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Candidate target genes in sepsis diagnosis and therapy: identifying hub genes with a spotlight on *KLRB1*

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Abstract

Background Sepsis, which causes systemic inflammation and organ failure, is one of the leading causes of death in the intensive care unit (ICU) and an urgent social health problem. However, the pathogenesis and molecular mechanism of sepsis are unclear. Therefore, this study aimed to identify candidate Hub genes during sepsis progression and the candidate target genes for sepsis diagnosis and treatment.

Methods GSE54514, GSE57065, GSE69528, GSE95233, and GSE131761 datasets were downloaded from public databases, and the differentially expressed genes (DEGs) between healthy and septic patients in each dataset were screened at adjusted *P*-value < 0.05 and| log2FC| ≥ 0.58. Subsequently, the obtained DEGs in each dataset were intersected to obtain the Hub genes. In addition, the DEGs between patients with better and poor prognoses in datasets GSE54514 and GSE95233 were analyzed after 28 days. The differential expression of Hub genes in septic patients with good and poor prognoses was detected at adjusted *P*-value < 0.05 and| log2FC| ≥ 0.58. Finally, real-time quantitative polymerase chain reaction (qRT-PCR) was used to verify the bioinformatics results.

Results In datasets GSE54514, GSE57065, GSE69528, GSE95233 and GSE131761, *RNASE2*, *RNASE3*, *CTSG*, *SLPI*, *TNFAIP6*, *PGLYRP1* and *BLOC1S1* were up-regulated in septic patients, and *RPL10A* and *KLRB1* were down-regulated compared to healthy controls. qRT-PCR confirmed the expression trend of the hub genes except *CTSG* (which was not differentially expressed). Compared to septic patients with good prognoses, the differential expression of *RNASE3* was higher in patients with poor prognoses. Furthermore, qRT-PCR revealed that *KLRB1* was the only differentially expressed hub gene with down-regulated expression in sepsis patients with poor prognosis.

Conclusions The candidate Hub genes closely related to sepsis include *KLRB1*, *RNASE2*, *RNASE3*, *CTSG*, *SLPI*, *TNFAIP6*, *PGLYRP1*, *BLOC1S1*, and *RPL10A*. *KLRB1* is the most relevant candidate hub gene among these hub genes in the molecular underpinnings of sepsis, which could be targeted for sepsis detection and treatment.

Keywords Bioinformatical analysis, Hub genes, *KLRB1*, Sepsis

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Introduction

Sepsis is a life-threatening organ dysfunction syndrome caused by the unbalanced response of the body to infection and is one of the main causes of death in critically ill medical patients [1, 2]. Each year, sepsis affects more than 30 million people globally [3]. As a result, it has become a global public health problem, given its high mortality, morbidity and huge economic burden [4]. Current treatments for sepsis, including antibiotics, fluid resuscitation, and organ support, are often insufficient and have significant limitations, highlighting the need for new therapeutic targets and biomarkers [5].

Given the complex and multifactorial nature of sepsis, understanding its molecular mechanisms is crucial. Studies have shown that the pathogenesis of sepsis involves a dysregulated immune response leading to widespread inflammation, tissue damage, and organ failure [1]. The identification of specific molecular targets that contribute to these processes could provide new avenues for treatment and improve patient outcomes.

The advent of gene chip technology and high-throughput sequencing has revolutionized the study of genetic and molecular mechanisms in various diseases, including sepsis [6]. Bioinformatics analysis allows for the comprehensive screening of gene-level mutations and the identification of differentially expressed genes (DEGs). These tools enable researchers to quickly pinpoint Hub genes that play central roles in disease pathways. Furthermore, integrated analyses, such as Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment, help in understanding the functional implications of these genes and their involvement in critical biological processes [7].

In our study, we aim to investigate the gene expression profiles in patients with sepsis using publicly available transcriptome datasets, including GSE54514, GSE57065, GSE69528, GSE95233, and GSE131761. These datasets are based on microarray technology, which allows for the analysis of differential gene expression between healthy individuals and sepsis patients. This approach provides valuable insights into the cellular and molecular changes that occur during sepsis, and we hope to uncover potential biomarkers and therapeutic targets that could lead to improved diagnostics and treatment strategies for sepsis.

Materials and methods

Transcriptomics data download and preprocessing

The transcriptomics datasets, including GSE54514, GSE57065, GSE69528, GSE95233, and GSE131761, used in this study, and their corresponding clinical information was downloaded from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database [8].

Dataset GSE54514 contained data from 18 healthy donors and 35 sepsis patients (including 26 sepsis patients with a good prognosis and 9 with a poor prognosis). Dataset GSE57065 contained transcriptomics data for 25 healthy donors and 28 sepsis patients. Dataset GSE69528 contained transcriptomics data from 27 healthy donors and 83 patients with sepsis. Dataset GSE95233 contained transcriptomics data for 22 healthy donors and 51 sepsis patients (including 34 sepsis patients with a good prognosis and 17 with a poor prognosis). The data from Day 1 samples (collected at admission) from sepsis patients were used for comparison with healthy controls and to assess gene expression changes based on 28-day survival status. Dataset GSE131761 contained transcriptomics data from 15 healthy donors and 81 sepsis patients (Supplementary Table 1).

The gene probe numbers were converted to gene symbols using the corresponding probe annotation files in each dataset. The final gene expression value was also determined by calculating the average expression value for multiple identical probes.

Identification of the differentially expressed genes (DEGs) and hub genes

The difference analysis was performed using "Limma" [9] package (version 3.57.9) in R software (version 4.3.2), and the DEGs were identified based on adjusted P-value < 0.05 and $|\log2FC| \geq 0.58$. To account for multiple comparisons, adjusted P-value were calculated using the Benjamini-Hochberg procedure.

The DEGs of each dataset were intersected, and the intersection genes were recorded as Hub genes.

Functional enrichment analysis

The DEGs enriched between healthy donors and patients with sepsis and between patients with better and worse prognosis were analyzed using the clusterProfiler package (version 4.9.4) in R software. The DEGs enriched in various GO terms, including biological processes (BP), cellular components (CC), and molecular function (MF) and KEGG pathways, were screened at adjusted P-value < 0.05 (Benjamini-Hochberg procedure).

The results of the enrichment analyses were visualized using the "ggplot2" package (version 3.4.3) in R software.

Statement

The study protocol was reviewed and approved by the ethics committee of the Lianyungang Clinical College of Nanjing Medical University (Approval number: LCYJ2022031801). A written informed consent to participate in the study was obtained from the patient's legal representatives before the beginning of the study. We confirm that all methods carried out in this study were performed in accordance with the relevant guidelines and

regulations. All experimental protocols were approved by the appropriate institutional and/or licensing committees. We have complied with all ethical standards in the treatment of human subjects, including obtaining informed consent.

Study design and participants

The study subjects were patients admitted to the Department of Surgical Critical Care Medicine of the First People's Hospital of Lianyungang City between January 2021 and February 2022. All patients were at least 18 years old and hospitalized in the intensive care unit (ICU) for more than 24 h. The observation period for all patients was 28 days, starting from ICU admission. Clinical outcomes, including 28-day survival status, were recorded during this period. The enrolled patients were screened according to the third international consensus definition of sepsis and septic shock (Sepsis 3.0). For enrollment, the patients must have had a clear source of infection or clinical suspicion of an infection. Subsequently, infection was confirmed through clinical assessment, laboratory tests, imaging, and organ dysfunction manifested by an increased Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score of 2 points or more [1]. After enrolment, the patient information, including age, gender, comorbidities, length of Intensive care unit (ICU) stay, SOFA scores (on the first day of ICU admission), 24-hour Acute Physiology and Chronic Health Evaluation II (APACHE II) score, duration of mechanical ventilation, length of hospitalization and 28-day mortality rate was collected. In addition, other clinical data collected, serum lactate, white blood cell (WBC) count, neutrophil count, neutrophil percentage, lymphocyte count, lymphocyte percentage, monocyte count, monocyte percentage, procalcitonin (PCT) level, and C-reactive protein (CRP) level were also assessed. Patient records/information was anonymized and deidentified before analysis.

For patients unable to provide informed consent due to incapacity or delirium, consent was obtained from their legal representatives or family members, in accordance with the ethical guidelines approved by the Institutional Review Board. All procedures adhered to the ethical guidelines set for critically ill patients.

A total of 30 samples were included in the study, consisting of 19 septic patients (Survivor: $n = 12$, Death: $n = 7$) and 11 healthy volunteers.

Blood samples collection

Blood samples (5–10 mL) were collected from all participants (ICU patients and healthy volunteers) using PAXgene® and serum separator tubes for subsequent qRT-PCR analyses and enzyme linked immunosorbent assay (ELISA), respectively. The blood samples were collected within 6 h of patient admission. Whole blood samples in PAX tubes were stored at -80℃ awaiting total RNA extraction and qRT-PCR analyses. On the other hand, blood samples in serum separator tubes were centrifuged at 3000 rpm for 10 min at 4℃. The supernatant was drawn into a cryopreservation tube and stored at -80℃, awaiting ELISA.

Multiple microsphere immunofluorescence assay

Serum inflammatory factors Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Interferon α (IFN-α), and Interferon γ (IFN-γ) were measured by a solid-phase sandwich assay using a commercially available enzyme-linked immunosorbent assay (ELISA, R&D Systems, Minneapolis, MN, USA), according to the manufacturer's protocol. Finally, the absorbance was measured at 450 nm, and the IL-2, IL-4, IL-6, IL-10, IFN-α, and IFN-γ levels were calculated from the serum concentration.

RNA extraction and qRT-PCR

Total cellular RNA was extracted from the whole blood samples in PAX tubes using the PAX Blood Total RNA Kit (BD, cat. no. 762165) and reverse transcribed using HiScript IIQ RT SuperMix for qPCR (RC211, Vazyme, China). Subsequently, qRT-PCR was performed using ChamQ SYBR qPCR Master Mix (Q331, Vazyme, China). Expression of the target genes was normalized to the level of β-actin [10].

The primer sequences corresponding to the Hub genes used in this study are presented in Supplementary Table 2.

Statistical analysis

For normally distributed data, comparisons between datasets were performed by t-test or one-way analysis of variance (ANOVA), with adjusted *P*-value calculated using the Benjamini-Hochberg procedure. The normality of the data was assessed using the Shapiro-Wilk test and the results were presented as mean ± standard deviation. In addition, Pearson correlation analysis was performed to establish the correlation between datasets.

The Mann-Whitney U test was performed for non-normally distributed data, and the results were expressed as median and interquartile range. Spearman correlation analysis was also performed for data correlation analysis.

For categorical variables, comparisons were made using the chi-square test or Fisher's exact test, and results were presented as numbers and percentages.

*Indicates $P < 0.05$, ** Indicates $P < 0.01$, *** Indicates $P < 0.001$, and **** Indicates $P < 0.0001$, ns Indicates No significance.

Results

Screening DEGs and identifying hub genes

Transcriptome data downloaded from the GEO public database, including GSE54514, GSE57065, GSE69528, GSE95233, and GSE131761 datasets, are presented in Supplementary Table 1 (Fig. 1A-E). From the DEGs in each dataset, seven up-regulated genes were identified, including ribonuclease A family member 2 (*RNASE2*), ribonuclease A family member 3 (*RNASE3*), cathepsin G (*CTSG*), secretory leukocyte peptidase inhibitor (*SLPI*), TNF alpha induced protein 6 (*TNFAIP6*), peptidoglycan recognition protein 1 (*PGLYRP1*), biogenesis of lysosomal organelles complex 1 subunit 1 (*BLOC1S1*), as well as two down-regulated genes, namely ribosomal protein L10a (*RPL10A*) and killer cell lectin like receptor B1 (*KLRB1*) (Fig. 1F and G). These nine genes were denoted as Hub genes, and their differential expression in the five transcriptome datasets is shown in Supplementary Table 3.

Enrichment analysis

In dataset GSE54514, 81 DEGs between healthy and septic patients were enriched in BPs, 15 in CCs and 1 in MFs. In dataset GSE57065, 885 DEGs were enriched in BPs, 190 in CCs, 25 in MFs and 59 in signal pathways. In dataset, GSE69528, 943 DEGs were enriched in BPs, 116 in CCs, 47 in MFs and 62 in signal pathways. In dataset GSE95233, 401 DEGs were enriched in BPs, 46 in CCs, 25 in MFs and 30 in signal pathways. In dataset GSE131761, 1038 DEGs were enriched in BPs, 158 in CCs, 88 in MFs and 53 in signal pathways (Fig. 2A-E).

Interestingly, *RNASE2* and *RNASE3*, which are mainly involved in storing and transporting substances, were found to be co-expressed with six DEGs enriched in CCs (Supplementary Table 4). Additionally, SLPI, which is primarily involved in the body's immune response and the storage and transport of substances, was co-expressed with seven DEGs, 2 enriched in BPs and 5 in CCs (Supplementary Table 5). *PGLYRP1*, also mainly involved in the body's immune response, storage and transport of substances, was regulated by 13 DEGs, 7 enriched in BPs and 6 in CCs (Supplementary Table 6).

Hub gene differences in sepsis patients with better versus poorer prognosis

The differences in hub genes between the patients with better and poorer prognoses (Survival Difference Differentially Expressed Genes, SD-DEGs) in datasets GSE54514 and GSE95233 are shown in Fig. 3A and B; Table 1. The expression of these hub genes was compared between patients with better prognosis (control group) and patients with poor prognosis (experimental group).

These results indicate that certain genes (such as *RNASE2*, *RNASE3*, *CTSG*, *SLPI*, and *BLOC1S1*) are more highly expressed in the better prognosis group, while *TNFAIP6*, *RPL10A*, and *KLRB1* are more highly expressed in the poor prognosis group. It is worth noting that some genes, such as *KLRB1* and *TNFAIP6*, showed inconsistent expression trends across the GSE54514 and GSE95233 datasets.

Enrichment analysis

In the dataset, GSE54514, 78 SD-DEGs were enriched in BPs, 29 in CCs, 23 in MFs and 3 in signal pathways. In dataset GSE57065, 160 SD-DEGs were enriched in BPs, 47 in CCs, 16 in MFs and 6 in signal pathways (Fig. 4A and B).

Notably, *RNASE3* involved in the organism's immune response was co-differentially expressed in patients with poorer prognosis and was co-expressed with 3 SD-DEGs enriched in BPs and 6 in CCs (Supplementary Table 7).

Verification of the differential expression of hub genes by qRT-PCR

The baseline data is shown in Supplementary Table 8. The average age of the sepsis patient group was 63.79, with 47.4% males. Among them, the average age of the patient group who died after 28 days was 64.29 years, with 57.1% males. The average age of the patient group who survived after 28 days was 65.50 years, with 41.7% males; and the average age of the healthy volunteer group was 31.09 years, with 45.5% males. In the general data, the age, concomitant chronic kidney injury, diabetes mellitus, and hypertension of the sepsis patients were statistically significant relative to that of the healthy volunteers (all P-values < 0.05); whereas the differences in the gender of the patients, as well as the types of underlying diseases other than chronic kidney injury, hypertension, and diabetes mellitus were not statistically significant compared with that of the healthy volunteer group (all p-values > 0.05). In the group of death and survival within 28 days, only the number of days of hospitalization was statistically significant ($P < 0.05$), while the rest were not statistically significant ($P > 0.05$).

Compared to healthy donors, 9 Hub genes except *CTSG* (no difference in expression) had the same differential expression trend in septic patients following qRT-PCR and bioinformatics analyses (Fig. 5A-I). Compared to septic patients with good prognosis, the high expression of *RNASE3* in patients with poor prognosis was not detected by qRT-PCR. However, *KLRB1* was lowly expressed in patients with poor prognosis consistent with bioinformatics analysis (Fig. 6A-I).

Correlation between the hub genes and clinical indicators

Correlation analysis revealed that *RNASE2* and *RPL10A* were negatively correlated with APACHE II score and lymphocyte count. Meanwhile, *TNFAIP6* was positively

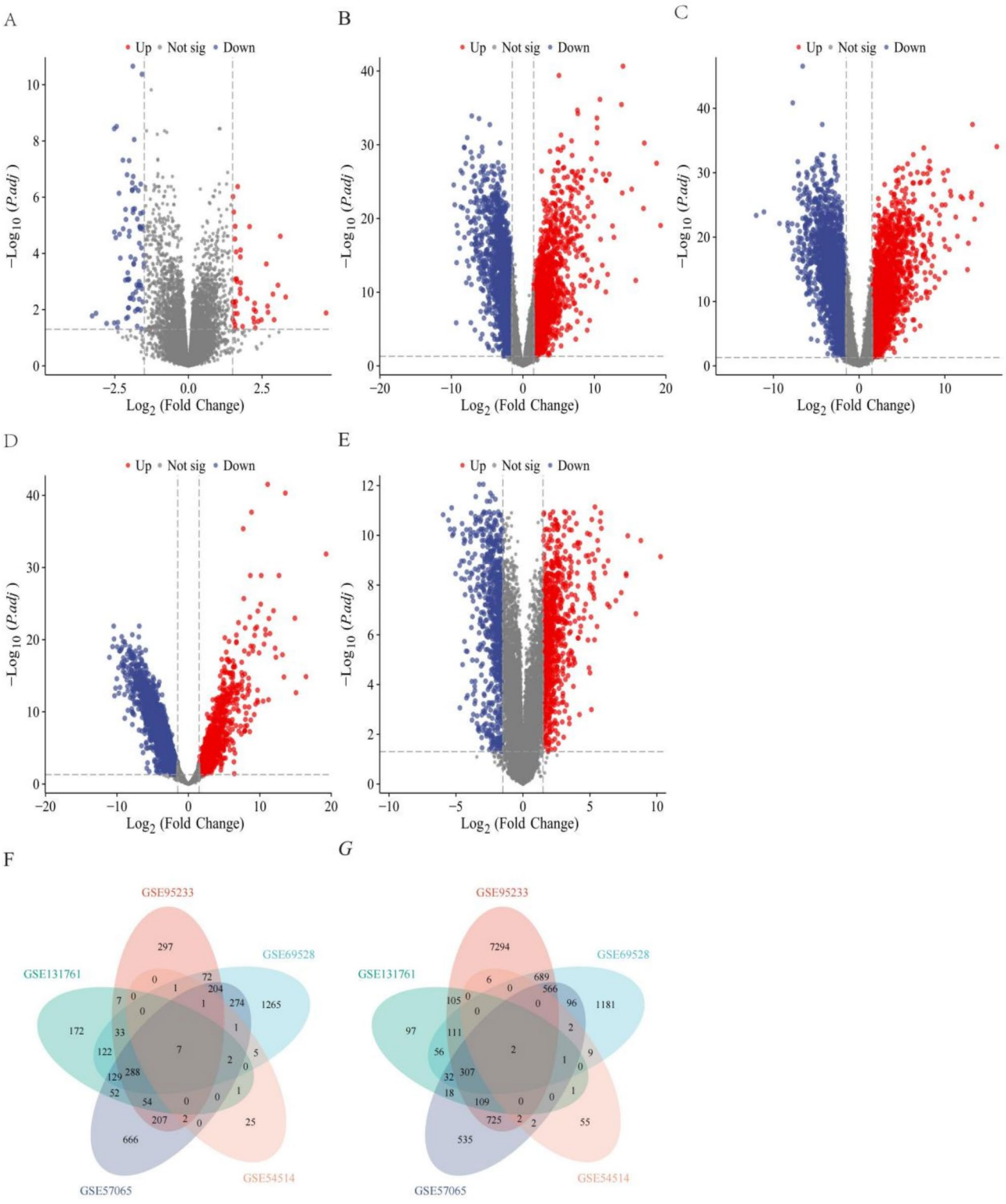

Fig. 1 Screening of DEGs and identification of Hub genes. Volcano plots of the DEGs in **(1A)** GSE54514, **(1B)** GSE57065, **(1C)** GSE69528, **(1D)** GSE95233, and **(1E)** GSE131761 transcriptome datasets. Red and blue dots indicate the up-and down-regulated DEGs, respectively. DEGs were identified based on adjusted *P*-values using the Benjamini-Hochberg procedure. Venn diagrams showing the co-differentially **(1F)** up-regulated, and **(1G)** down-regulated DEGs across the five datasets

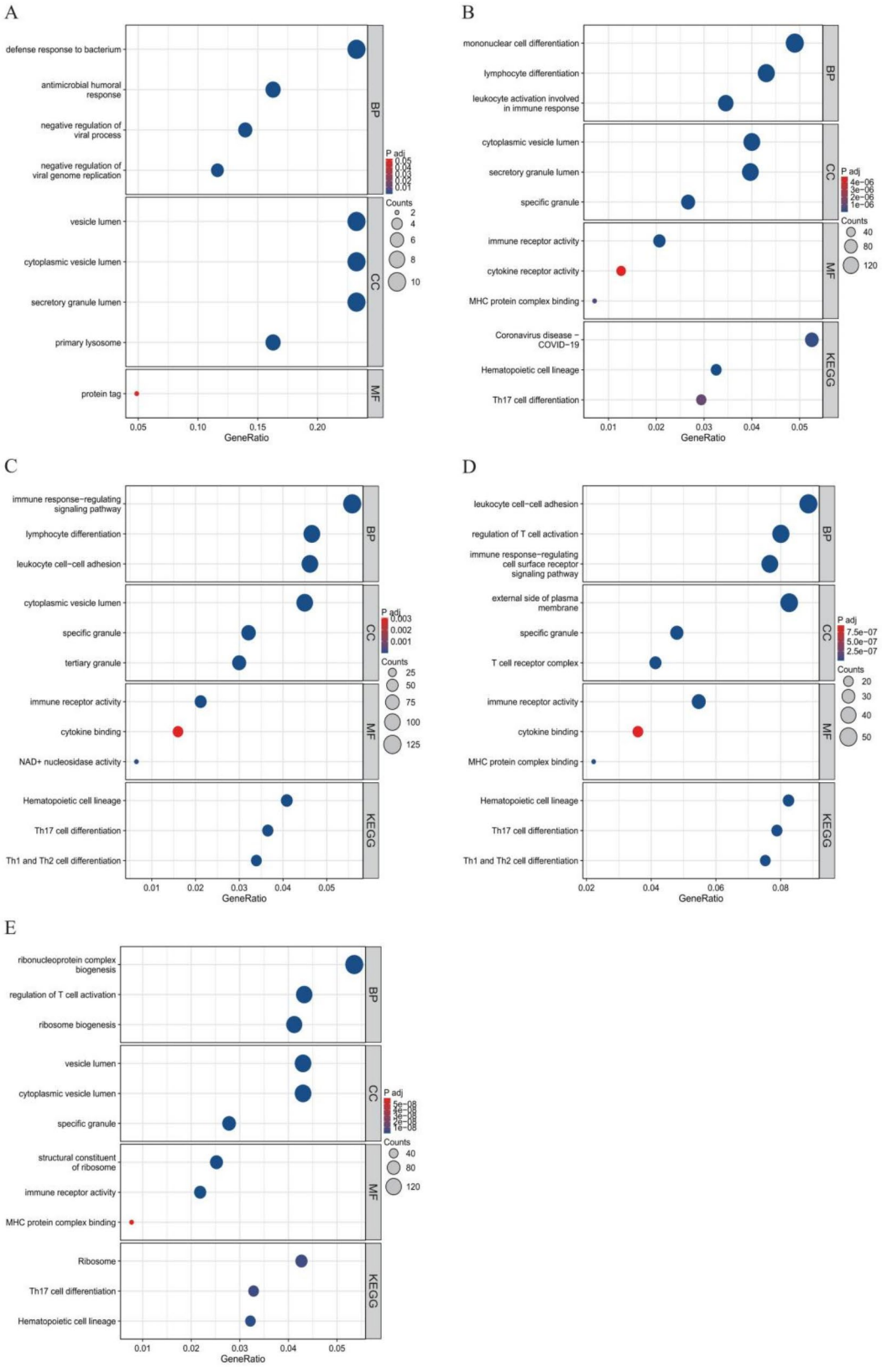

Fig. 2 (A-E) Bubble plots showing the enrichment of DEGs in biological processes (BP), cellular components (CC), molecular functions (MF), and KEGG pathways across different datasets. The X-axis represents the gene ratio, and the Y-axis shows the specific GO terms or pathways. Bubble size indicates the number of genes enriched in each term/pathway, and color represents the statistical significance (adjusted *P*-value). See Results for detailed information on the specific DEGs enriched in each term and dataset (Supplementary Tables 4–6)

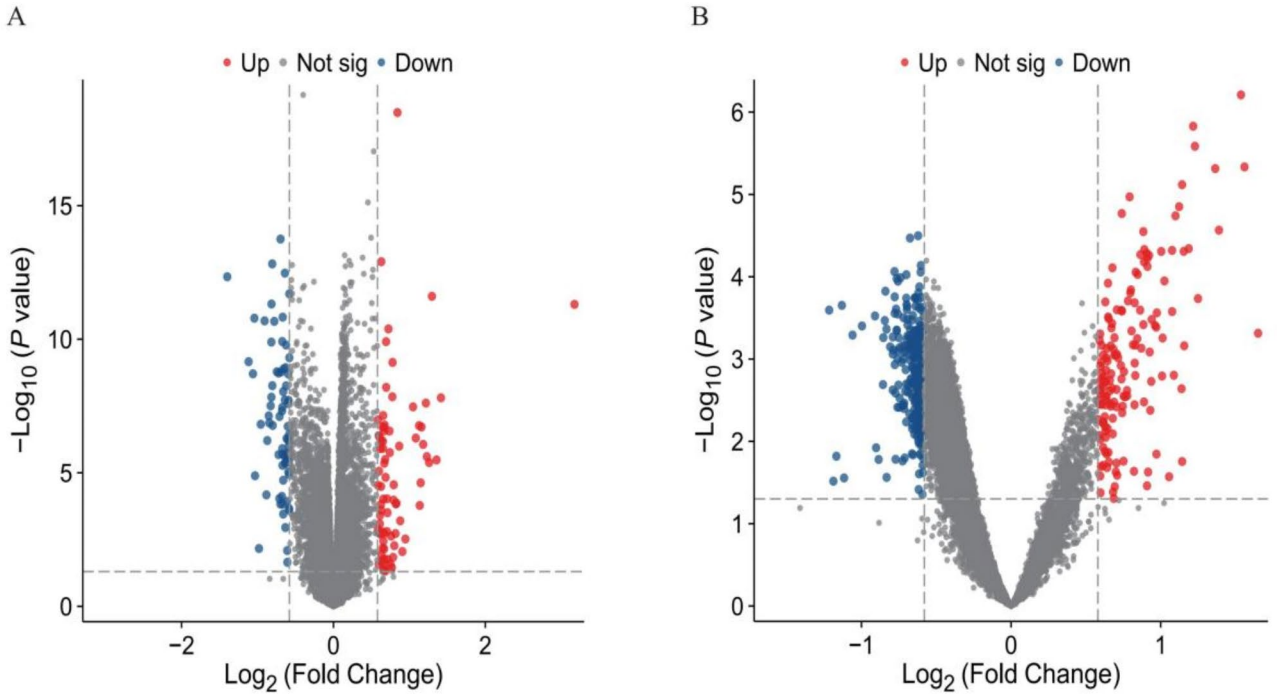

Fig. 3 Volcano plots Of the SD-DEGs between patients with better prognosis (control group) and patients with poor prognosis (experimental group) in **(3A)** GSE54514 and **(3B)** GSE95233 datasets. Red and blue dots indicate the up-regulated and down-regulated SD-DEGs, respectively. DEGs were identified based on adjusted *P*-values using the Benjamini-Hochberg procedure

Table 1 Differential expression of hub genes between patients with better and poor prognosis in GSE54514 and GSE95233 datasets

ID	GSE54514		GSE95233	
	log2FC	*P*-value	log2FC	*P*-value
RNASE2	0.393	0.027	0.546	0.546
RNASE3	0.668	0.007	0.971	< 0.001
CTSG	0.641	0.051	0.852	0.065
SLPI	0.221	0.235	0.127	0.636
TNFAIP6	-0.306	0.086	0.465	0.029
PGLYRP1	0.077	0.692	0.064	0.749
BLOC1S1	0.339	0.005	0.035	0.793
RPL10A	-0.274	0.101	-0.298	0.067
KLRB1	0.075	0.615	-0.374	0.110

correlated with the number of ICU days during the hospital stay and the duration of mechanical ventilation. Additionally, *RNASE3* was negatively correlated with WBC and monocyte count. *BLOC1S1* was negatively correlated with lymphocyte count. *CTSG* was negatively correlated with PCT, positively correlated with lymphocyte count, and negatively correlated with lymphocyte percentage. Meanwhile, *SLPI* and *TNFAIP6* were negatively correlated with monocyte and lymphocyte counts.

Moreover, *RNASE2* was negatively correlated with IL-2, IL-4, TNF-α, and IFN-γ. *BLOC1S1* was negatively correlated with IL-2 and IFN-γ and positively correlated with TNF-α. At the same time, *RPL10A* was negatively correlated with IL-2, IL-4, TNF-α, and IFN-γ. *SLPI* was negatively correlated with IL-2, =TNF-α, and IFN-γ and positively correlated with IL-6. Besides, *TNFAIP6* was negatively correlated with TNF-α (Supplement Fig. 1).

Discussion

With the development of medicine, researchers have done much research on sepsis. However, the mortality rate of septic patients is still very high. In 2017, approximately 48.9 million sepsis cases were reported worldwide, leading to 11 million sepsis-related deaths, accounting for nearly 20% of all global deaths, which accounted for approximately 22.5% of the global sepsis-related mortality rate. Sepsis incidence and mortality rates vary significantly across different regions, with the majority of cases and deaths occurring in low- and middle-income countries [11]. Currently, sepsis diagnosis and prediction mainly depend on clinical indexes. Although many studies have been on sepsis-related biomarkers, none yielded the accuracy required in clinical practice [12]. Therefore, exploring the molecular mechanism underlying sepsis occurrence and unravelling new biomarkers is important to provide new insights into sepsis prevention and management.

In this study, *RNASE2*, *RNASE3*, *CTSG*, *SLPI*, *TNFAIP6*, *PGLYRP1* and *BLOC1S1* were differentially highly expressed in septic patients. Additionally, *RPL10A* and *KLRB1* were differentially expressed in

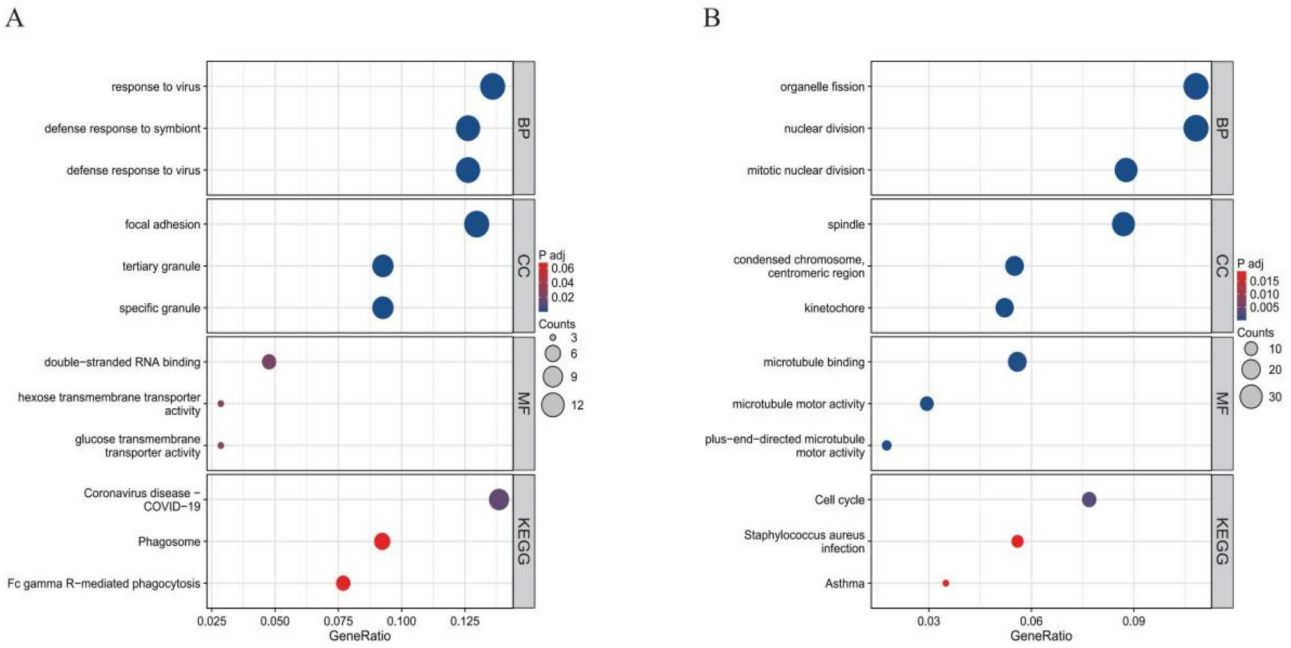

Fig. 4 **(A-B)** Bubble plots showing the distribution of DEGs enriched in BP, CC, MF and KEGG pathways in septic patients. The x-axis represents the gene ratio, and the y-axis represents the GO term

septic patients based on datasets GSE54514, GSE57065, GSE69528, GSE95233 and GSE131761. *RNASE2* and *RNASE3*, mainly related to substance storage and transport, were regulated by 6 DEGs enriched in CCs. Additionally, 3 DEGs enriched in BPs and 6 DEGs enriched in CCs regulated the co-differential expression of *RNASE3*, which is mainly related to immune responses in the body in patients with poor prognosis. Enrichment analysis was not performed in this study. However, based on existing literature, *RNASE2* has been identified as an endogenous ligand for Toll-like receptor 2 (TLR2), which drives the T helper 2 (Th2) response [13–15]. TLR2 is a key molecule in modulating the immune system and is involved in the sepsis mechanism. Besides, *RNASE3* is mainly involved in nucleolysis, cell binding, lipid instability, cytotoxicity, and antimicrobial activity, and it plays an immunomodulatory role in host defence [16, 17].

On the other hand, *SLPI*, mainly involved in the body immune response, substance storage and transport, was regulated by 2 DEGs enriched in BPs and 5 DEGs enriched in CCs. *SLPI* reduces excessive inflammatory response and may be used as a therapeutic strategy against sepsis [18]. *PGLYRP1*, also mainly involved in immune response, substance storage and transport, was regulated by 7 and 6 DEGs enriched in BPs and 6 CCs, respectively. *PGLYRP1* recognizes and binds the peptidoglycan of Gram-positive bacteria, the outer membranes of Gram-negative bacteria, and lipopolysaccharides (LPS), where it plays an important role in regulating the innate immune response [19]. Although there are no reports on the relationship between *RNASE3*, *PGLYRP1*, and the sepsis mechanism of action, previous studies have revealed the function of these two genes, which suggests some relationship between them and the sepsis mechanism of action.

Furthermore, qRT-PCR confirmed the bioinformatics analysis of 9 Hub genes except *CTSG* (no difference in expression), which were differentially expressed between healthy donors and septic patients. Interestingly, *RNASE3* was not highly expressed in patients with poor prognoses compared to those with good prognoses. However, *KLRB1* expression was lowly expressed in patients with poor prognosis. *KLRB1* (Killer cell lectin-like receptor subfamily B1), also known as killer cell lectin-like receptor B1, is a gene encoding CD161, which is mainly expressed on the surface of granulocytes and mononuclear cells [20]. In NK cells, it serves as an inhibitory receptor, inhibiting the cytotoxicity of NK cells by binding its ligand LLT1 (*CLEC2D*), which prevents the release of IFN-γ, potentially related to inflammation. The function of *KLRB1* is especially important in the context of sepsis, particularly during the "late stage", where it is associated with immune suppression. In this stage, the number of T cells and NK cells is decreased, and apoptosis is increased, accompanied by decreased production of IFN-γ, leading to secondary infection and even death [21, 22].

Furthermore, the downstream signals associated with CD161 activation produce different effects in NK and T cells. On one hand, the intracellular domain

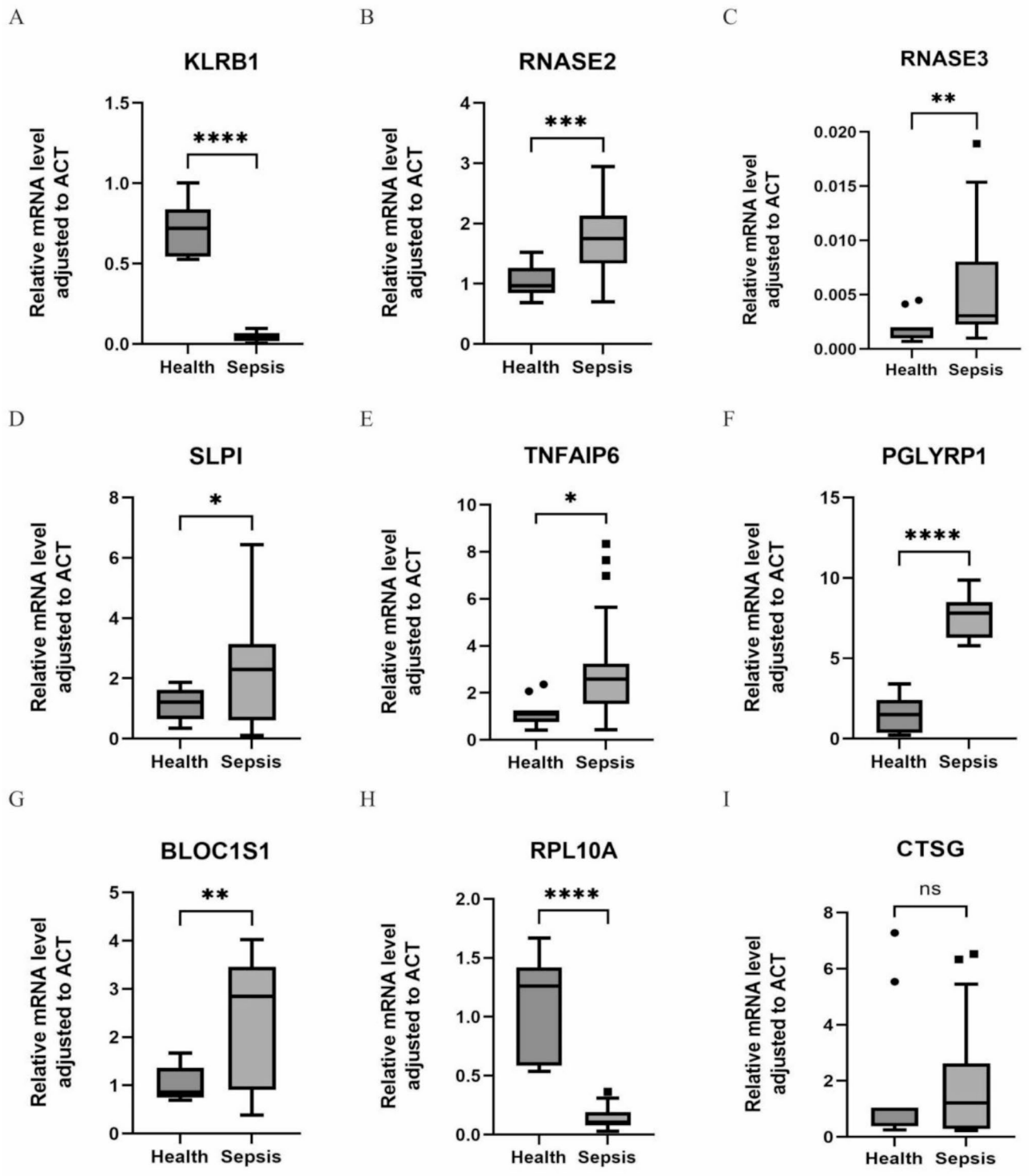

Fig. 5 **(A-I)** Box line diagrams showing the qRT-PCR validation of the differential expression of 9 Hub genes between healthy donors and septic patients. **(5J)**: Box line plot of the qRT-PCR validation of the differential expression of 9 Hub genes between patients with better and poorer prognoses. *Indicates $P < 0.05$, ** Indicates $P < 0.01$, *** Indicates $P < 0.001$, and **** Indicates $P < 0.0001$

activates neurophospholipase (ASM, SMPD1) by combining with CD39 and up-regulating the intracellular ceramide through STAT3 and mTOR. On the other hand, it activates AKT1/PKB, RPS6KA1/RSK1 kinases and downstream signals [23]. For example, mitochondrial STAT3 exacerbates LPS-induced sepsis by promoting CPT1α-mediated fatty acid oxidation [24]. Besides, there are significant changes in glycolysis and mTOR/

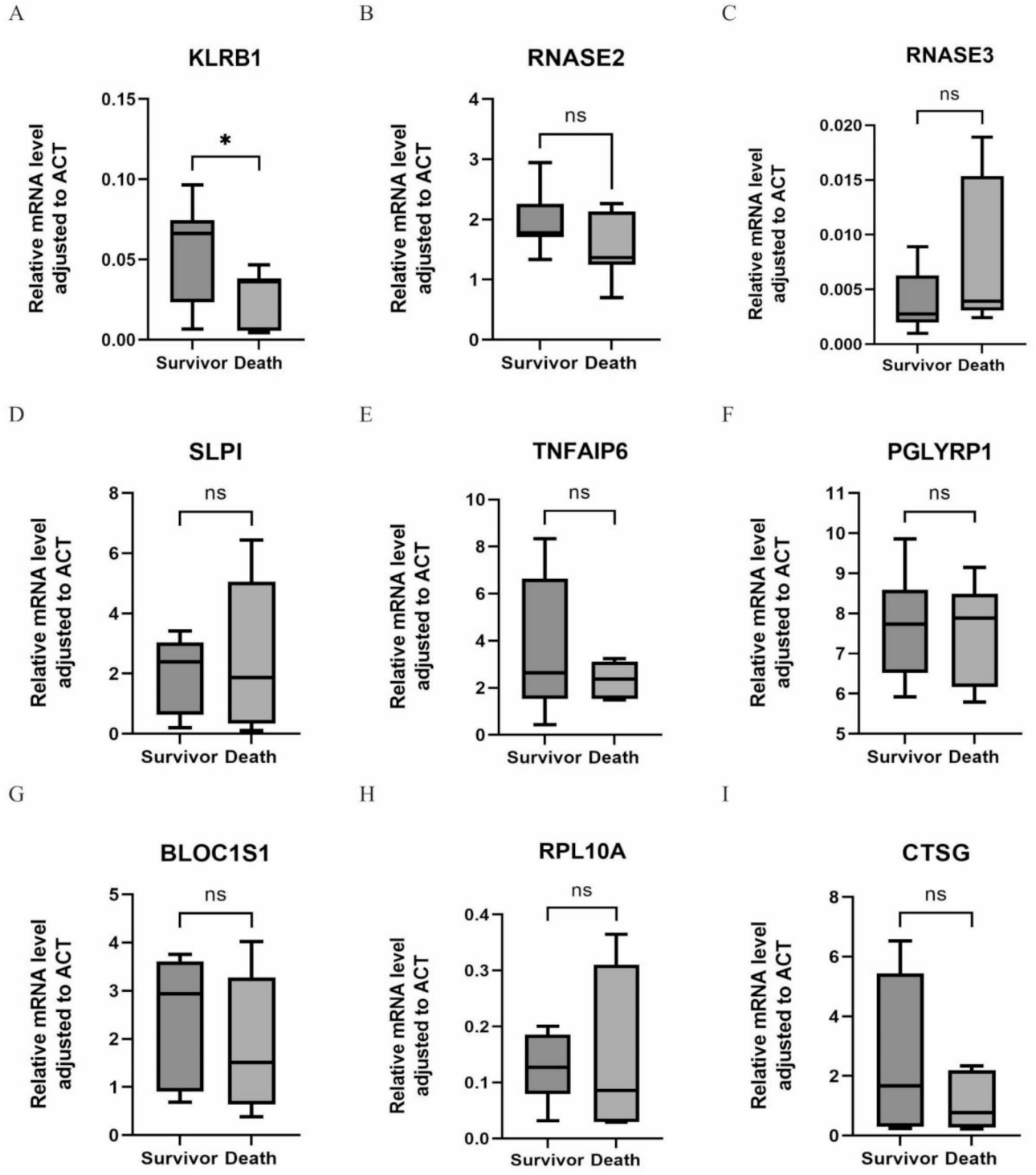

Fig. 6 **(A-I)** Box line plot of the qRT-PCR validation of the differential expression of nine Hub genes between patients with better and poorer prognoses. *Indicates $P < 0.05$, ns Indicates No significance

HIF-1α signalling pathway in septic patients [25]. At the same time, the ceramide kinase (*CERK*) gene knockout increases the serum levels of several IIR molecules (including cytokines and chemokines) and aggravates sepsis-like response in septic model mice treated with LPS [26]. However, *KLRB1* shows reduced expression in sepsis [27], there is no detailed description of the *KLRB1* mechanism of action in sepsis. Besides, there is no report on the relationship between *KLRB1*, *STAT3*, *mTOR*,

ceramide and sepsis, which should be the focus of future studies.

APACHE II score, ICU hospitalization days, mechanical ventilation time, laboratory infection index and cytokine concentration are related to the severity and prognosis of sepsis [28, 29]. Herein, the motifs related to clinical indexes were negatively correlated with most clinical indexes. At the same time, *TNFAIP6*, *RNASE3*, *BLOC1S1* and *SLPI* were positively correlated with some clinical indexes. However, given the small sample size, this correlation can only be used as a reference.

Additionally, here were some limitations in this study. Firstly, *KLRB1* was differentially expressed between septic patients with better and poorer prognosis. However, its function and pathway were not established in this study. Therefore, future experiments should explore the function and pathway of this gene in the pathology of sepsis, both in vitro and in vivo. Secondly, the correlation analyses conducted in this study were exploratory in nature and based on a limited sample size. As such, these findings should be considered preliminary, and further validation with larger cohorts is necessary to confirm these associations. Thirdly, given that this is a single-center study with a small sample size, the conclusions drawn should be treated as preliminary, and further validation with larger cohorts is required. The current findings should be cautiously interpreted, as the sample size may not provide sufficient statistical power. A larger cohort of sepsis patients is essential to confirm the diagnostic and therapeutic value of the identified genes.

Future studies should further investigate the specific function of *KLRB1* in sepsis through in vivo or in vitro experiments. This includes verifying the mechanism by which *KLRB1* regulates IFN-γ release via the STAT3/mTOR signaling pathway and its specific role in immune response and cell survival. Additionally, it is important to elucidate the broader functional network involving *KLRB1* and other genes identified in this study to better understand their collective impact on sepsis pathology. Although our current findings suggest the differential expression of *KLRB1*, *RNASE2*, *RNASE3*, and other hub genes, future research is necessary to confirm these interactions and their regulatory mechanisms.

Conclusion

Nine Hub genes (*KLRB1*, *RNASE2*, *RNASE3*, *CTSG*, *SLPI*, *TNFAIP6*, *PGLYRP1*, *BLOC1S1* and *RPL10A*) closely related to sepsis were detected from the DEGs intersection of each data set. The Hub genes identified in this study, particularly *KLRB1*, outline the molecular underpinnings of sepsis and candidate gene targets for sepsis detection and treatment.

Abbreviations

ICU	Intensive care unit
DEGs	Differentially expressed genes
qRT-PCR	Real-time quantitative polymerase chain reaction
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
SD-DEGs	Survival Difference Differentially Expressed Genes
GEO	Gene Expression Omnibus
BP	Biological processes
CC	Cellular components
MF	Molecular function
SOFA	Sequential [Sepsis-related] Organ Failure Assessment
APACHE II	Acute Physiology and Chronic Health Evaluation II
WBC	White blood cell
PCT	Procalcitonin
CRP	C-reactive protein
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-10	Interleukin-10
IFN-α	Interferon-α
IFN-γ	Interferon-γ
ANOVA	One-way analysis of variance
RNASE2	Ribonuclease A family member 2
RNASE3	Ribonuclease A family member 3
CTSG	Cathepsin G
SLPI	Secretory leukocyte peptidase inhibitor
TNFAIP6	TNF alpha induced protein 6
PGLYRP1	Peptidoglycan recognition protein 1
BLOC1S1	Biogenesis of lysosomal organelles complex 1 subunit 1
RPL10A	Ribosomal protein L10a
KLRB1	Killer cell lectin like receptor B1
TLR2	Toll-like receptor 2
Th2	T helper 2
CERK	Ceramide kinase

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-10818-5.

Supplementary Material 1

Author contributions

W.C. conceived the idea for the study, conducted the experiments, analyzed the data, created Figs. 5 and 6, and wrote and revised the manuscript. C.H.R. analyzed the data, created Figs. 1, 2, 3 and 4, and Table 1, and participated in the writing and revision of the manuscript. D.J.Q and T.X.Y. provided guidance on the experimental methods. Y.D. provided guidance on the statistical analysis. X.Y.P. and L.X.M. offered guidance on the manuscript and provided funding for the study.

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Data availability

All transcriptomic data and single cell transcriptome data and their corresponding clinical information used in this study can be obtained in Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database. GSE54514 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54514), GSE57065 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57065), GSE69528 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE69528), GSE95233 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE95233), and GSE131761 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE131761 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE167363).

Declarations

Ethics approval and consent to participate
The study protocol was approved by the ethics committee of Lianyungang Clinical College of Nanjing Medical University (approval number: LCYJ2022031801). The study was conducted in compliance with the Declaration of Helsinki. Written informed consent was obtained from the legal representatives of all patients prior to the commencement of the study. Patient records and information were anonymized and de-identified before analysis.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Patient consent for publication
Written informed consent was obtained from the legal representatives of all patients prior to the commencement of the study. Patient records and information were anonymized and de-identified before analysis.

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