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LINC02363: a potential biomarker for early diagnosis and treatment of sepsis

Linghan Leng[1], Hao Wang[2], Yingchun Hu[3*] and Li Hu[3*]

Abstract

Background Sepsis remains a leading cause of global morbidity and mortality, yet early diagnosis is hindered by the limited specificity and sensitivity of current biomarkers.

Aim The aim of this study was to identify lncRNAs that play a key role in sepsis and provide potential biomarkers for the diagnosis and treatment of sepsis.

Methods Transcriptomic data from sepsis patients were retrieved from the Chinese National Genebank (CNGBdb). Differential expression analysis identified 2,348 LncRNAs and 5,125 mRNAs ($|FC| \geq 2$, $FDR < 0.05$). Weighted gene co-expression network analysis (WGCNA) and meta-analysis were applied to screen core genes. Gene set enrichment analysis (GSEA) explored functional pathways, while single-cell sequencing and qPCR validated cellular localization and expression patterns.

Results WGCNA identified three key genes: LINC02363 (LncRNA), DYNLT1, and FCGR1B. Survival and meta-analyses revealed strong correlations between these genes and sepsis outcomes. GSEA highlighted LINC02363's involvement in "herpes simplex virus type 1 infection," "tuberculosis," and ribosome pathways. Single-cell sequencing showed FCGR1B's broad distribution across immune cells, while DYNLT1 localized predominantly in macrophages. qPCR confirmed significant upregulation of LINC02363 ($p < 0.01$), FCGR1B ($p < 0.05$), and DYNLT1 ($p < 0.05$) in sepsis patients compared to controls.

Conclusion LINC02363 may serve as a new biomarker for the diagnosis and treatment of sepsis.

Keywords Sepsis, ScRNA-seq, QPCR, LncRNA, Prognosis

Introduction

Sepsis, a critical condition of organ dysfunction caused by an uncontrolled host response, manifests most severely as septic shock [1]. Sepsis remains the leading cause of morbidity and mortality worldwide [2]. Early detection and timely management of sepsis has been shown to improve prognosis [3]. Biomarkers for the diagnosis of sepsis may enable early intervention and may reduce the risk of death [4]. Currently, the identification of sepsis primarily depends on clinical symptoms and traditional biomarkers, such as C-reactive protein (CRP) and procalcitoninogen (PCT), which have insufficient specificity and sensitivity for early intervention and treatment [5].

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Therefore, there is an urgent need to seek more effective biomarkers to improve the diagnostic accuracy and prognostic assessment of sepsis.

In recent years, the role of long non-coding RNAs (lncRNAs) in a variety of biological processes has gained increasing attention, including their functions in immune regulation and inflammatory responses [6]. LncRNAs, which are generally longer than 200 nucleotides and do not encode proteins, regulate cellular functions and behaviors by modulating gene expression at the epigenetic, transcriptional, and post-transcriptional levels, thus playing a key role in the regulation of immune responses and inflammation [7]. Relatively few studies have been conducted on LINC02363, which are potentially crucial in the pathological process of sepsis or serve as a novel biomarker. While the precise mechanism of LINC02363 remains unclear, drawing on the findings from current research, it may be involved in the inflammatory response by affecting the function of immune cells or regulating specific signal transduction pathways. The exploration of these mechanisms not only contributes to the understanding of the role of LINC02363 in sepsis, but also offers the possibility of further developing new therapeutic strategies.

The aim of this study was to investigate the possibility of LINC02363 as a new biomarker for sepsis and whether it can provide more accurate and earlier diagnostic information than existing biomarkers. In addition, investigating the function of LINC02363 could help to reveal new molecular mechanisms of sepsis and offer the possibility of developing new therapeutic targets. This study lays the foundation for further research on the expression pattern, regulatory mechanism and its clinical significance of LINC02363 in sepsis. The study's flow chart is depicted in Fig. 1.

Methodology

Data source

The raw data of sepsis were obtained from the National Genebank of China (CNGBdb), database number CNP0002611.

Between December 2018 and December 2019, venous blood samples were collected from 23 sepsis patients and 10 healthy volunteers at the Affiliated Hospital of Southwest Medical University. The study was approved by the hospital ethics committee and followed the Declaration of Helsinki. All participants signed an informed consent form. Blood samples were collected within 24 h of admission, while healthy volunteers had their blood samples collected during a routine physical examination during the same period. Patients with severe organ failure, immune system or haematological disorders, and pregnant and lactating women were excluded. (Ethical approval number: ky2018029, clinical trial number: ChiCTR1900021261. Registration Date: December 27, 2018).

Screening of differentially expressed RNAs

In transcriptome data analysis using the online tool iDEP97 (http://bioinformatics.sdstate.edu/idep97/), data accuracy was first ensured through a rigorous data quality control process [8]. Principal component analysis (PCA) was used for further dimensionality reduction processing and identification of outlier samples, which ensured the analysed data completeness and consistency. Finally, differential expression analysis was performed by the DEseq2 method, establishing the thresholds with a minimum fold change (FC) of 2 and a false discovery rate (FDR) below 0.05, to screen out significant differentially expressed RNAs and LncRNAs.

WGCNA and the identification of core genes

In this study, a weighted gene co-expression network analysis [9] (WGCNA) approach was used to explore the co-expression patterns among genes to identify core genes that may be involved in specific biological processes. First, a gene expression data matrix was constructed and correlations between genes were calculated, which were then converted into a weighted neighbour-joining matrix. The connection strength of highly correlated gene pairs is enhanced by choosing an appropriate soft threshold β. A topological overlap matrix (TOM) is generated using the weighted neighbour-joining matrix to analyse the indirect interactions between genes to improve the stability of module detection. Identify multiple densely connected gene clusters (modules) that represent different sets of co-expressed genes by hierarchical clustering. Using the tool of OmicShare (http://www.omicshare.com/tools), a free online data analysis platform, genes within the key modules were analysed by intra-group correlation analysis to screen the key genes with LncRNAs, and finally, Spearman's correlation analysis was conducted with R language version 4.3.2 to further reveal the expression relationship between LncRNA and key genes, and scatter plots were drawn.

Survival curves and analysis of key gene expression

This study analysed the impact of core genes on the prognosis of patients with sepsis to reveal their prognostic value, based on the public dataset GSE65682 [10]. The GSE65682 dataset contains whole-blood transcriptome analyses from critically ill patients, and is designed to differentiate between infectious and non-infectious sources of critical etiology. In this study, genes were classified based on their expression levels in the dataset GSE65682. The top 50% of genes, which exhibited the highest expression levels, were categorized as the 'high expression group', while the bottom 50%, exhibiting the lowest expression levels, were categorized as the 'low expression

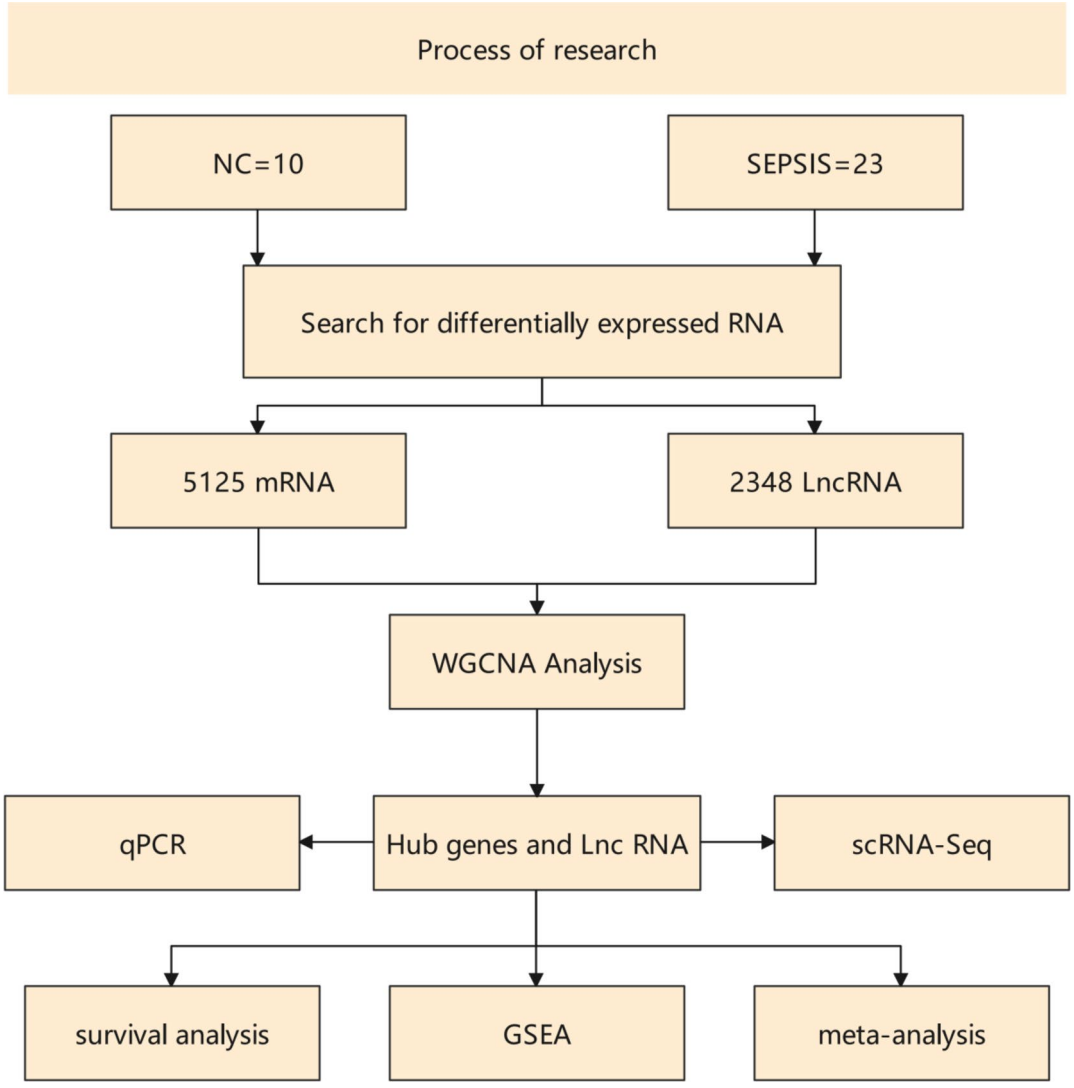

Fig. 1 Flowchart of this study. Firstly, sepsis data were obtained from the Chinese National Genebank (CNGBdb) and screened for LncRNAs and mRNAs by differential expression analysis. Key genes were identified from them using weighted correlation network analysis (WGCNA) and subjected to meta-analysis, survival analysis, gene-set enrichment analysis (GSEA), and single-cell sequencing, and finally validated by qPCR experiments

group'. This division was based on the median expression value of each gene across all samples. The upper 50% of the dataset, including 293 genes, was labeled as the high expression group, and the remaining 50% was identified as the low expression group. To ensure robustness, statistical analyses were conducted, and a p-value of 0.05 or lower was considered statistically significant. Additionally, this study compared the expression levels of key genes in the sepsis and non-sepsis groups using RNA sequencing data, aiming to identify specific markers associated with sepsis progression. A detailed examination of gene expression differences between these groups was performed to highlight the potential prognostic biomarkers for sepsis. The analysis was carried out using R version 4.3.2, and 'edgeR' package were used for differential gene expression analysis. For visualization, we utilized the 'ggplot2' package to generate box diagrams to present the gene expression patterns and the statistical significance of the results.

Meta-analysis

A systematic Meta-analysis approach was used in this study to assess the differences in biomarkers between sepsis survivors and non-survivors [11]. Differential expression of DYNLT1, FCGR1B in septic patients was assessed by combining datasets GSE54514 [12], GSE63042 [13], and GSE95233 [14]. The study was carefully screened and data such as sample size, mean and standard deviation were extracted from it. Meta-analysis was done to synthesise the data by using fixed effect model and random effect model, while inter-study

heterogeneity was assessed and measured using I^2 statistic and tau^2.

GSEA enrichment analysis

In this study, gene set enrichment analysis of long noncoding RNA (LINC02363) was performed using R language 4.3.2. First, genes and pathways associated with changes in LINC02363 expression were identified using gene ranking and arithmetic analysis. Next, Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed to identify the roles of these genes in biological processes, cellular composition, and molecular function [15–17]. Gene set enrichment analysis (GSEA) was used to identify key pathways enriched in upstream and downstream effects of LINC02363. The gene set enrichment analysis was performed to identify key pathways associated with LINC02363 expression changes [18].

Single-cell sequencing

In this study 10× single-cell sequencing was employed to analyse five peripheral blood samples (including 2 healthy controls, 1patients with systemic inflammatory response syndrome and 2sepsis) to determine the expression of specific target genes in different cell types. This method was performed in strict accordance with the manufacturer's protocol [19]. Quality control of the high-throughput sequencing data was performed by Cell Ranger software, while the Seurat software package was used for data downscaling, visualisation, and identification of marker genes [19]. The present study utilised single-cell transcriptome data to reveal the specific expression of key genes in cellular subpopulations, providing detailed information on cellular localisation for further functional studies.

Q-PCR experiments

The human monocytic leukaemia cell line THP-1 was selected to determine the trend of core gene expression in sepsis and for further in vitro experiments. Firstly, an appropriate amount of sample was mixed with RNAiso Plus reagent and left at room temperature for 5 min, emulsified with chloroform and then centrifuged at 4 °C. The supernatant was transferred to a new tube, isopropanol was added, centrifuged and the precipitate was washed with ethanol, dried and dissolved in RNA-free water. Next, the RNA samples were used for reverse transcription, and after adding gDNA Eraser to remove genomic DNA, PrimeScript™ RT reaction mixture was added and incubated at 37 °C to form cDNA. cDNA was used for qPCR, the reaction system was set up and predenatured and cyclic amplification was carried out, and finally, gene expression was quantified by the $2^{-\Delta\Delta Ct}$ method. The results of the experiments were visualised by drawing histograms using R language 4.3.2.

Results

Screening for differentially expressed RNA

PCA analysis showed clustering between healthy and sepsis groups with less overlap and no abnormal samples, and no outliers were found (Fig. 2A). Screening under|FC|≥2 and selected FDR < 0.05, we identified 2348 differentially expressed LncRNAs, comprising 351 that were up-regulated and 1997 that were down-regulated, along with 5125 differentially expressed mRNAs, of which 847 were up-regulated and 4278 were down-regulated. Figure 2B shows the map of the volcano distribution with the results of the differential expression analysis. The results of the difference screening are shown in Table 1.

Identification of WGCNAs and core genes

Selecting a soft threshold of 7 (Fig. 3C), WGCNA analysis showed a total of 5 modules of size 50 were detected, which were clustered based on similarity in gene expression patterns, with the brown module being significantly associated with the sepsis phenotype (Fig. 3A). Then, correlation analysis of the first 40 genes within this module

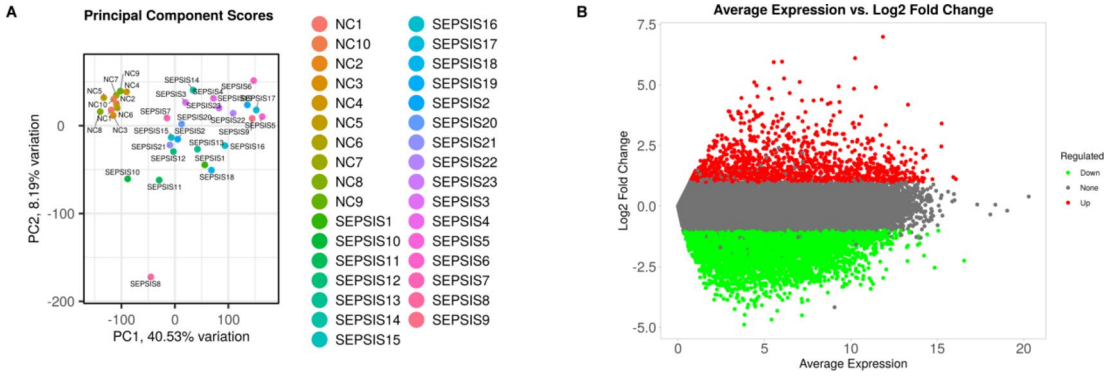

Fig. 2 Screening of differentially expressed RNAs. **A** clear clustering between NC and SEPSIS groups with less overlap to exclude outlier samples. **B** volcano plot depicting RNAs that are up-regulated (red) and down-regulated (green)

Table 1 Some differentially expressed genes

Symbol	Regulation	Ensembl ID	log2 Fold Change	Adj.Pval
KIR2DL2	Up	ENSG00000278731	4.583151861	4.40E-05
LRRC26	Up	ENSG00000184709	4.124426731	6.06E-12
KRT5	Up	ENSG00000186081	3.980313378	7.51E-10
PRSS40A	Up	ENSG00000183292	3.850634167	8.17E-06
NRCAM	Up	ENSG00000091129	3.848788633	2.28E-11
COL26A1	Up	ENSG00000160963	3.840018305	2.12E-03
KIR2DS2	Up	ENSG00000278152	3.746480352	1.06E-05
SHANK1	Up	ENSG00000161681	3.699594095	9.95E-11
SIAH3	Up	ENSG00000215475	3.67812245	2.65E-10
ZMAT4	Up	ENSG00000165061	3.675662444	1.06E-16
FCGBP	Up	ENSG00000281123	3.650036737	5.37E-18
CYP4F29P	Up	ENSG00000228314	3.609721105	9.56E-16
GAB4	Up	ENSG00000215568	3.589955411	2.62E-07
LINC02363	Up	ENSG00000180712	2.400287369	4.89E-08
SLC7A3	Up	ENSG00000165349	3.422025204	1.63E-06
PLD4	Up	ENSG00000166428	3.414740501	2.83E-18
EPHA2	Up	ENSG00000142627	3.377932793	7.07E-19
AJAP1	Up	ENSG00000196581	3.370309319	8.18E-11
HTR3A	Up	ENSG00000166736	3.348434461	2.07E-11
CLEC4F	Up	ENSG00000152672	3.288149579	3.40E-06
PRSS35	Up	ENSG00000146250	3.265263218	1.32E-06
KRT86	Up	ENSG00000170442	3.238981016	2.23E-09
FCGR1B	Up	ENSG00000198019	3.053445344	6.15E-19
DYNLT1	Up	ENSG00000146425	2.010380124	1.22E-13
IGFBP2	Down	ENSG00000115457	−3.705621596	7.24E-09
HPGD	Down	ENSG00000164120	−3.699902523	6.92E-09
SLCO4A1	Down	ENSG00000101187	−3.692964644	3.43E-11
RGL4	Down	ENSG00000159496	−3.690657135	1.33E-17
TWIST2	Down	ENSG00000288335	−3.676075729	2.96E-05
IL10	Down	ENSG00000136634	−3.674901635	2.99E-12
HLA-J	Down	ENSG00000243336	−3.672514436	5.08E-03
TYMSOS	Down	ENSG00000176912	−3.661096681	1.31E-04
OPLAH	Down	ENSG00000178814	−3.637983149	2.87E-14
TNFAIP6	Down	ENSG00000123610	−3.617129131	7.95E-23
KCNE1B	Down	ENSG00000276289	−3.606469024	3.68E-11
NDNF	Down	ENSG00000173376	−3.583404025	1.02E-03
CA1	Down	ENSG00000133742	−3.580295933	8.97E-06
HS3ST5	Down	ENSG00000249853	−3.53435731	1.13E-04
FCGR1A	Down	ENSG00000150337	−3.52457649	5.35E-22
HPD	Down	ENSG00000158104	−3.519246462	2.91E-05
LRRN1	Down	ENSG00000175928	−3.518913963	6.01E-17
CACNA1E	Down	ENSG00000198216	−3.514025203	5.42E-15
SAMSN1	Down	ENSG00000155307	−3.513932728	1.41E-16
DACH1	Down	ENSG00000276644	−3.506047036	1.66E-19
FCGR1CP	Down	ENSG00000265531	−3.501567968	9.37E-18
CDK1	Down	ENSG00000170312	−3.49479646	2.56E-08

showed that LINC02363 exhibited strong correlation with DYNL1 and FCGR1B (Fig. 3B). Spearman correlation analysis showed that the expression of LINC02363 was positively correlated with that of DYNLT1 (Fig. 4A) (Spearman $R = 0.58$, P-value = 0.000372) and a stronger positive correlation (Spearman $R = 0.75$, P-value = 5.43e-07) with the expression of FCGR1B (Fig. 4B).

Survival curve analysis

To delve deeper into the connection between the crucial genes and the prognosis of patients with sepsis, this

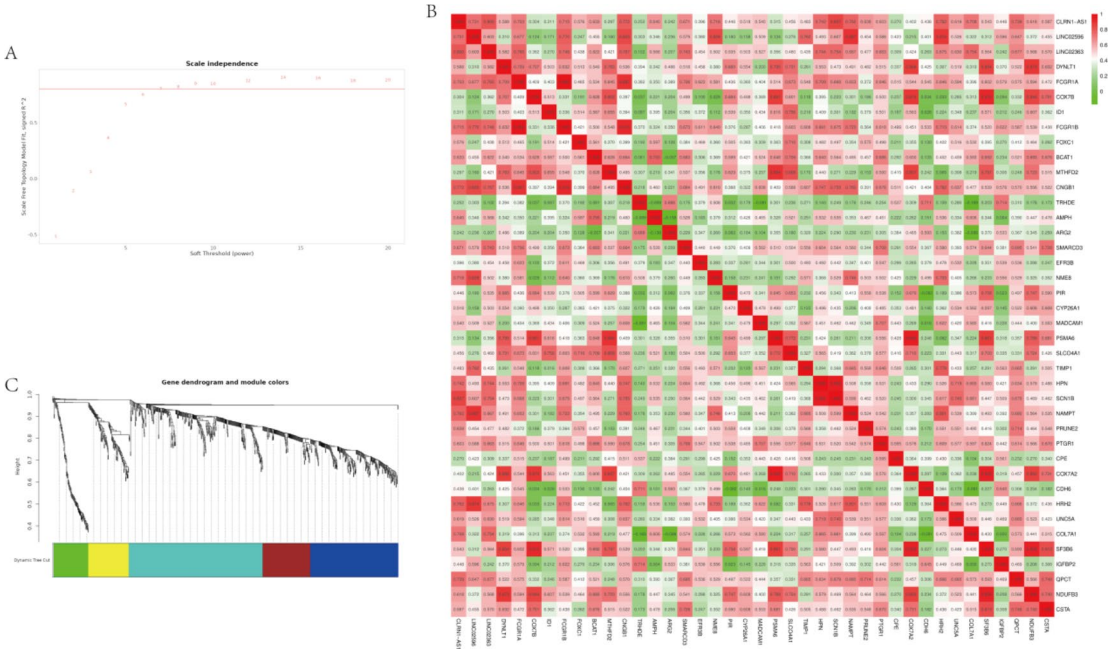

Fig. 3 WGCNA. **A** A soft threshold of 7 was chosen. **B** Correlation analysis between the top 40 genes in the brown module. Pearson's coefficient is usually used to calculate the correlation for continuous data, and Pearson's correlation coefficient of > 0.5 represents a significant correlation. **C** Modules based on overlapping topologies of co-expression of different coloured mRNAs (module size = 50). The brown module was significantly associated with clinical features of sepsis

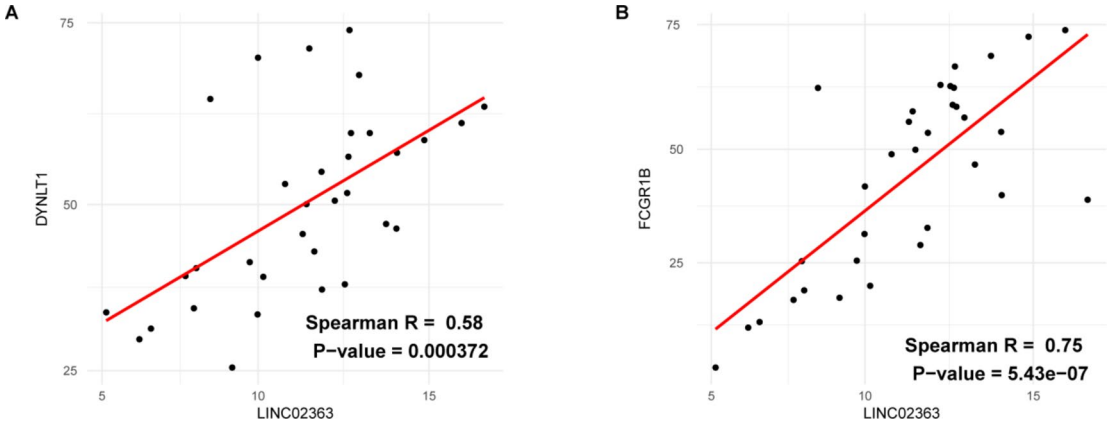

Fig. 4 Scatter plot. **A**, **B** LINC02363 showed positive correlation with the expression of DYNLT1 and FCGR1B, where *P*-value < 0.05

study conducted survival curve analysis of the above key genes in conjunction with the public database GSE65682 (Fig. 5A, B) [20]. DYNLT1 expression was negatively correlated with survival, indicating poor prognosis, whereas FCGR1B expression was positively correlated with survival, suggesting a potential protective role. These two genes may serve as biomarkers for predicting survival in sepsis patients. In addition, DYNLT1, FCGR1B were highly expressed in the sepsis group (Fig. 5C, D).

Meta-analysis

Meta-analysis of DYNLT1, FCGR1B showed that these genes exhibited different expression patterns between sepsis survivors and non-survivors (Fig. 6A, B). DYNLT1 expression was relatively higher in sepsis non-survivors, with a standardised mean difference (SMD) of −0.28 under the random-effects model with a 95% confidence interval of [−0.64, −0.90], and FCGR1B expression was higher in sepsis survivors, with an SMD for the difference in expression of 0.44 and a 95% confidence interval of [−0.02, 0.90]. Overall, these results indicate that these two genes may play different roles in biomarker studies of sepsis.

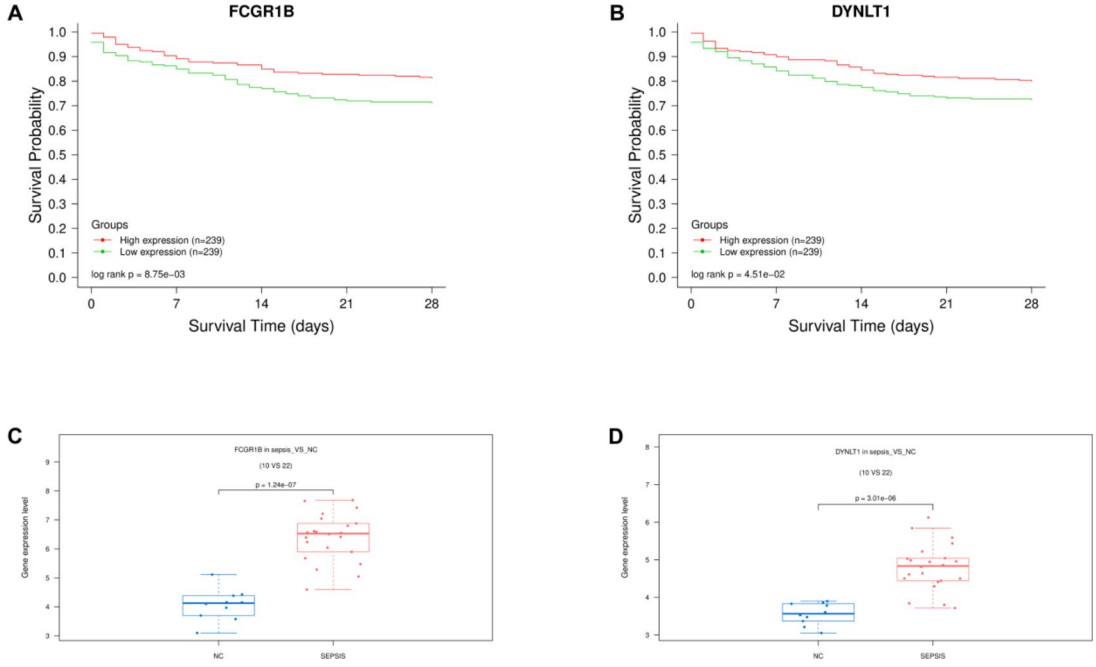

Fig. 5 Prognostic analysis and expression of key genes. **A**, **B** Survival time in days is shown on the horizontal axis and survival rate on the vertical axis. The green line corresponds to low mRNA samples while the red line represents high mRNA samples. **C**, **D** Box line plots show that DYNLT1, FCGR1B were highly expressed in the sepsis group

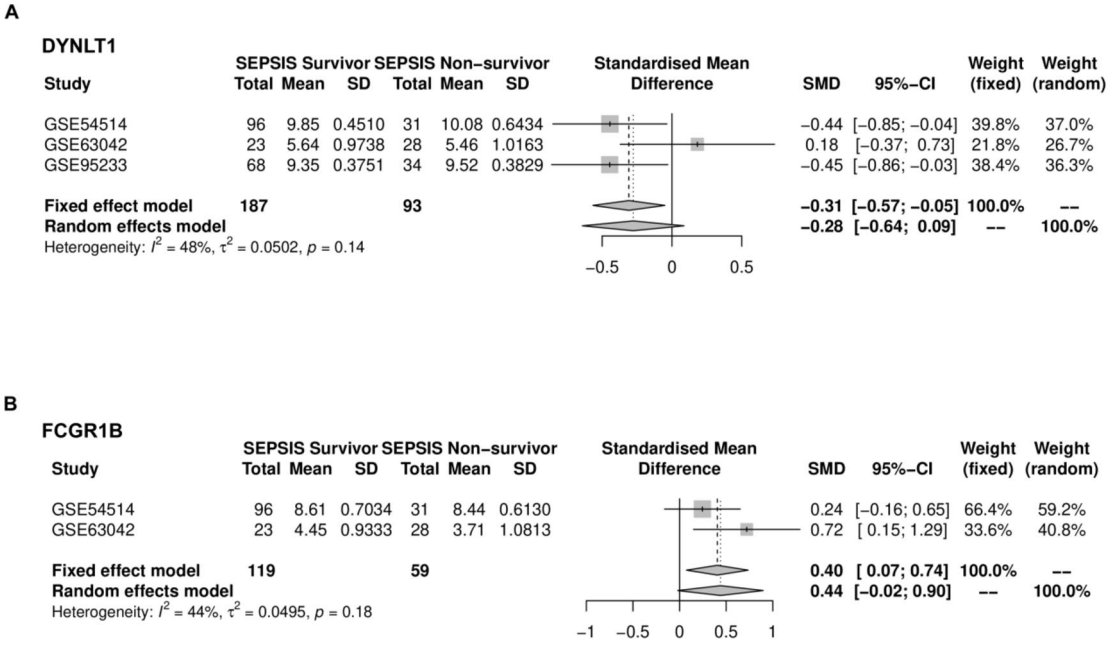

Fig. 6 Meta-analysis results. **A**, **B** meta-analysis of the expression of DYNLT1, FCGR1A in the sepsis group versus the normal group in the GSE54514, GSE95233 and GSE63042 datasets

GSEA enrichment analysis

In this study, GSEA enrichment analysis of LINC02363 showed that this LncRNA was significantly expressed in the gene sets related to 'Herpes simplex virus 1 infection' and 'Tuberculosis'. The significant enrichment of this LncRNA in 'Herpes simplex virus 1 infection' and 'Tuberculosis'-related gene sets suggests that LINC02363 may act a part in the regulation of inflammation and immune responses, and its significant enrichment in ribosomal function and oxidative phosphorylation pathways suggests that LINC02363 plays a regulatory role in cellular stress responses and energy metabolism (Fig. 7A,

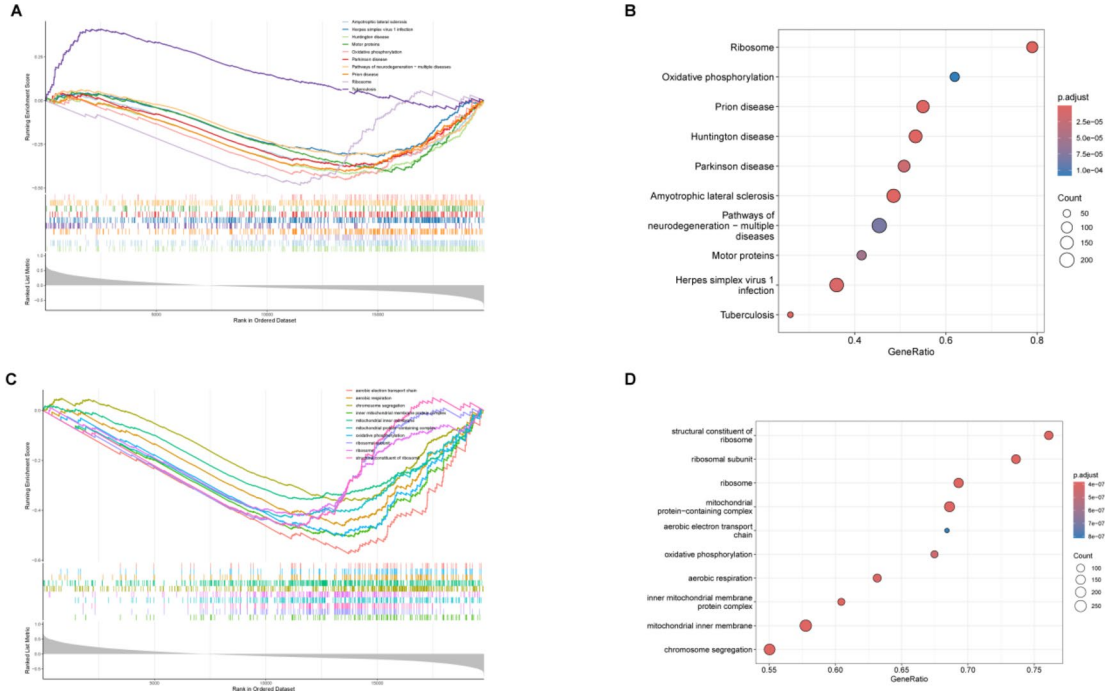

Fig. 7 Results of GSEA enrichment analysis. **A**, **C** Spikes in the enrichment score (ES) curves reveal the tendency of these gene sets to concentrate in the sequential gene list, suggesting their potential activity in the study state. **B**, **D** Bubble plots further corroborate the results of the enrichment analysis, GeneRatio shows the proportion of significant genes in a given functional term, p.adjust is a *p*-value corrected for multiple hypothesis testing to control for false positive rates, and Count represents the number of significant genes in a given functional term

B). In addition, a significant positive enrichment of gene sets linked to cellular component, bioprocess, and molecular functions was observed in the GSEA enrichment analysis (Fig. 7C, D). These findings reveal that LINC02217 may play an important role in inflammatory response, immune regulation, and regulation of ribosomal function.

Single-cell sequencing

Based on the transcriptome sequencing analyses of the five samples described above, nine groups of cells were screened after dimensionality reduction clustering and the expression of the nine groups of cells was plotted as a template, with each region corresponding to a different cell type (Fig. 8A). Figure 8B shows the percentage composition of different cell populations in the five samples. The height of each bar represents the proportion of each cell cluster (clusters) in that sample, and the color corresponds to the different cell clusters, the specific cell cluster numbers are shown in the figure. The proportion of each cell cluster reflects the distribution of different cell populations in the sample. Next, cellular localisation of DYNLT1 and FCGR1B was performed, and DYNLT1 was found to be widely distributed in all immune cells, and FCGR1B was mainly localised in macrophage cell lineage (Fig. 8C, D).

Q-PCR experiments

In quantitative polymerase chain reaction (qPCR) experiments, the present study compared the gene expression differences between the sepsis group and the healthy control group. qPCR experiments showed that the expression levels of FCGR1BP, LINC02363, and DYNLT1 were significantly up-regulated in the sepsis group as compared to the control group (Fig. 9). This suggests that FCGR1BP, LINC02363 and DYNLT1 expression regulation might have a significant function in the pathological process of sepsis. Table 2 displays the specific primer sequences.

Discussion

Sepsis is a complex clinical syndrome involving multiple immune responses and molecular mechanisms [21]. Epidemiological data show that the morbidity and mortality of sepsis remain high worldwide [22]. Existing studies have highlighted the importance of early diagnosis and prompt treatment of sepsis, noting that prompt treatment significantly improves the survival of patients with sepsis [23]. However, the diagnosis of sepsis is a complex process and early diagnosis of sepsis is made particularly difficult by the non-specificity of early symptoms of sepsis. There is still a lack of clinical biomarkers that can rapidly and accurately diagnose sepsis. Previous studies have found that long non-coding RNAs play key roles

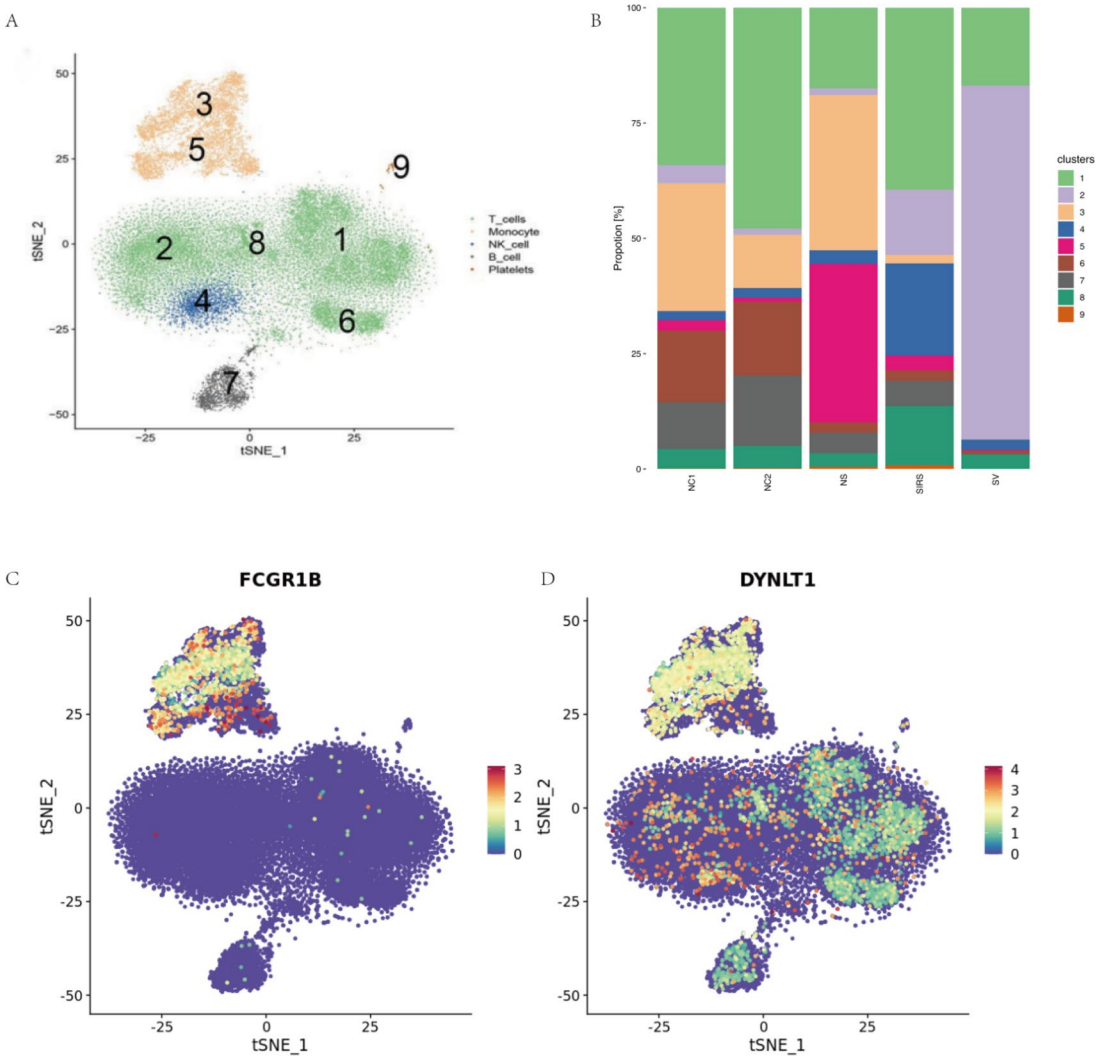

Fig. 8 Cell lineage localisation of key genes. **A** Groups 3 and 5 are macrophages, Group 4 represents natural killer cells, Groups 1, 2, 6 and 8 are T cells. B cells represent Group 7 and Group 9 represents platelets. **B** illustrates the differences in the distribution of cell populations in each sample. **C**, **D** show that DYNLT1 is widely distributed in all immune cells and that FCGR1B is predominantly localised in macrophage cell lineages

in immune regulation and inflammatory responses [24], suggesting that LncRNAs have the potential to be biomarkers for the prompt detection of sepsis.

In the diagnosis and management of sepsis, the application of biomarkers has been an important way to improve diagnostic accuracy and patient prognosis assessment [25, 26]. Currently, procalcitoninogen (PCT) and C-reactive protein (CRP) are commonly used clinical biomarkers for sepsis, with different advantages and limitations in terms of time, sensitivity, specificity and clinical application, respectively [27, 28]. PCT, as a rapid and sensitive marker of infection, is more prominent in its diagnostic performance in sepsis and septic shock, especially in the case of bacterial infection, with sensitivity and specificity [29]. The sensitivity and specificity were 74.4% and 86.7%, respectively. In contrast, CRP, as a traditional inflammatory marker, can be elevated in both infectious and noninfectious inflammation and lacks sufficient specificity, but has shown high sensitivity in some studies, especially in the early diagnosis of neonatal sepsis, with a sensitivity of 89.4% [30, 31]. Although PCT and CRP play an important role in the diagnosis of sepsis, their diagnostic efficacy is dependent on the patient's clinical status and stage of the disease and may show low sensitivity in early immune dysregulation. Therefore, the search for new biomarkers, especially those that can provide earlier diagnosis and have higher specificity, remains an important direction in sepsis research.

The potential of LINC02363, DYNLT1 and FCGR1B as emerging biomarkers in sepsis is being gradually explored. Unlike PCT and CRP, the combined detection of LINC02363, DYNLT1 and FCGR1B may provide an earlier and more specific diagnostic capability for sepsis, especially in the early stages of immune regulation

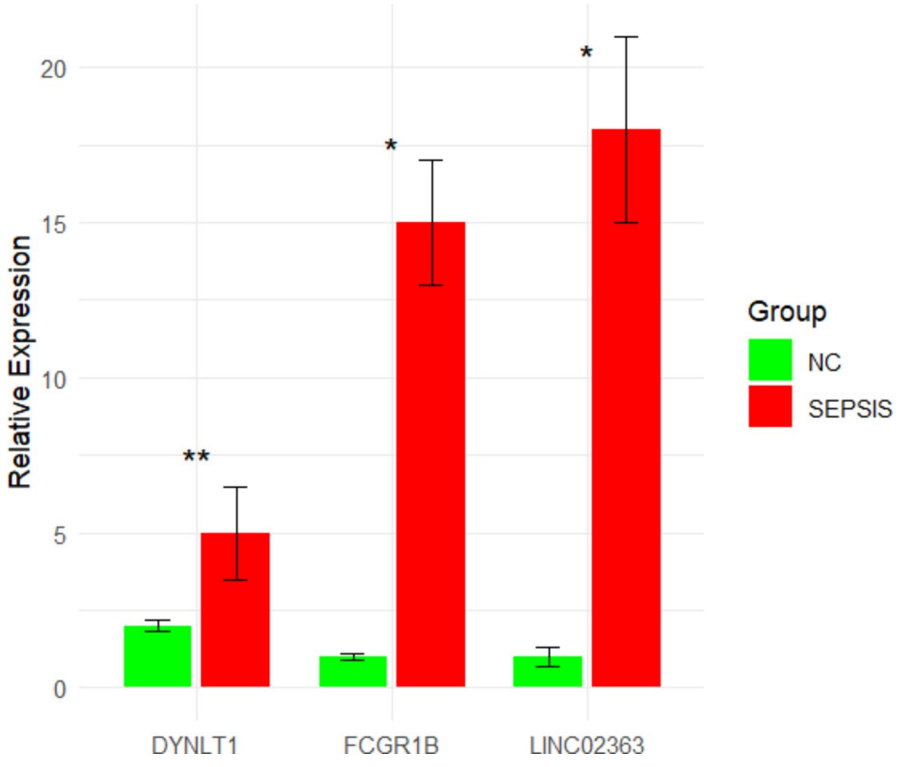

Fig. 9 Results of qPCR experiments. qPCR detects the expression of three core genes in the sepsis cell model. Green indicates control group and red indicates sepsis group. An asterisk (*) indicates a *p*-value less than 0.05; two asterisks (**) denote a *p*-value less than 0.01

Table 2 Primer sequences

Primer Name	Sequence(5′–3′)
LINC02363-F	CCTCCCTAATCCCTTCCAGTC
LINC02363-R	ATTGCCTCTTCTAAGTGCCTGTT
FCGR1B-F	GTCAAGCCTAGCCTGATAATCCT
FCGR1B-R	TACTATTCCCTCTTGTCTCCCTGA
DYNLT1-F	TGAACCAGTGGACCACAAATGT
DYNLT1-R	GTCTTATTCTCCCATCGCACAGT

and immune response, and the changes in the expression of LINC02363 may reflect the onset of sepsis earlier. LINC02363 was found to be significantly enriched in multiple pathways associated with immune response and inflammation by gene set enrichment analysis (GSEA), which provides preliminary evidence for its role in immune dysregulation in sepsis. However, studies directly comparing LINC02363, DYNLT1 and FCGR1B with the traditional markers PCT and CRP are lacking. In future work, we plan to evaluate the sensitivity, specificity, and effect of combining these new markers with PCT and CRP in the diagnosis of sepsis through multicenter clinical trials. In particular, the time window of these markers in diagnosis will be compared at different stages of sepsis (e.g., early, intermediate, and severe) to evaluate their potential application in clinical management. Through these studies, it is expected to further validate the clinical utility of LINC02363, DYNLT1, and FCGR1B and provide additional rationale for early diagnosis and personalized treatment of sepsis.

Two genes, DYNLT1 (dynamin light chain Tctex type 1) and FCGR1B (Fcγ receptor 1B), play important roles in regulating immune responses and inflammatory processes. Studies have shown that DYNLT1 is expressed in a wide range of tumours and correlates with disease prognosis, especially in breast cancer, where its expression level is strongly linked to the proliferation, recurrence, metastatic potential of tumour cells. In addition, prognostic analyses of the whole cancer species have shown that DYNLT1 may influence the immune response by regulating immune cell-mediated response processes [32]. Meanwhile, FCGR1B, as a predominantly expressed on leukocytes, FCGR1B is crucial in combating infections and managing inflammatory reactions by initiating the immune response through recognition of antibody-antigen complexes in vivo [33]. Studies have shown that Fcγ receptors (FcγRs) play an important role in the regulation of cytokine production and immune responses. FcγRs play a key role in infection by binding to the Fc region of immunoglobulin G (IgG) antibodies and regulating tissue- and pathogen-specific cytokine production [34]. In sepsis, activation of FcγRs may lead to selective amplification or inhibition of specific cytokines, thereby affecting the intensity and nature of the inflammatory response.

In addition, the interaction of FcγRs with other pathogen-recognizing receptors has also been suggested to play a role in immunomodulation in sepsis. For example, the interaction of FcγRIIa with TLRs induces specific cytokine responses that are essential for antimicrobial immunity [35]. This interaction is not limited to dendritic cells but also includes a variety of myeloid antigen-presenting cells such as monocytes and macrophages [36]. Single-cell sequencing revealed that DYNLT1 is widely distributed in all immune cells, and that FCGR1B is predominantly localised in macrophage lineages. The expression of both DYNLT1 and FCGR1B was upregulated in sepsis patients, but they acted in different directions in the survival analysis, suggesting that they may play roles in different immunomodulatory mechanisms, respectively.DYNLT1 may play a protective role by maintaining immune homeostasis, whereas FCGR1B may be associated with a poor prognosis by augmenting the inflammatory response. These findings highlight the importance of these two genes in disease progression and immune regulation, providing direction for further research.

In contrast to previous studies that have focused on inflammatory mediators and other small molecule biomarkers [37]. The present study demonstrates the potential role of long non-coding RNAs in diagnosing sepsis. In this research, the expression pattern of long non-coding RNA (LINC02363) as well as DYNLT1 and FCGR1B in macrophages was verified by qPCR experiments. They were all significantly highly expressed in the sepsis group, which was statistically significant ($p < 0.05$) compared to healthy controls. This finding suggests that LINC02363, DYNLT1 and FCGR1B may be potential biomarkers for sepsis.

In this study, we integrated datasets from multiple sources (GSE65682, GSE54514, GSE63042, GSE95233); however, the heterogeneity of these datasets may affect the comparability of results. There may be differences in patient populations, sample processing methods, and data normalization across datasets, such as differences in age, gender, and clinical subtypes, differences in sample collection and processing techniques, and different sequencing platforms and normalization methods used. These factors may introduce batch effects and technical variability that affect the comparison of gene expression data. Therefore, future studies should strengthen the unification of data standardization and normalization methods and apply batch effect correction methods to improve the reliability and comparability of results. In addition, we established a preliminary correlation between LINC02363 and sepsis through multidimensional data analysis and hypothesized that it may play a role in immune regulation. Through weighted gene co-expression network analysis (WGCNA), we found a significant correlation between LINC02363 and key immune genes such as DYNLT1 and FCGR1B, a finding that provides preliminary evidence for the possibility of LINC02363 as an immune regulator in sepsis. Combined with the results of survival analyses, the expression levels of these genes were strongly correlated with the prognosis of sepsis patients, further supporting the potential role of LINC02363 in the immune response. Nevertheless, the current study has not yet validated the specific role of LINC02363 in the immune response in sepsis by direct functional experiments. At this stage, all speculations are mainly based on the results of gene expression analyses and enrichment analyses, showing that LINC02363 may modulate sepsis-associated immune dysregulation through pathways involved in immune response, inflammatory response, and cellular stress response. However, these speculations still lack functional validation and thus cannot directly prove the causal role of LINC02363 in the immune response to sepsis. Future studies will focus on functional validation of LINC02363, especially through cellular experiments, such as RNA interference and CRISPR-Cas9 technology, to delve into the regulatory effects of LINC02363 on immune cell function and clarify its immune regulatory mechanism in sepsis. In addition, experiments in animal models will be an important means to verify the specific function of LINC02363 in sepsis. Although the current findings provide preliminary support for LINC02363 as a potential biomarker, more in-depth functional studies are needed to validate its role in sepsis in order to establish its application in the clinical setting.

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Authors' contributions
H.W and L.L wrote the main manuscript text, L.H and Y.H prepared Figs. 1, 2, 3, 4, 5, 6, 7, 8 and 9. All authors reviewed the manuscript.

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Data availability
We intend to share individually identified participant data. The peripheral blood drna sequencing data from 23 sepsis patients and 10 healthy individuals can be found in the China National Gene Bank Database (CNGBdb): db.cngb.org/, log on: CNP000261 1, you can access it now and it will be valid forever, (https://db.cngb.org/search/project/CNP0002611/).

Declarations

Ethics approval and consent to participate
Each patient and their family members voluntarily participated in this study and signed an informed consent form. The study was approved by the Ethics Committee of the Affiliated Hospital of Southwest Medical University (No. 1. ky2018029), Clinical Trial No.: ChiCTR1900021261, Registration Date: February 4, 2019.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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