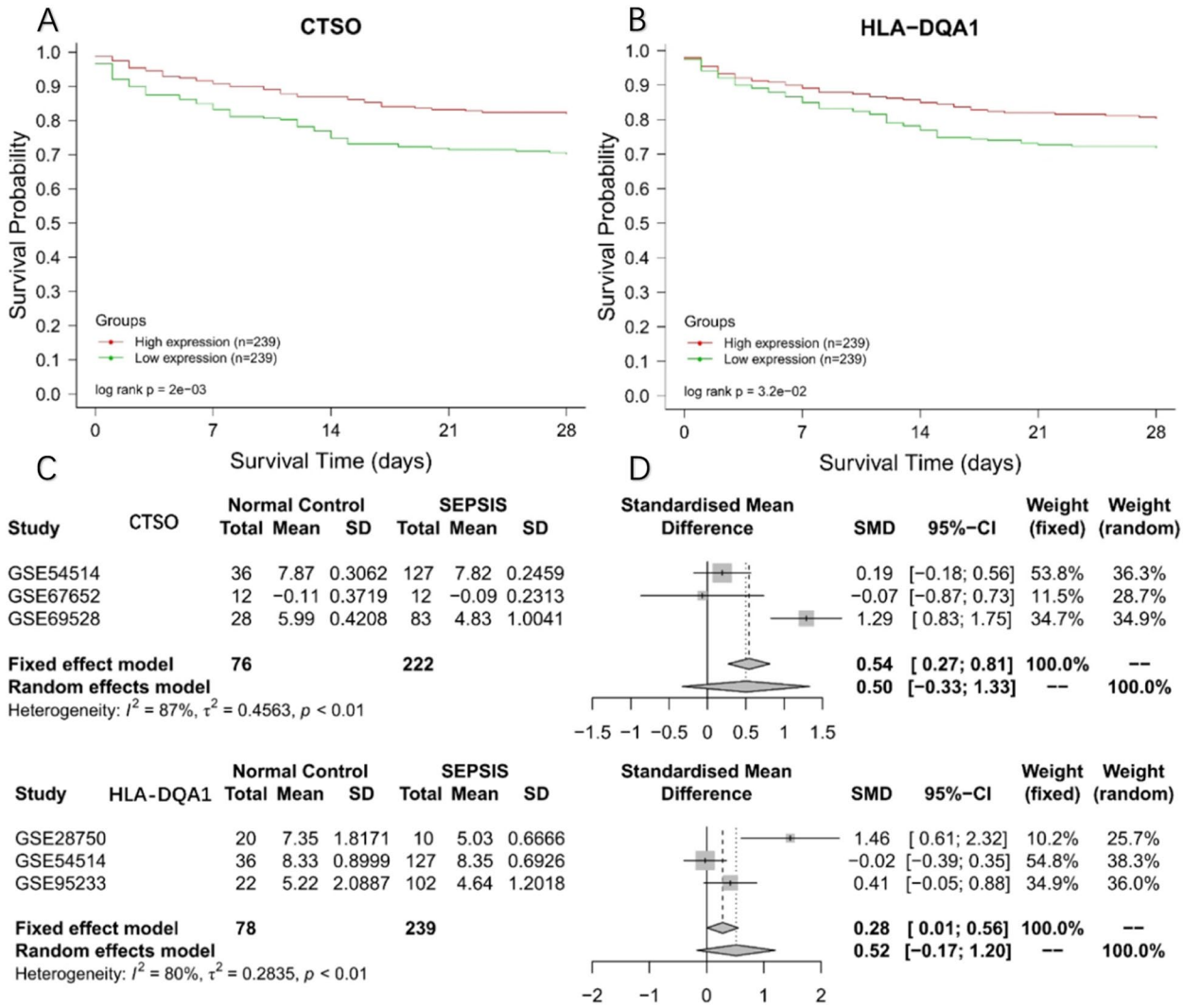

Fig. 9 Meta-analysis. **A**-**D** refer to genes CSTO and HLA-DQA1 in the GSE28750 and GSE69528 datasets for sepsis and NC groups. Furthermore, there was a conspicuous augment in both genes in the sepsis group compared to the healthy group (adj $P < 0.05$)

consequences, acute lung injuries stand out as a prevalent type of organ damage that can result from sepsis [43, 44]. Nevertheless, the specific mechanisms of the disease still need urgent exploration, so as to ameliorate patient prognosis. As a consequence, by integrating various bioinformatics approaches to gather insights into sepsis-related differentially expressed genes (DEGs), this research believe it would be intriguing to probe deep into the interplay among these three factors: between genes and lysosomal metabolism and immune infiltration inflammation. As suggested by numerous studies, iysosomal dysfunction is accompanied by a multitude of cellular events, which is paramount to understand the oxidative stress reaction, mitochondrial function, and mitochondrial structure [45, 46]; its dysfunction can give rise to a multitude of diseases, such as atherosclerosis, neurodegenerative diseases, pancreatitis, autoimmune diseases, etc.

ARDS is a disease that poses a serious risk of death. Immune mechanisms are complex in this organism, which usually can be ascribed to the combined effects of inflammation, coagulation, and injury [47–50]. Drawing upon these findings from the study, we can deduce the following conclusions to analyze the role of lysosomal metabolism and immune disturbances in the progression of sepsis, and to delve into potential therapeutic targets. ARDS is better understood when lysosomal metabolism, immune response, and inflammation are combined. Nowadays, there exists a notable scarcity of research fixing attention on lysosome-associated genes in the context

of sepsis. In this study, lysosome-related genes were obtained from the lysosome GO database. Additionally, 6 hub DEGs strongly associated with SEPSIS or ARDS were identified. Lysosomal metabolic disorders can worsen SEPSIS, while CTSO and HLA-DQA1 are essential for lysosomal functional metabolism. Other than participating in the degradation of intracellular proteins, Cathepsin O (CTSO) not only participates in protein turnover [51] and activates the degradation of extracellular proteins, but also facilitates the reprogramming of macrophages [52].

As already illustrated by multiple studies, it has potential therapeutic value in anti-cancer and various cancer drugs are resistant to treatment [53–56]. On top of that, it has been found that CTSO can regulate various transcription factors, such as ZNF423 [57], MTDH [58, 59] and PABPC4L [60]. Nevertheless, sepsis has failed to capture enormous academic attention on its action mechanism. On that account, it is imperative to conductfurther experiments to confirm its effectiveness.

It is part of the MHC family and is located on chromosome 6p21 as HLA-DQA1 (human leukocyte antigen-DQA1). Abnormal expression can bring about autoimmune diseases [61–63]. As one of the genes bound up with iysosomes, several years have passed since it was discovered that genes responsible for psoriasis are differentially expressed. More importantly, the human leukocyte antigen-C, the human leukocyte antigen-B, and human leukocyte antigen-DPB1 are found in just a few of the examples cited above [64–66]. However, the precise mechanism by which it interacts with T cells in the context of sepsis remains unclear.

In general, patients in an immunosuppressed state have a higher risk of death [67, 68]. Apart from that, surviving the early stages of sepsis while still being immunosuppressed is no small feat state can also lead to death in late-stage sepsis is quite an accomplishment. In advanced sepsis, this state may also lead to fatality [69, 70]. Other than presenting antigens,

B cells also secrete chemicals that regulate immune responses antibodies and a diverse array of cytokines [71, 72]. As a result, immunosuppression continues to develop when B cells are in a state of depletion. Moreover, exploring immune activation therapy in immunosuppressed states can help save the lives of late-stage sepsis patients.

Definitely, this study is imperfect in certain aspects: Above all, despite the fact that it has probed into the potential biomarkers correlated with sepsis-ARDS, this research failed to explore the specific biomarkers in subgroups of sepsis populations. For this reason, it is more preferable for future work to revolve around this aspect. Regarding the exploration of heterogeneity in sepsis populations, it first involves the significant differences in clinical symptoms, pathophysiological processes, treatment responses, and prognosis among sepsis patients attributable to various factors. From an immunological standpoint, the initial stage of sepsis involves the activation of the host's innate immune system by pathogen infection, which induces the expression of pattern recognition receptors on the surface of immune cells. These receptors recognize pathogen-associated molecular patterns and damage-associated molecular patterns, initiating the secretion and release of inflammatory factors, potentially resulting in a cytokine storm. This immune response manifests as early uncontrolled inflammatory reactions and subsequent immunosuppression, although both are present throughout the pathological process, their dominant roles vary at different stages. Immune cell function is associated with their energy metabolism. During the acute inflammatory response phase, glycolysis, fatty acid oxidation, oxidative phosphorylation, and the tricarboxylic acid cycle are activated within immune cells, while the latter two processes are suppressed [73–75]. In contrast, glycolysis, fatty acid oxidation, oxidative phosphorylation, and the tricarboxylic acid cycle are all inhibited during the immunosuppressive phase. In addition, from a macroscopic perspective of the host, the diversity in clinical manifestations of sepsis arises from differences in patient age, gender, comorbidities, genetic factors, and the infecting pathogen. For instance, patients with different ages may exhibit varying tolerances and responses to sepsis, with differences in their immune systems, organ functions, and other aspects compared to adults, potentially bringing about iverse symptoms and development processes when sepsis occurs. Elderly individuals, ascribable to declining physical functions and multiple underlying diseases, are more prone to organ dysfunction and poorer prognosis when sepsis is triggered by infection. Patients of different genders may differ in hormone levels and physiological structures, thereby affecting the manifestation and progression of sepsis. With regard to underlying diseases, patients with diabetes may experience exacerbation of glucometabolic disorders when suffering from sepsis, complicating the condition and affecting treatment efficacy; those with cardiovascular diseases may be more susceptible to severe complications when circulatory changes triggered by sepsis occur. The type and virulence of the infectious pathogens also lead to differences in sepsis. For instance, sepsis stemmed from Gram-positive and Gram-negative bacterial infections may differ in the degree of inflammatory response and drug sensitivity, which can be validated by the research progress on the heterogeneity of sepsis population [76, 77].

Aside from that, the scale of our research population is insufficient, and our mining of the dataset is superficial, clearly lagging behind existing published articles [78]: To start with, we studied the dataset by utilizing a single transcriptomics approach, which may trigger inadequate data analysis, thereby overlooking pivotal details. In contrast, multi-omics joint analysis (transcriptomics-proteomics) can sufficiently mine the information in the dataset. Apart from that, our data mining centered around exploring biomarkers, neglecting the specific mechanisms and modes of action of immune cells (mainly white blood cells) in sepsis, as pointed out in the literature. Our discussion of the relationship between transcriptomic genes and immune cells was cursory, failing to delve into the mechanisms by which they activate and elicit the release of inflammatory mediators, or the implications of the crucial innate immune barrier system, namely the complement system, on sepsis. Because in the literature, it is pointed out that the occurrence and development of sepsis are bound up with endothelial function and vascular barrier integrity. The destruction of these can give riseo t increased vascular permeability and glycocalyx injury, which are crucial to the pathogenesis of sepsis and its clinical sequelae. These significant findings underline the complex interplay between gene expression, endothelial biology, and hemodynamic perturbations observed in sepsis patients, thus making this a focal point for future research; thirdly, the deficiency of relevant practical operations in the clinical management of patients in this study, such as the research on fluid strategies mentioned multiple times in the literature, leaves adequate room for future research. Attributable to the absence of data on specific types of fluids, whether balanced or unbalanced, employed for resuscitation, the choice of fluid could impact clinical outcomes, particularly in sepsis, where fluid management is a critical component of treatment. For this reason, the applicability of these results to other populations or settings may be limited on account of variations in patient demographics, comorbidities, and clinical practices. It is pressing to conduct future research to validate the performance of this model across different populations and explore its suitability in various clinical scenarios.

By employing bioinformatics analysis methods, this study examined the interactions between lysosome-related genes and immune microenvironment changes, which was based on the screening and validation of two genes. The identification of potential molecular targets has been completed by carrying out in-depth research on the immune metabolism of sepsis. In spite of the above findings and contributions, inherent limitations still exist. Notwithstanding the composition of lysosome-related genes in the immune system microenvironment and multiple organs has been studied through bioinformatics analysis. Nonetheless, data from experiments are still lacking on genes linked with lysosomes and their function.

As a result, researchers should look into the mechanisms that regulate the immune system during sepsis so as to put forward innovative, feasible and productive methodologies for future studies.

Conclusion

The difference in lysosomal-related genes has been identified through the combination of various bioinformatics methods and immune cell infiltration between SEPSIS and NC. Our understanding has deepened regarding the correlation between lysosomal metabolism and immune system infiltration in sepsis, leading us to identify and validate two genes that exhibit upregulation in this condition. This is one of the most pivotal findings. There is a positive correlation between CTSO and B-cell margins, whereas a negative association is observed between CTSO and dendritic cells (DCs). Additionally,

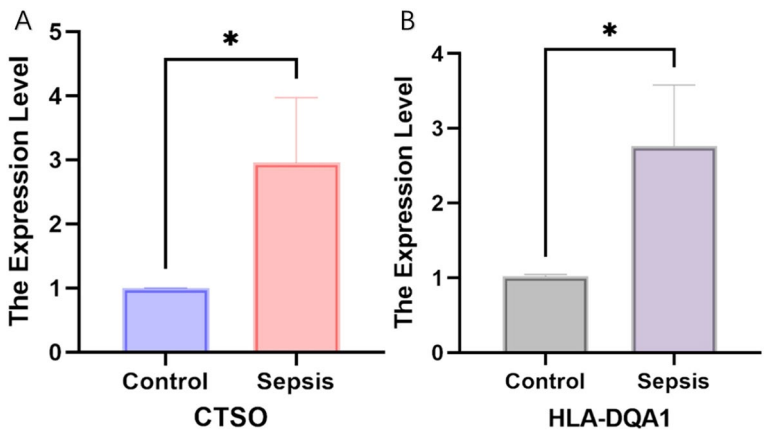

Fig. 10 RT-qPCR. **A**, **B** RT-qPCR detection of the expression of core genes in the sepsis cell model. Blue symbolizes normal, and red denotes sepsis. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

HLA-DQA1 displays a positive connection with natural killer T (NKT) cells. This suggests that CTSO and HLA-DQA1 are immune metabolic regulatory molecules in SEPSIS.

RT-qPCR was employed to detect the expression of CTSO和HLA-DQA1 in RAW264.7 cells in vitro. In accordance with the independent samples t-test, CTSO([−3.585—−0.3290]; t = 3.337, $p < 0.0289$); HLA-DQA1 ([−3.062—−0.4255]; t = 3.673, $p < 0.0213$) was up-regulated in a negative direction in contrast to normal cells. As indicated in Fig. 10A and B, CTSO和HLA-DQA1 exhibits high expression in sepsis and low expression in the control group, with statistically significant differences between the two groups ($p < 0.05$).

Abbreviations

ARDS	Acute Respiratory Distress Syndrome
DEGs	Differential expression genes
GSEA	Gene Set Enrichment Analysis
WGCNA	Weighted Gene Co-Expression Network Analysis
ROS	Reactive Oxygen Species
TNF-a	Tumor necrosis factor
IL-1	Interleukin1
SCCM	Society of Critical Care Medicine
ESICM	European Society of Intensive Care Medicine
TF	Transcription Factors
MM	Module membership
GS	Gene significance
FBS	Fetal bovine serum

Acknowledgements
We thank BGI for instructing RNA sequencing.

Informed consent
Informed consent was obtained from all individuals included in this study.

Authors' contributions
SYZ, LHL, YYD, LY, HYC designed the study. LHL, YYD and LY performed the bioinformatics analysis and interpretation of the data. SYZ wrote the manuscript. HYC revised the manuscript and gave final approval of the version to be published. All authors read and approved the final manuscript. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Funding
1. This study was funded by Sichuan Provincial Key clinical Specialty Construction Project.
2. [Luzhou Municipal People's Government—Southwest Medical University Science and Technology Strategic Cooperation Applied Basic Research (NO.2021LZXNYD-J13)].
3. Mechanism Study on 18α-Glycyrrhetinic Acid Improving Sepsis Prognosis via CDK2-CCND1.(2024LZXNYDJ038)

Data availability
We intend to share individual deidentified participant data. Peripheral blood RNA sequencing data from 19 patients with sepsis and10 Healthy people are available in the China National GeneBank DataBase (CNGBdb) and can be found below: https://db.cngb.org/, under the accession: CNP0002611, you can access it now and it's valid forever.

Declarations

Ethics approval and consent to participate
The study was conducted in strict accordance with the rules of the Declaration of Helsinki. The study protocol has been approved by the ethics committee of the Affiliated Hospital of Southwest Medical University (Ethical Approval No. ky2018029). The Registration Number was ChiCTR1900021261.

Consent for publication
Written informed consent for publication was obtained from all participants.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Emergency Medicine, The Affiliated Hospital of Southwest Medical University, Lu Zhou, People's Republic of China.

Received: 10 April 2024 Accepted: 27 February 2025
Published online: 07 March 2025

References

1. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA. 2016;315(8):801–10.
2. Hu B, Ji W, Bo L, Bian J. How to improve the care of septic patients following "Surviving Sepsis Campaign: international guidelines for management of sepsis and septic shock 2021"? J Intensive Med. 2022;3(2):144–6.
3. Rudd KE, Johnson SC, Agesa KM, et al. Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study. Lancet. 2020;395(10219):200–11.
4. Wasyluk W, Zwolak A. Metabolic alterations in sepsis. J Clin Med. 2021;10(11):2412.
5. Puleston DJ, Villa M, Pearce EL. ancillary activity: beyond core metabolism in immune cells. Cell Metab. 2017;26(1):131–41.
6. Van Wyngene L, Vandewalle J, Libert C. Reprogramming of basic metabolic pathways in microbial sepsis: therapeutic targets at last? EMBO Mol Med. 2018;10(8):e8712.
7. Lee MKS, Al-Sharea A, Shihata WA, et al. Glycolysis is required for LPS-induced activation and adhesion of human CD14+CD16- monocytes. Front Immunol. 2019;10:2054.
8. Peck TJ, Hibbert KA. Recent advances in the understanding and management of ARDS. F1000Res. 2019;8:F1000.
9. Guillen-Guio B, Lorenzo-Salazar JM, Ma SF, et al. Sepsis-associated acute respiratory distress syndrome in individuals of European ancestry: a genome-wide association study. Lancet Resp Med. 2020;8(3):258–66.
10. Wheeler AP, Bernard GR. Acute lung injury and the acute respiratory distress syndrome: a clinical review. Lancet. 2007;369(9572):1553–64.
11. Lim CY, Zoncu R. The lysosome as a command-and-control center for cellular metabolism. J Cell Biol. 2016;214(6):653–64.
12. Perera RM, Zoncu R. The lysosome as a regulatory hub. Annu Rev Cell Dev Bi. 2016;32:223–53.
13. Roczniak-Ferguson A, Petit CS, Froehlich F, et al. The transcription factor TFEB links mTORC1 signaling to transcriptional control of lysosome homeostasis. Sci Signal. 2012;5(228):ra42.
14. Settembre C, Zoncu R, Medina DL, et al. A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mTOR and TFEB. EMBO J. 2012;31(5):1095–108.
15. Faverio P, Stainer A, De Giacomi F, et al. Molecular pathways and respiratory involvement in lysosomal storage diseases. Int J Mol Sci. 2019;20(2):327.
16. Sluyter R. The P2X7 receptor. Adv Exp Med Biol. 2017;1051:17–53.
17. Orioli E, De Marchi E, Giuliani AL, et al. P2X7 receptor orchestrates multiple signalling pathways triggering inflammation, autophagy and metabolic/trophic responses. Curr Med Chem. 2017;24(21):2261–75.
18. Lin YC, Huang DY, Wang JS, et al. Syk is involved in NLRP3 inflammasome-mediated caspase-1 activation through adaptor ASC phosphorylation and enhanced oligomerization. J Leukocyte Biol. 2015;97(5):825–35.
19. Adinolfi E, Giuliani AL, De Marchi E, et al. The P2X7 receptor: a main player in inflammation. Biochem Pharmacol. 2017;151:234–44.
20. Savio LEB, de Andrade Mello P, da Silva CG, et al. The P2X7 receptor in inflammatory diseases: angel or demon? Front Pharmacol. 2018;9:52.

21. Bhatia M, Moochhala S. Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. J Pathol. 2004;202(2):145–56.
22. Fumeaux T, Pugin J. Role of interleukin-10 in the intracellular sequestration of human leukocyte antigen-DR in monocytes during septic shock. Am J Resp Crit Care. 2002;166(11):1475–82.
23. Latifi SQ, O'Riordan MA, Levine AD. Interleukin-10 controls the onset of irreversible septic shock. Infect Immun. 2002;70(8):4441–6.
24. Zhu H, Santo A, Jia Z, et al. GPx4 in bacterial infection and polymicrobial sepsis: involvement of ferroptosis and pyroptosis. React Oxyg Species (Apex). 2019;7(21):154–60.
25. Wang Y, Dong Z, Zhang Z, et al. Postconditioning with irisin attenuates lung ischemia/reperfusion injury by suppressing ferroptosis via induction of the Nrf2/HO-1 signal axis. Oxid Med Cell Longev. 2022;2022:9911167.
26. Nichols DP, Caceres S, Caverly L, et al. Effects of azithromycin in Pseudomonas aeruginosa burn wound infection. J Surg Res. 2013;183(2):767–76.
27. Zhang YF, Sun CC, Duan JX, et al. A COX-2/sEH dual inhibitor PTUPB ameliorates cecal ligation and puncture-induced sepsis in mice via anti-inflammation and anti-oxidative stress. Biomed Pharmacother. 2020;126:109907.
28. Savina A, Jancic C, Hugues S, et al. NOX2 controls phagosomal pH to regulate antigen processing during crosspresentation by dendritic cells. Cell. 2006;126(1):205–18.
29. Mantegazza AR, Savina A, Vermeulen M, et al. NADPH oxidase controls phagosomal pH and antigen cross-presentation in human dendritic cells. Blood. 2008;112(12):4712–22.
30. Luo Y, Yang X, Shi Q. The cytochrome P450 inhibitor SKF-525A disrupts autophagy in primary rat hepatocytes. Chem Biol Interact. 2016;255:55–62.
31. Engoren M, Seelhammer T, Freundlich RE, et al. A comparison of sepsis-2 (Systemic Inflammatory Response Syndrome Based) to Sepsis-3 (Sequential Organ Failure Assessment Based) Definitions-A Multicenter Retrospective Study. Crit Care Med. 2020;48(9):1258–64.
32. Grasselli G, Calfee CS, Camporota L, et al. ESICM guidelines on acute respiratory distress syndrome: definition, phenotyping and respiratory support strategies. Intensive Care Med. 2023;49(7):727–59.
33. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. P Natl Acad Sci USA. 2005;102(43):15545–50.
34. Chen D, Xie J, Fiskesund R, et al. Chloroquine modulates antitumor immune response by resetting tumor-associated macrophages toward M1 phenotype. Nat Commun. 2018;9(1):873.
35. Szklarczyk D, Gable AL, Nastou KC, et al. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res. 2021;49(D1):D605–12.
36. Davis AP, Grondin CJ, Johnson RJ, et al. Comparative toxicogenomics database (CTD): update 2021. Nucleic Acids Res. 2021;49(D1):D1138–43.
37. Janky R, Verfaillie A, Imrichová H, et al. iRegulon: from a gene list to a gene regulatory network using large motif and track collections. Plos Comput Biol. 2014;10(7):e1003731.
38. Dweep H, Sticht C, Pandey P, et al. miRWalk–database: prediction of possible miRNA binding sites by "walking" the genes of three genomes. J Biomed Inform. 2011;44(5):839–47.
39. Miao YR, Xia M, Luo M, et al. ImmuCellAI-mouse: a tool for comprehensive prediction of mouse immune cell abundance and immune microenvironment depiction. Bioinformatics. 2022;38(3):785–91.
40. Sutherland A, Thomas M, Brandon RA, et al. Development and validation of a novel molecular biomarker diagnostic test for the early detection of sepsis. Crit Care. 2011;15(3):R149.
41. Pankla R, Buddhisa S, Berry M, et al. Genomic transcriptional profiling identifies a candidate blood biomarker signature for the diagnosis of septicemic melioidosis. Genome Biol. 2009;10(11):R127.
42. Wang Y, Liu Z, Zhang M, et al. Mucosa-associated lymphoid tissue lymphoma translocation protein 1 exaggerates multiple organ injury, inflammation, and immune cell imbalance by activating the NF-κB pathway in sepsis. Front Microbiol. 2023;14:1117285.
43. Cecconi M, Evans L, Levy M, et al. Sepsis and septic shock. Lancet. 2018;392(10141):75–87.
44. Fiorenza MT, Moro E, Erickson RP. The pathogenesis of lysosomal storage disorders: beyond the engorgement of lysosomes to abnormal development and neuroinflammation. Hum Mol Genet. 2018;27(R2):R119–29.
45. Parenti G, Medina DL, Ballabio A. The rapidly evolving view of lysosomal storage diseases. Embo Mol Med. 2021;13(2):e12836.
46. Thompson BT, Chambers RC, Liu KD. Acute respiratory distress syndrome. New Engl J Med. 2017;377(6):562–72.
47. Matthay MA, Zemans RL, Zimmerman GA, et al. Acute respiratory distress syndrome. Nat Rev Dis Primers. 2019;5(1):18.
48. Bos LDJ, Ware LB. Acute respiratory distress syndrome: causes, pathophysiology, and phenotypes. Lancet. 2022;400(10358):1145–56.
49. Gorman EA, O'Kane CM, McAuley DF. Acute respiratory distress syndrome in adults: diagnosis, outcomes, long-term sequelae, and management. Lancet. 2022;400(10358):1157–70.
50. Ingle JN, Liu M, Wickerham DL, et al. Selective estrogen receptor modulators and pharmacogenomic variation in ZNF423 regulation of BRCA1 expression: individualized breast cancer prevention. Cancer Discov. 2013;3(7):812–25.
51. Zucker S, Cao J, Chen WT. Critical appraisal of the use of matrix metalloproteinase inhibitors in cancer treatment. Oncogene. 2000;19(56):6642–50.
52. Gocheva V, Joyce JA. Cysteine cathepsins and the cutting edge of cancer invasion. Cell Cycle. 2007;6(1):60–4.
53. Jedeszko C, Sloane BF. Cysteine cathepsins in human cancer. Biol Chem. 2004;385(11):1017–27.
54. Wang W, Long L, Wang L, et al. Knockdown of Cathepsin L promotes radiosensitivity of glioma stem cells both in vivo and in vitro. Cancer Lett. 2015;371(2):274–84.
55. Gangoda L, Keerthikumar S, Fonseka P, et al. Inhibition of cathepsin proteases attenuates migration and sensitizes aggressive N-Myc amplified human neuroblastoma cells to doxorubicin. Oncotarget. 2015;6(13):11175–90.
56. Thirkettle HJ, Mills IG, Whitaker HC, et al. Nuclear LYRIC/AEG-1 interacts with PLZF and relieves PLZF-mediated repression. Oncogene. 2009;28(41):3663–70.
57. Menssen A, Hermeking H. Characterization of the c-MYC-regulated transcriptome by SAGE: identification and analysis of c-MYC target genes. P Natl Acad Sci USA. 2002;99(9):6274–9.
58. Deo RC, Bonanno JB, Sonenberg N, et al. Recognition of polyadenylate RNA by the poly(A)-binding protein. Cell. 1999;98(6):835–45.
59. Alvarenga MP, Fernandez O, Leyva L, et al. The HLA DRB1*03:01 allele is associated with NMO regardless of the NMO-IgG status in Brazilian patients from Rio de Janeiro. J Neuroimmunol. 2017;310:1–7.
60. Yamasaki K. HLA-DPB1* 0501-associated opticospinal multiple sclerosis: clinical, neuroimaging and immunogenetic studies. Fukuoka Igaku Zasshi. 2000;91(10):243–5.
61. Brill L, Mandel M, Karussis D, et al. Increased occurrence of anti-AQP4 seropositivity and unique HLA Class II associations with neuromyelitis optica (NMO), among Muslim Arabs in Israel. J Neuroimmunol. 2016;293:65–70.
62. Muñiz-Castrillo S, Vogrig A, Honnorat J. Associations between HLA and autoimmune neurological diseases with autoantibodies. Auto Immun Highlights. 2020;11(1):2.
63. Ogawa K, Okuno T, Hosomichi K, et al. Next-generation sequencing identifies contribution of both class I and II HLA genes on susceptibility of multiple sclerosis in Japanese. J Neuroinflammation. 2019;16(1):162.
64. Xie JL, Liu J, Lian ZY, et al. Association of GTF2IRD1-GTF2I polymorphisms with neuromyelitis optica spectrum disorders in Han Chinese patients. Neural Regen Res. 2019;14(2):346–53.
65. Wang QS, Xiao HQ, Chen HX, et al. The single nucleotide polymorphism site of aquaporin-4 gene in patients with neuromyelitis optica. Exp Ther Med. 2017;14(6):6017–21.
66. Wang X, Buechler NL, Woodruff AG, et al. Sirtuins and immuno-metabolism of sepsis. Int J Mol Sci. 2018;19(9):2738.
67. Otto GP, Sossdorf M, Claus RA, et al. The late phase of sepsis is characterized by an increased microbiological burden and death rate. Crit Care. 2011;15(4):R183.
68. Torres LK, Pickkers P, van der Poll T. Sepsis-Induced Immunosuppression. Annu Rev Physiol. 2021;84:157–81.

69. Liu D, Huang SY, Sun JH, et al. Sepsis-induced immunosuppression: mechanisms, diagnosis and current treatment options. Mil Med Res. 2022;9(1):56.
70. Cyster JG, Allen CDC. B cell responses: cell interaction dynamics and decisions. Cell. 2019;177(3):524–40.
71. Pieper K, Grimbacher B, Eibel H. B-cell biology and development. J Allergy Clin Immun. 2013;131(4):959–71.
72. Dong X, Liu Q, Zheng Q, et al. Alterations of B cells in immunosuppressive phase of septic shock patients. Crit Care Med. 2020;48(6):815–21.
73. Keskinidou C, Vassiliou AG, Dimopoulou I, Kotanidou A, Orfanos SE. Mechanistic understanding of lung inflammation: recent advances and emerging techniques. J Inflamm Res. 2022;15(15):3501–46.
74. Aki T, Unuma K, Uemura K. The role of peroxiredoxins in the regulation of sepsis. Antioxidants (Basel). 2022;11(1):126.
75. SanzCodina M, Zeitlinger M. Biomarkers predicting tissue pharmacokinetics of antimicrobials in sepsis: a review. Clin Pharmacokinet. 2022;61(5):593–617.
76. Wasyluk W, Zwolak A. Metabolic alterations in sepsis. J Clin Med. 2021;10(11):2412.
77. Vulczak A, Catalão CHR, Freitas LAP, Rocha MJA. HSP-target of therapeutic agents in sepsis treatment. Int J Mol Sci. 2019;20(17):4255.
78. Zhang Z, Chen L, Sun B, et al. Identifying septic shock subgroups to tailor fluid strategies through multi-omics integration. Nat Commun. 2024;15(1):9028.

Publisher's Note