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S100P is a core gene for diagnosing and predicting the prognosis of sepsis

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Sepsis, characterized as a severe systemic inflammatory response syndrome, typically originates from an exaggerated immune response to infection that gives rise to organ dysfunction. Serving as one of the predominant causes of death among critically ill patients, it's pressing to acquire an in-depth understanding of its intricate pathological mechanisms to strengthen diagnostic and therapeutic strategies. By integrating genomic, transcriptomic, proteomic, and metabolomic data across multiple biological levels, multi-omics research analysis has emerged as a crucial tool for unveiling the complex interactions within biological systems and unraveling disease mechanisms in recent years. Samples were collected from 23 cases of sepsis patients and 10 healthy volunteers from January 2019 to December 2020. The protein components in the samples were explored by independent data acquisition (DIA) analysis method, while Circular RNA (circRNA) categories were usually identified by RNA sequencing (RNA-seq) technology. Subsequent to the above steps, data quality monitoring was performed by employing software, and unqualified sequences were excluded, and conditions were set for differential expression network analysis (protein group and circRNA group were separately used $\log_2$ |FC|≥1 and $\log_2$ |FC|≥2, P < 0.050). Gene Ontology (GO) enrichment analysis and gene set enrichment analysis (GSEA) analysis were performed on common differentially expressed proteins, followed by protein–protein interaction between common differentially expressed genes and cytoscape software enrichment analysis, and subsequently its association with associated diseases (Disease Ontology (DO)) was investigated in an all-round manner. Afterwards, the distribution distinction of common differentially expressed genes in sepsis group and healthy volunteer group was displayed by heat map after Meta-analysis. Subsequent to the above procedures, pivotal targets with noticeable survival curve distinctions in two states were screened out after Meta-analysis. At last, their potential value was verified by in vitro cell experiment, which provided reference for further discussion of the diagnostic value and prognostic effect of target gene. A total of 174 DEPs and 308 DEcircRNAs were identified in the proteomics analysis, while a total of 12 common differentially expressed genes were identified after joint analysis. The protein–protein interaction (PPI) network suggested the degree of interaction between the dissimilar genes, and the heat map demonstrated their specific distribution in distinct groups. Through enrichment analysis, these proteins predominantly participated in a sequence of crucial processes such as intracellular material synthesis and secretion, changes in inflammatory receptors and immune inflammatory response. The meta-analysis identified that S100P is highly expressed in sepsis. As illustrated by the ROC curve, this gene has high clinical diagnostic value, and utimately confirmed its expression in sepsis through in vitro cell experiments. In these two groups of healthy people and septic patients, S100P demonstrated a more obvious trend of differential expression; Cell experiments also proved its value in diagnosis and prognosis judgment in sepsis; As a result, they may become diagnostic and prognostic markers for sepsis in clinical practice.

Keywords S100P, Sepsis, RNA sequencing, Proteomics, Survival analysis

Abbreviations

GO	Gene ontology
PCA	Principal component analysis
FBS	Fetal bovine serum

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ROC	Receiver operating characteristic
GEO	Gene expression omnibus
DIA	Data independent acquisition
DO	Disease ontology
miRNA	MicroRNA
circRNA	Circular RNA
PPI	Protein–protein interaction
RNA-seq	RNA sequencing
DEPs	Differentially expressed proteins
DEcircRNAs	Differentially expressed proteins circRNAs
GSEA	Gene set enrichment analysis
Q-PCR	Quantitative PCR
SCCM	Society of critical care medicine
ESICM	European Society of Intensive Care Medicine
SD	Standard deviation

Sepsis is an organ dysfunction disease spawned from a variety of internal and external infection factors, which has become one of the most dangerous and pivotal problems in critical care medicine[1,2]. In accordance with incomplete statistics, sepsis-associated deaths account for 16.8% of all hospitalized patients worldwide[3], which has become a major hidden danger to human health. Notwithstanding the fact that with the increasing number of antibiotics, the inflammatory storm of sepsis has been controlled, but it remains essential to elevate the efficacy as high as possible[4]. Aside from that, this disease progresses stealthily, lacks appropriate specific diagnostic means, so that the treatment research ROC of sepsis is delayed[5]. Consequently, it's imperative to find the possible target points correlated with the diagnosis and treatment of sepsis to elevate its cure rate. Generally speaking, biomarkers can distinguish the nature of infection, i.e., microorganism and non-microorganism, which provides a trustworthy and speedy approach for early detection of sepsis and realizes risk level division, evaluation of prognosis change and amelioration of antibiotic use standard policy[6]. This provides a possible reference basis for continuously updating the treatment plan[7].

For high-throughput data, gene expression analysis is generally performed by adopting RNA-seq approaches, which offer valuable insights into understanding underlying mechanisms[8]. As previous studies have demonstrated, this approach has been applied to transcriptomic data associated with immune and inflammatory responses in sepsis in both mice and humans[9]. Conversely, proteomics relies on biochemical techniques to detect and quantify distinct proteins within biological samples. Conducting proteomic analyses on samples from patient groups and subsequently matching them with control groups reveals distinct patterns of alterations associated with protein-dependent diseases, ultimately furnishing pertinent biomarkers for prognostic assessments[10].

circRNA is a type of covalently closed circular molecule, which has neither a 5' nor a 3' end. In line with its translatable properties, it is grouped into non-coding circular RNA and coding circular RNA[11]. Some parts of it can not only function as " microRNA (miRNA) sponges", but also can competitively bind to miRNA, thereby regulating the expression of miRNA target genes[12].

With the dramatic development of omics tools, it has become possible to carry out comprehensive assessment of molecular changes in cells, surpassing the capabilities of traditional targeted analysis. Transcriptomics has become an immensely employed omics analysis approach, providing a wider biological coverage, higher signal-to-noise ratio, and cost-effectiveness in small sample sizes for studying cell characteristics[13–15]. Furthermore, it has become a conventional analysis method for in vitro and in vivo toxicology studies. More importantly, it is continuously evolving with next-generation RNA-seq approaches[16–18].

This study is principally intended to use a combination of RNA-seq technology and DIA proteomics technology to identify target genes for the diagnosis and treatment of sepsis, and to clarify the transcriptional expression levels of core genes, thereby laying a theoretical basis for subsequent mechanistic research.

The analytical steps of this study are as follows, as illustrated in Fig. 1: (1) Recruitment of subjects and volunteers for transcriptomic sequencing and proteomic mass spectrometry analysis, selecting 23 sepsis patients and 10 healthy volunteers; (2) Data analysis conducted through the IDEP0.93 website, utilizing Principal Component Analysis(PCA) to screen outlier samples, with volcano plots and heatmaps representing the distribution of differential data across multi-omics samples; (3) GO enrichment analysis of differentially expressed genes from the transcriptome via the Shiny GO website, categorized as BP, CC, and MF, and enrichment of differentially expressed proteins from the proteome by adopting GSEA-relevant enrichment software; (4) Intersection analysis of the two datasets, visualized by utilizing Venn diagrams to depict overlapping areas and PPI networks to illustrate interconnectedness; (5) Further analysis of differential genes or proteins, with heatmaps showing distribution characteristics between sepsis and healthy groups, and enrichment patterns of differential components explored by utilizing the metascape software; (6) Validation of S100P: External datasets were adopted to validate the findings, with Meta analysis summarizing the distribution trends and evidence-based medicine characteristics of the selected genes in five external clinical validation datasets, ROC curves illustrating the diagnostic efficacy of S100P across these datasets in terms of sensitivity and specificity, survival curves depicting how 100P expression levels correlate with clinical patient outcomes in large clinical samples, and Real-time Quantitative PCR Detecting System (Q-PCR) results verifying the association between S100P and sepsis.

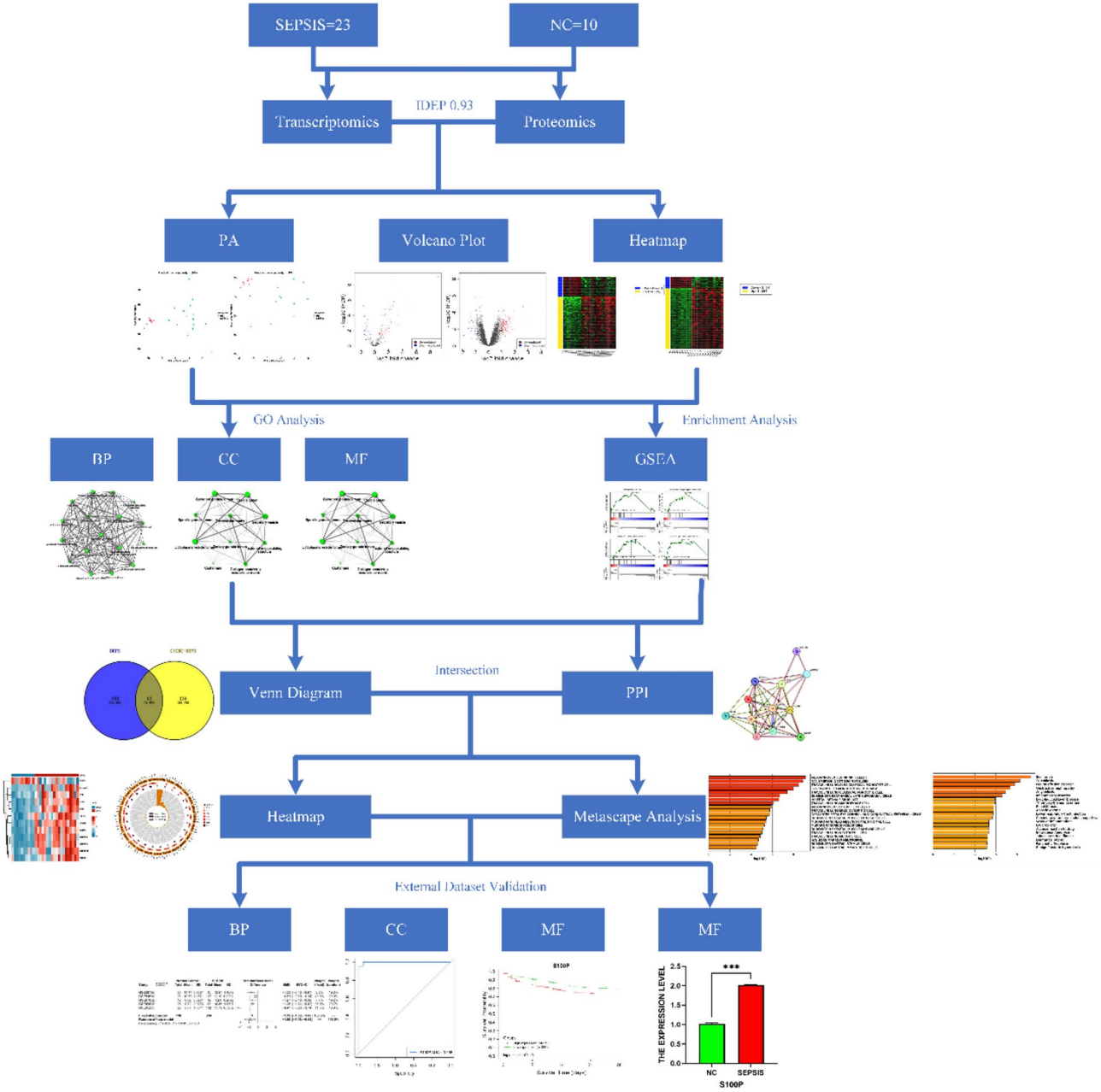

Fig. 1. The timeline of this study is outlined as follows: (1) Selection of appropriate sample data; (2) Data analysis by employing the IDEP0.93 website; (3) Enrichment analysis of multi-omics differential genes or proteins through the Shiny GO website; (4) Intersection processing of the two datasets, with PPI adopted to demonstrate the degree of association between them; (5) Analysis of differential genes or proteins; (6) Verification of S100P by utilizing external validation datasets and Q-PCR.

Materials and methods

Subject Recruitment and Blood Collection

In the previous period (from January 2019 to December 2020), our research group utilized 23 cases of sepsis diagnosis in patients treated in the Emergency Intensive Care Unit of the Affiliated Hospital of Southwest Medical University, and recruited 10 cases of healthy individuals as a control group (Ethical approval No. ky2018029). The inclusion criteria for patients first required compliance with the diagnostic definition of sepsis 3.0 and adherence to the diagnostic criteria published by the Society of Critical Care Medicine (SCCM) and the European Society of Intensive Care Medicine (ESICM). Aside from that, the age of the patients was generally between 16 and 70 years old. At last, the study participants, whether subjects or legal guardians, voluntarily entered the research group and signed a written informed consent form.

The corresponding exclusion criteria are: 1. Age, if the patient is under 16 years old and does not have a legal guardian or if they do not consent to this study; 2. If the subject is currently participating in another ongoing clinical trial study, the above two situations are the main exclusion criteria.

Proteomic analysis

Proteomics typically employs DIA digital processing approaches. In this procedure, we utilized the Q-Exactive HF instrument (Thermo Fisher Science, San Jose, CA) to obtain accurate and highly reproducible quantitative proteomic data. Afterwards, the raw mass spectrometry data underwent processing to convert it into a substantial and trustworthy set of quantitative results.

Differential protein and gene selection on the basis of common disparities

Logarithmic transformation was applied to all data. The consistency of samples was assessed by utilizing box plots and principal component analysis. DEcircRNAs and Differentially Expressed Proteins (DEPs) were filtered by adopting the online tool Idep (version 0.93; URL: https://idep.cloud) with criteria set at $\log_2|FC| \geq 2$ and$\log_2|FC| \geq 1$ and $P < 0.050$. The common differentially expressed genes from both sets were submitted to the online platform STRING (version: 12.0; URL: https://string-db.org/), establishing an association network.

GO analyses

In an effort to further understand the changes of functional expression of DEPs and DEcircRNAs in sepsis, GO enrichment analysis was performed by employing Shiny GO (version: 0.77 ; URL: http://bioinformatics.sdstate.edu/go/) and metascape (version: 3.5.20240901; URL: https://metascape.org), separately. It demonstrates the biological processes in which DEPs may be involved.

GSEA analysis

This tool was adopted to further understand the specific expression patterns of core target genes in the sepsis and normal groups. GSEA (version: 4.1.0; URL: https://metascape.org) was utilized for enrichment analysis. This enables us to gain a deeper understanding of the differential expression of target genes.

DO analysis

Genes are often bound up with the functionality of diseases. We dig into the network of correlations between gene functions and changes in diseases by adopting the online Omicshare tool (version: 2025; URL: https://www.omicshare.com/tools). This approach is advantageous for us to pertinently assess whether there are other diseases aside from sepsis that can exert influence on gene expression.

Cumulative analysis

With an aim to delve into the expression disparities of genes at the transcript level in distinct groups, we downloaded a dataset of human peripheral blood sepsis samples (GSE9523[19]) from the public database gene expression omnibus (gene expression omnibus (GEO)) (URL: https://www.ncbi.nlm.nih.gov/geo/). Subsequent to the data homogenization, it was categorized into two groups, namely the sepsis group and the control group. Afterwards, meta-analysis was performed on individual genes to validate the reliability of the expression trends of core targets in this study.

External dataset validation

(1) Meta-analysis validation.

To further assess the expression of the core genes associated with sepsis across dissimilar populations, we validated our findings by adopting publicly available data. We retrieved the sepsis datasets GSE28750[20], GSE54514[21], GSE69528[22], GSE67652[23] and GSE95233[19] from the GEO database. The patients were categorized into sepsis and normal groups. A forest plot was created after a meta-analysis of the core genes' expression levels.

(2) Survival analysis.

To probe deep into the critical functions of potential core genes selected by employing the PPI approach in determining patient prognosis in sepsis, we downloaded the public dataset GSE28750 on the association between genes and prognosis. GSE28750 contains gene expression values and clinical outcome data for 27 patients with sepsis and 20 normal. We conducted survival analysis by adopting GraphPad Prism 8 software, with a log-rank test where $P < 0.050$.

(3) Receiver operating characteristic ROC curve.

The ROC curve was utilized in the dataset GSE54514 from the GEO database. The dataset was submitted in June 2015 and consisted of a sepsis group (n = 26) and a normal control.

(4) Single-cell sequencing.

The Process of Single-Cell Sequencing.

1. Preparation and sequencing of single cell library

By encapsulating beads with Cell Barcodes and cells in droplets, capturing droplets with cells and lysing cells within the droplets with microfluidic technology, the 10 × genomics platform has enabled the connection of mRNAs in cells with Cell Barcodes on the beads to create Single Cell GEMs. The droplets were utilized to carry out the reverse transcription reaction for creating a library of cDNAs, with the sample index on the sequence library employed to identify the source of the target sequence.

2. 10 × sequencing data preprocessing

As per information from 10 × Genomics, visit https //support. 10xgenomics. com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger. The raw sequencing data has been investigated by utilizing CellRanger (URL:https://support.10xgenomics.com/single-cell-gene-expression/software/downloads/lates; version: v2.2.0). Simply put, the "cellranger mkfastq" function has first been adopted to convert the sequencing raw BCL file to an FASTQ file, which has been processed by virtue of the 'cell ranger count' wrapper function. Apart from that, the sample has been resized with the '-force-cells¼ 7000' argument. The cell cursor counting function has employed the wrapped STAR software to compare sequences to the reference genome. The output file has contained the gene expression matrix and barcode information for the CellRanger pipeline, which is subsequently utilized for downstream visual analysis.

3. Characteristics of cell clusters

The 10 × genomics official software Cell Ranger was used for quality control of samples, with the integration of the STAR software. Grounded in the comparison of the reads to the reference genome, the quality control results were obtained from the original data, encompassing the high-quality cell number, gene number and genome comparison rate, so as to assess the quality of each sample. With regard to cell clustering, we refer to the Seurat(URL: https://satijalab.org/seurat/; version: v5) . Moreover, the CellRanger-generated "filtered gene bc matrices" was employed as the input file for Seurat. The quality control criteria are as follows: elimination of cells with fewer than 200 genes, less than 1000 UMIs, log10GenesPerUMI below 0.7, mitochondrial UMI proportion exceeding 10%, and red blood cell gene proportion surpassing 5% to identify high-quality cells. Afterwards, the DoubletFinder software was utilized for double cell elimination and subsequent analysis. Subsequent to normalization, the genes specific to each dataset were computed for the subsequent clustering analysis.
To start with, the MNN algorithm was utilized to remove the batch effect by identifying mutual nearest neighbors. Afterwards, the UMAP algorithm was applied to visualize the single cell cluster founded on the dimension abatement outcomes from MNN. The clustering algorithm adopted SNN (spatial clustering algorithm on the basis of sharing the nearest neighbor density) to utimately get the optimal cell cluster. To some content, the number of cell clusters can be adjusted by setting the resolution parameters. In the later stage, it is decided whether to adjust the clusters in accordance with the cell type identification. Generally, the default is resolution = 0.4.

④ In an effort to assess the quality of duplicate samples or their individual discrepancies, the dissimilar cells between a variety of groups are evaluated for further analysis.
⑤ To identify the specifically expressed genes in the cluster, this study has adopted the presto test approach to test the distinctions between the designated cell populations and all other cell populations. To identify all maker genes in each cell population, the screening criteria are set as follows: $Log_2|FC|$. threshold had to be above 0.0 and min.pct (the percentage of genes expressed in all cells) had to exceed 0.25.
⑥ With an aim to comprehensively examine the cell clustering and the development of downstream differentiation paths, this paper has utilized the SingleR tool(version: 3.2; URL: https://bioconductor.org/packages/release/bioc/html/SingleR.html) to label the cells with a shared dataset. The FindMarkers function of the Seurat package was employed to screen all cells in each sample group for differentially expressed genes between groups. Moreover, the default presto disparity test from an opensource software library was adopted to reinforce the data analysis speed in bioinformatics.
⑦ Enrichment analysis of constructed marker genes.

The hypergeometric distribution test was utilized to determine whether the function set was conspicuously enriched in the list of differential protein coding genes, which were selected from a background list of all protein coding genes. Subsequently, the resulting p value was adjusted with the Benjamini & Hochberg multiple tests to obtain the value.

KEGG[24] serves as the primary public repository for Pathway information. Pathway analysis (combined with KEGG annotation results) of differential protein encoding genes was performed by KEGG database, and the significance of differential gene enrichment in each Pathway entry was calculated by hypergeometric distribution test.

The STRING https://string-db.org/ refers to a database of functional correlations between protein predictions. Genomic correlation among protein encoding genes can frequently illustrate functional linkages. Genes required for specific functions typically exhibit comparable species coverage, which are tightly situated in the genome (especially in prokaryotes) and are prone to result in gene fusion. As a precomputed global resource, the STRING database was adopted to browse and probe deep into these dependencies. On this basis, the analysis

was strengthened by utilizing the STRING database to identify the interaction patterns among dissimilar genes and create a network diagram.

Visualization analysis of single cell localization the cell line localization of single target gene was detected by 10 × scRNA-seq technique, whose particular procedure was carried out in accordance with the company's operational guidelines. The collected blood samples were subjected to high-throughput sequencing after mixing to generate raw data in fastq format. The quality of the raw data was assessed by employing the official CellRanger software from 10 × genome. The Seurat software package was utilized for additional data quality control after visiting 10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger. Gene expression data was adopted to conduct linear dimensionality decrement analysis, which has subsequently been visualized in two dimensions by adopting UMAP. FindAllMarkers function was employed to identify marker genes, while VlnPlot and FeaturePlot function to identify specific genes. SingleR software can determine how cell expression profiles associate with reference dataset, so as to create a sepsis-relevant single cell library by assigning the most correlated cell type. The main gene identified in the previous research was encompassed in a single-cell library to delve into the positioning of the specific genes within the cell line.

(5) In vitro cell experiment.

RAW264.7 cells were selected to identify the expression of target genes in sepsis, and further in vitro experiments were conducted in a systematic manner. RAW264.7 cells were cultured in complete culture medium containing 10% fetal bovine serum (FETAL BOVINE SERUM(FBS)) in a 5% CO2, 37 °C cell culture incubator. After 72 h of culture, when the adherent cells covered 70%-80% of the culture dish or bottle, the supernatant was discarded and the cells were collected. Subsequently, the cells were cultured in a medium containing 3 ml of 10% FBS and no penicillin–streptomycin for 24 h, followed by stimulation with lipopolysaccharide (LPS) (100 ng/ml) to induce sepsis in the cells for 6 h. The control cells were cultured in medium without any other treatment at the same time point. Afterwards, cell Q-PCR experiments were performed, and the primer design and synthesis were completed by Beijing Qing Ke Biological Technology Co., Ltd. Chengdu Branch. The specific primer sequences are as follows: S100P: forward primer GTCAAGGATTGGCCCGATAG, reverse primer TACCAC ATCACAGGGGACCT. RNA extraction was performed by adopting a reagent kit (Oriscience Biotechnology Co., Ltd) to extract total RNA from the cells, and the concentration and purity were determined by employing a spectrophotometer. The RNA was then reverse-transcribed into cDNA by utilizing an RT kit (Seq-Hunt Biotechnology, China, CA01). Subsequently, Q-PCR was performed by employing the SYBR Green reagent kit (Seq-Hunt Biotechnology, China, A01), and melt curve analysis was carried out in a systematic manner. The results were explored by adopting 2^-ΔΔCt approach.

Statistical analysis

Numerical analysis was conducted by utilizing GraphPad Prism 9.0 software to perform statistical analysis on clinical data of the patients. Continuous variables were presented as mean ± standard deviation (SD) or median (interquartile range). distinctions between continuous variables were explored by adopting unpaired Student's t-test. When comparing data between two groups that adhered to normal distribution and homogeneity of variance, an independent samples t-test was employed. The significance threshold was set at $P < 0.050$.

Results

Data quality control

We obtained relative transcription levels of 308 genes and data on 184 commonly differentially expressed proteins. Figure 2A presents 10 groups of sepsis populations and 19 groups of normal populations, while Fig. 2B shows 8 groups of sepsis populations and 12 groups of normal populations. Figure 2C reveals 174 differentially expressed genes, and Fig. 2D discloses 308 differentially expressed proteins. Figure 2E exhibits the upregulation of 126 elements and the downregulation of 48 elements, whereas Fig. 2F demonstrates the upregulation of 254 elements and the downregulation of 54 elements.

GO and GSEA enrichment

Functional enrichment analysis revealed that the differentially expressed proteins (DEPs) were primarily involved in protein synthesis and metabolism, immune inflammatory responses, cellular metabolism, secretion, and cellular activation processes (Fig. 3A–C).

Gene set enrichment analysis (GSEA) illustrated that these DEPs were tremendously associated with pathways such as inflammatory complement cascade, protein metabolism and synthesis, biotin metabolism, and processes associated with leukocyte transendothelial migration and bacterial infection establishment (Fig. 4A–D).

Intersection analysis

Through cross-analysis of circRNA-associated proteins, a total of 12 common associated genes were identified, specifically comprising LDHA, GSTO1, S100A9, B2M, S100A11, AHNAK, S100A8, ORM1 (Fig. 5A), LYZ, TGFBI, S100P, and LCN2. The protein–protein interaction network is illustrated in Fig. 5B.

Target enrichment analysis

Functional enrichment analysis was conducted through all manner of protein expression profiles. Figure 6A illustrates the distribution disparities of 12 target genes between septic and normal groups. Figure 6B underscores the close association of these proteins with disorders in immune system function, cardiovascular diseases, and certain cancers. Figure 6C and D further elucidate the involvement of immune cell activation, dysregulated release of inflammatory mediators, and alterations in the fibrotic system.

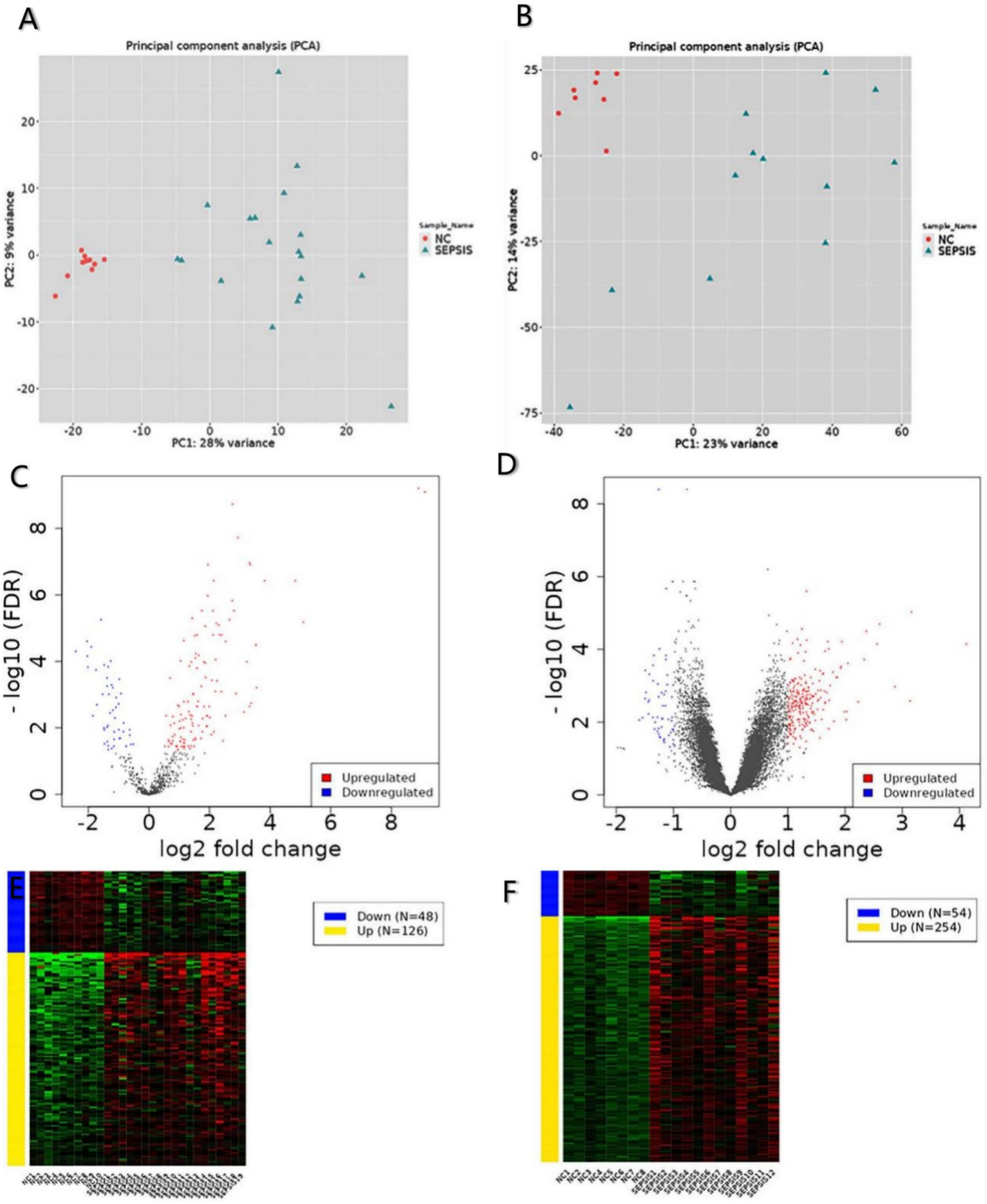

Fig. 2. Data processing. (**A**–**B**) PCA plots of the proteome and circRNA groups. (**C**, **D**) Volcano plots of differentially expressed genes in circRNA and proteome groups. (**E**, **F**) The heat map distributions of the proteome and circRNA.

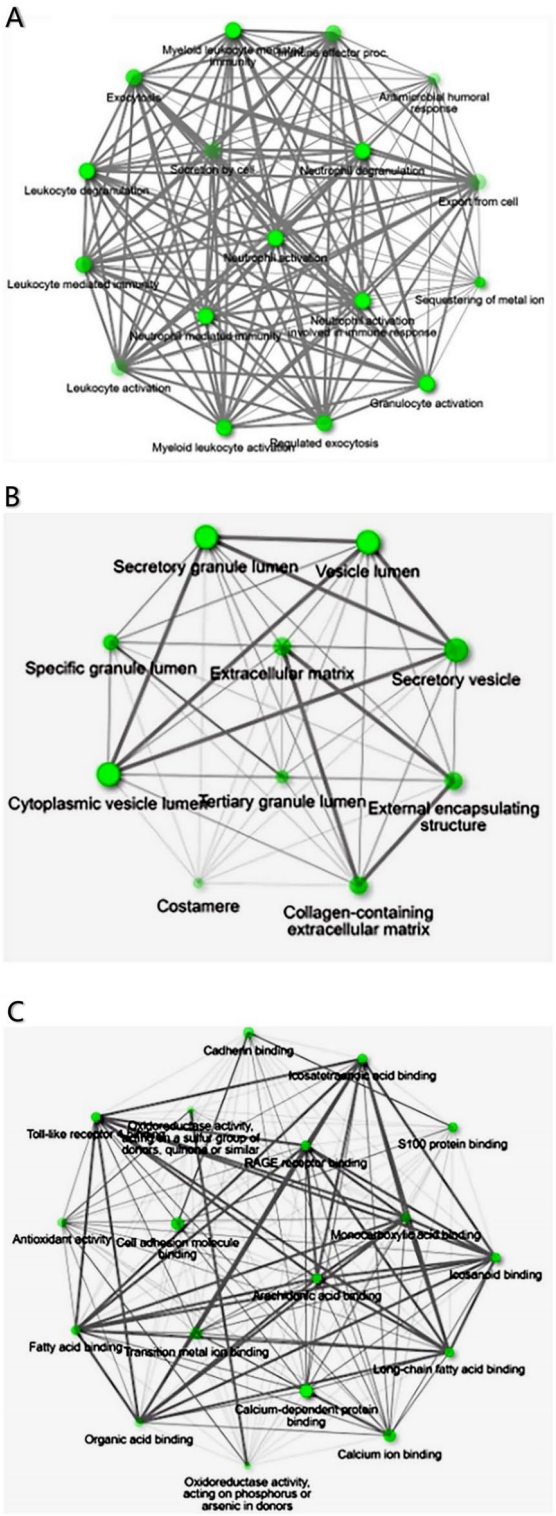

Fig. 3. Enrichment analysis of DEGs by employing the Shiny GO 0.77 tool. (**A**–**C**) Enrichment analysis typically involves three biological processes, namely biological process, Cellular Component and Molecular Function.

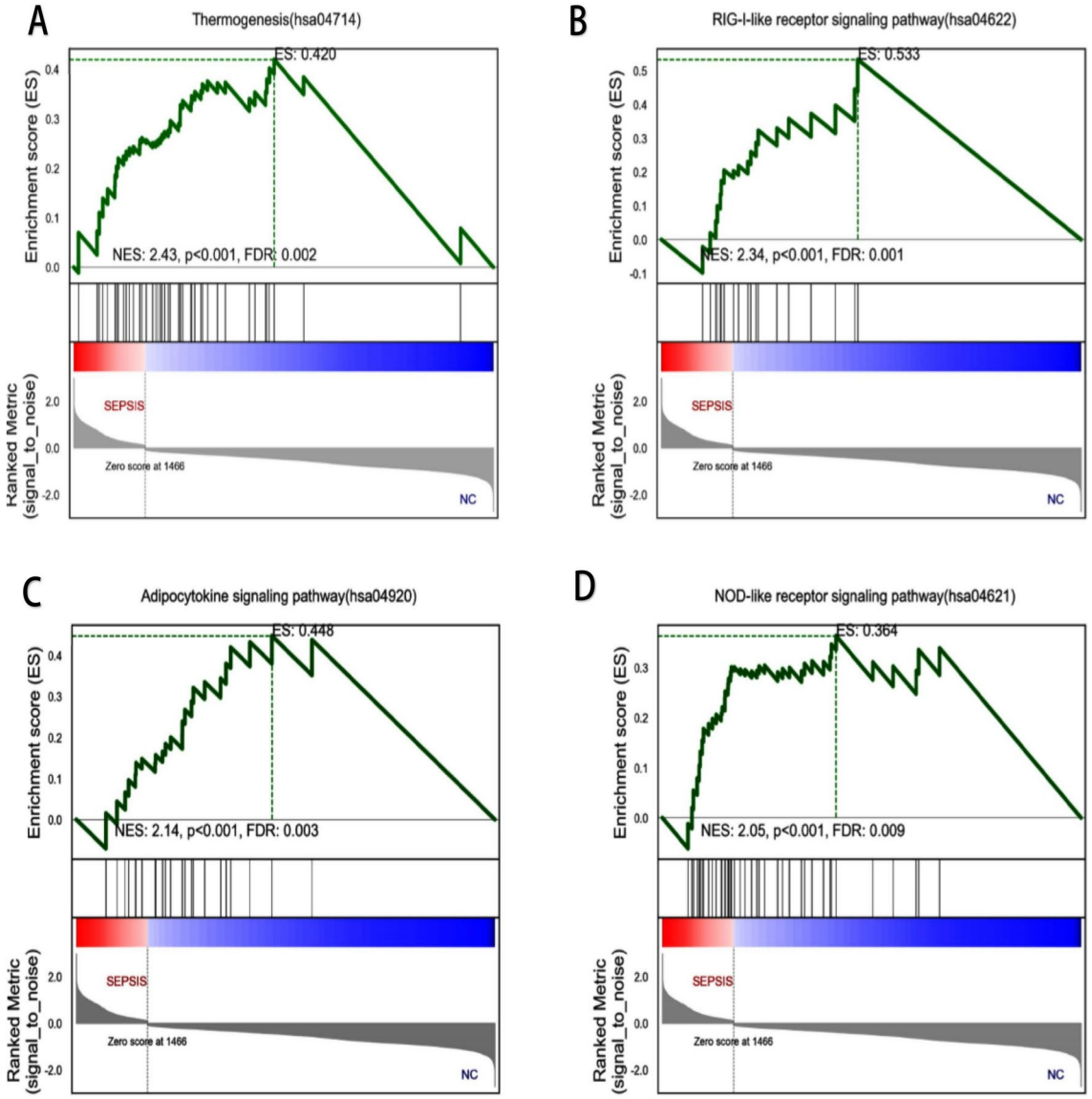

Fig. 4. GSEA Enrichment Analysis. (**A**–**D**) Represents the clustering range in the two groups, severally.

Meta-analysis validation

As illustrated in Fig. 7, a meta-analysis of multiple datasets, demonstrates that S100P is highly expressed in sepsis, whereas it is expressed at lower levels in normal samples.

Identification of core genes for prognostic and diagnostic value in sepsis

In survival analysis, as illustrated in Fig. 8A, patients with lessened levels of S100P demonstrated a negative correlation between protein levels and survival rate in septic patients. ROC curve analysis revealed that S100P exhibited high sensitivity and specificity, with an area under the curve (AUC) value of 0.998 (Fig. 8B).

Q-PCR

Independent sample t-test results suggest that S100P is noticeably upregulated compared to normal cells (0.9994 [0.9288–1.073]; $t = 59.56$, $p = 0.003$). All discrepancies were statistically striking ($p < 0.05$) (Fig. 9).

Single-cell RNA sequencing analysis

This report is grounded in the analysis pipeline of five single-cell transcriptomic sequencing samples. As depicted in Fig. 10A, the default resolution was set. Figure 10B–C illustrate the clustering conditions of the sample/population through clustering or cellular proportion statistics; Fig. 10D reflects discrepancies in cellular distribution among samples. Figure 10E displays the cell types identified. Figure 10F depicts the results of cell type annotation. Figure 10G–H suggest that S100P is predominantly expressed in the third and fifth cell clusters, which belong to the macrophage lineage.

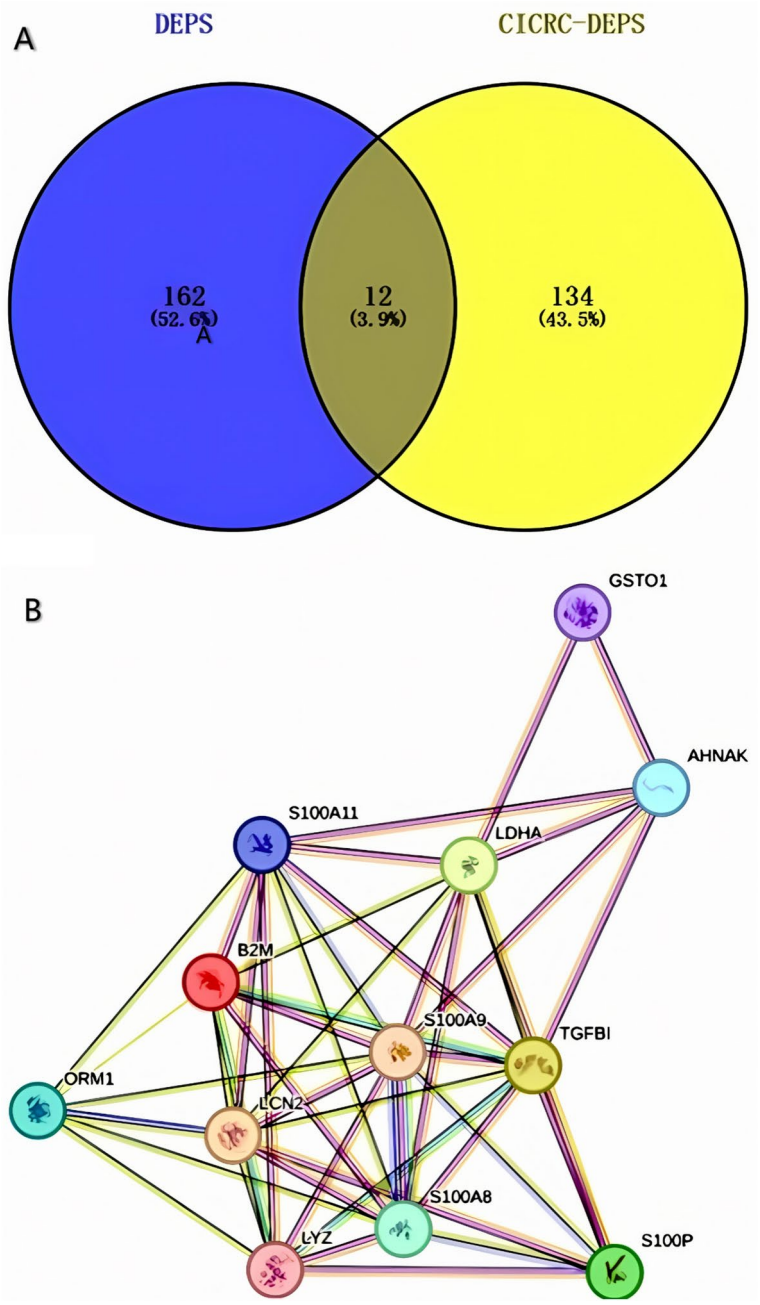

Fig. 5. Cross-linking of differentially expressed proteins (DEP) with circular RNA-associated proteins. (**A**) The twelve shared proteins formed in the intersection of both; (**B**) The interconnections between co-expressed differentially regulated proteins in the two datasets.

Discussion

In recent decades, medical workers have gained a wealth of frontier knowledge about the pathogenesis of sepsis, but the current treatment effects suggest that the mortality rate remains high[25]. For this reason, the key lies in early diagnosis and treatment[26]. Exploring stable and sustained diagnostic biomarkers contributes to the betterment of the disease. In this study, a total of 14 common differentially expressed genes were screened in sepsis and normal samples. Enrichment analysis illustrated that these DEGs were principally concentrated in biological processes linked with immune cell infiltration and inflammation. Last but not least, through experimental validation, a core target was screened out, which may become a potential regulatory network to guide the clinical diagnosis and prognosis of sepsis as a biomarker.

circRNA is a new type of endogenous RNA molecule with a covalently closed loop structure. Many circular RNAs act as "miRNA sponges" and play paramount regulatory roles in the transcription and post-transcription levels of genes through binding protein interactions or self-translation[27]. Another definition of CircRNA is grounded in the genetic origin of CircRNA sequence classification, which can be grouped into several types,

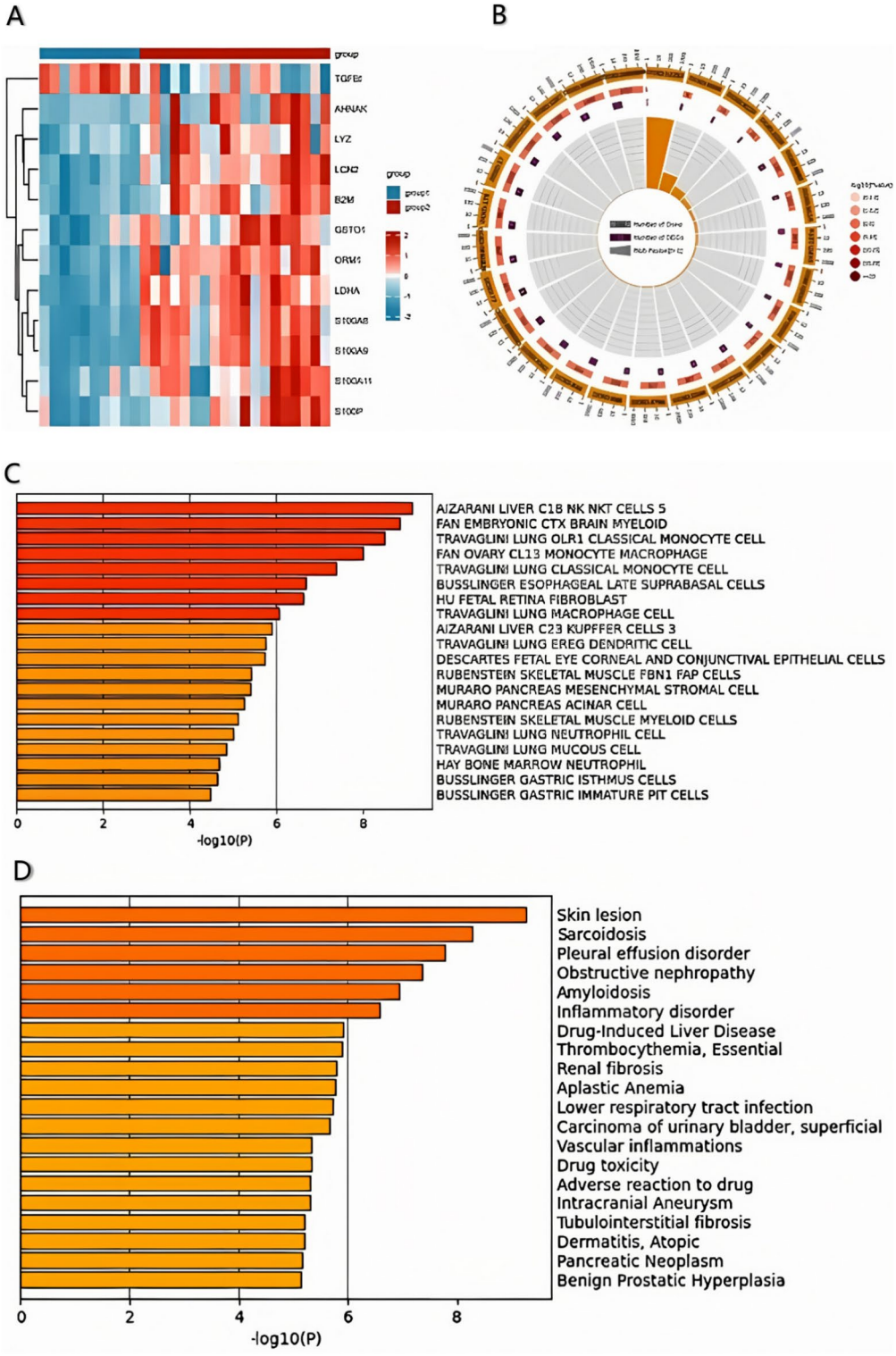

Fig. 6. The functional analysis of differentially expressed proteins. Section (**A**) demonstrates the distribution properties of diverse proteins; (**B**) elucidates their associations with respective diseases; sections (**C**–**D**) discuss the functional characteristics of distinct proteins from the perspective of functional effects.

encompassing exotic CircRNA, intronic CircRNA, antisense CircRNA, and sense overlapping CircRNA[28–30]. As a consequence of its natural structural features, it has characteristics that other non-coding RNAs do not possess, such as high stability and natural resistance to RNase R, which may change correspondingly with the progression of the disease[31,32]. On that account, circRNA conducts a paramount role in the future development of diagnostic biomarkers and corresponding therapeutic interventions.

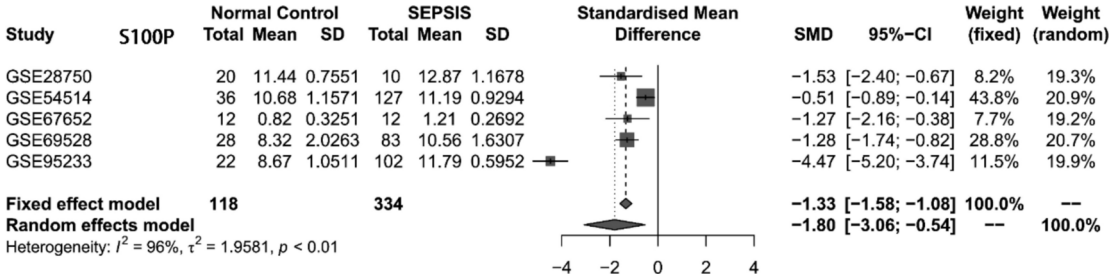

Fig. 7. S100P was expressed at a high level in the sepsis group on the basis of meta-analysis (P < 0.010).

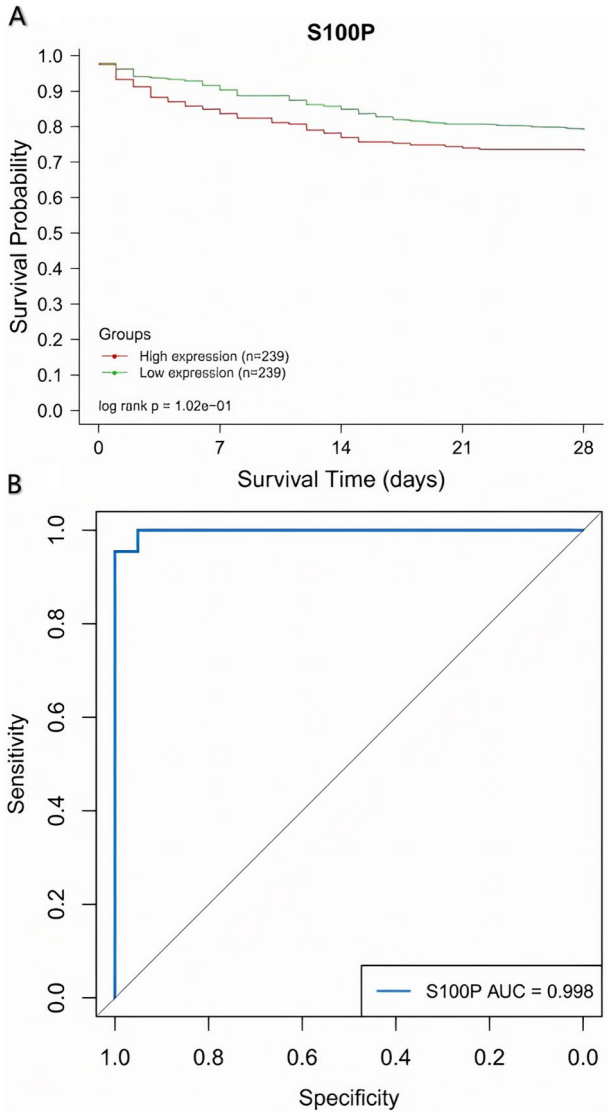

Fig. 8. Survival Analysis and ROC Curve for Core Genes. (**A**) In the survival curve, patients with low S100P expression demonstrate a higher 28-day survival rate compared to those with high expression; (**B**) The ROC curve reveals high sensitivity and diagnostic specificity of S100P.

As depicted in Fig. 3A, there is a potential link between sepsis-associated differentially expressed genes and the pathogenesis of leukemia. By comparing with previous machine learning studies on leukemia-associated biomarkers, we put forth the following standpoints: ① Identifying biomarkers to uncover potential targets for diagnosis and treatment; ② Revealing leukemia stem cell characteristics by calculating stem cell indices, which helps elucidate the stem cell properties in leukemia cells, deepening our understanding of the molecular

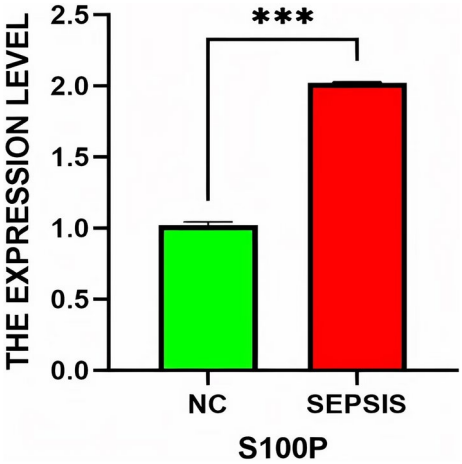

Fig. 9. Q-PCR. Expression levels of S100P mRNA, with green representing the normal control group and red representing the sepsis group. *, $p < 0.050$* *, $p < 0.010$. * * *, $p < 0.001$.

mechanisms of leukemia, particularly the pathological processes bound up with leukemia stem cells; ③ Establishing potential applications for diagnosis and treatment: the identified biomarkers offer new standpoints for the diagnosis, prognosis assessment, and treatment of acute leukemia; ④ Determining the reference value of research approaches: machine learning algorithms can analyze gene expression data to unveil the molecular mechanisms of cancer, providing potential targets for cancer diagnosis and treatment[33].

The potential mechanisms between the 12 sepsis-linked differentially expressed genes/proteins and leukemia we identified may involve: Lactate Dehydrogenase A (LDHA) conducts an irreplaceable role in cancer metabolism, particularly in the aerobic glycolysis process of tumor cells. Research demonstrates that high expression of LDHA is associated with aggressive progression in multifarious cancers, encompassing leukemia. In acute myeloid leukemia (AML), LDHA is considered a potential therapeutic target. Inhibition of LDHA activity can effectively suppress the proliferation and survival of AML cells, thereby demonstrating anti-leukemic effects[34]. Moreover, the role of LDHA in T-cell acute lymphoblastic leukemia (T-ALL) has also been explored. Relevant studies reveal that LDHA activity is remarkably elevated in the serum of T-ALL patients, which demonstrates its underlying involvement in leukemia progression. Targeting LDHA can notably inhibit the proliferation of T-ALL cells and trigger their apoptosis, offering a promising therapeutic strategy for T-ALL[34].

GSTO1 (Glutathione S-transferase omega 1) is found to be upregulated in multifarious cancers, yet its specific mechanisms in leukemia remain inadequately understood. In non-small cell lung cancer, GSTO1 reinforces cell proliferation, migration, and invasion while inhibiting apoptosis by modulating the JAK/STAT3 signaling pathway[35]. As this mechanism illustrates, GSTO1 might similarly accelerate cancer cell growth and survival in leukemia by affecting cellular signaling pathways. Aside from that, GSTO1 is correlated with drug resistance. In lung adenocarcinoma, GSTO1 affects the NPM1 protein through deglutathionylation, activating the AKT/NF-κB signaling pathway, thereby exacerbating resistance to EGFR-TKIs and tumor metastasis[36]. This resistance mechanism may also exist in leukemia, where GSTO1 could potentially regulate leukemia cell responses to chemotherapy drugs through similar signaling pathways. Polymorphisms in GSTO1 may also influence an individual's sensitivity to environmental toxins. For instance, the GSTO1 polymorphism is correlated with arsenic-induced genotoxicity, with individuals carrying the mutated GSTO1 genotype exhibiting greater sensitivity to arsenic's genotoxic effects[37]. This genetic polymorphism may conduct a role in the development of leukemia by affecting cellular responses to environmental toxins, thereby altering the risk of leukemia.

S100A9 protein conducts a striking role in all manner of diseases, particularly in its underlying mechanistic connections within leukemia. As a calcium-binding protein, S100A9 was demonstrated to contribute to inflammation and tumor progression. In leukemia, the expression of S100A9 may be bound up with disease progression and treatment resistance. Specifically, as relevant studies have illustrated, overexpression of S100A9 is bound up with glucocorticoid resistance in acute lymphoblastic leukemia (ALL). Specifically, in infant ALL with MLL rearrangements, high expression of S100A9 is linked with an inability to induce intracellular calcium ion concentration elevation and resistance to prednisolone[38]. Moreover, S100A9 expression may contribute to rapid resistance to the drug by inhibiting prednisolone-induced calcium ion flux[38]. Aside from that, S100A9 has also been implicated in the maintenance of disease in AML. As correlated studies have suggested, the HOXA9-SAFB chromatin complex is correlated with the suppression of S100A8 expression, which is one of the homologous proteins to S100A9.This inhibitory effect may be associated with the differentiation and apoptosis of AML cells, thereby imposing certain influence on disease progression[39]. In the end, the role of S100A9 in leukemia may also involve its function within the immunosuppressive tumor microenvironment. S100A9 regulates the immunosuppressive functions of myeloid-derived suppressor cells (MDSCs) through the RAGE and TLR4 signaling pathways, which may conduct a role in the immune evasion of leukemia[40].

B2M (β2-microglobulin) conducts a paramount role in a diverse array of diseases, particularly in hematologic disorders such as leukemia. As associated studies demonstrate, the expression of B2M is bound up with the progression and prognosis of leukemia. In AML, overexpression of B2M is considered a common aberration

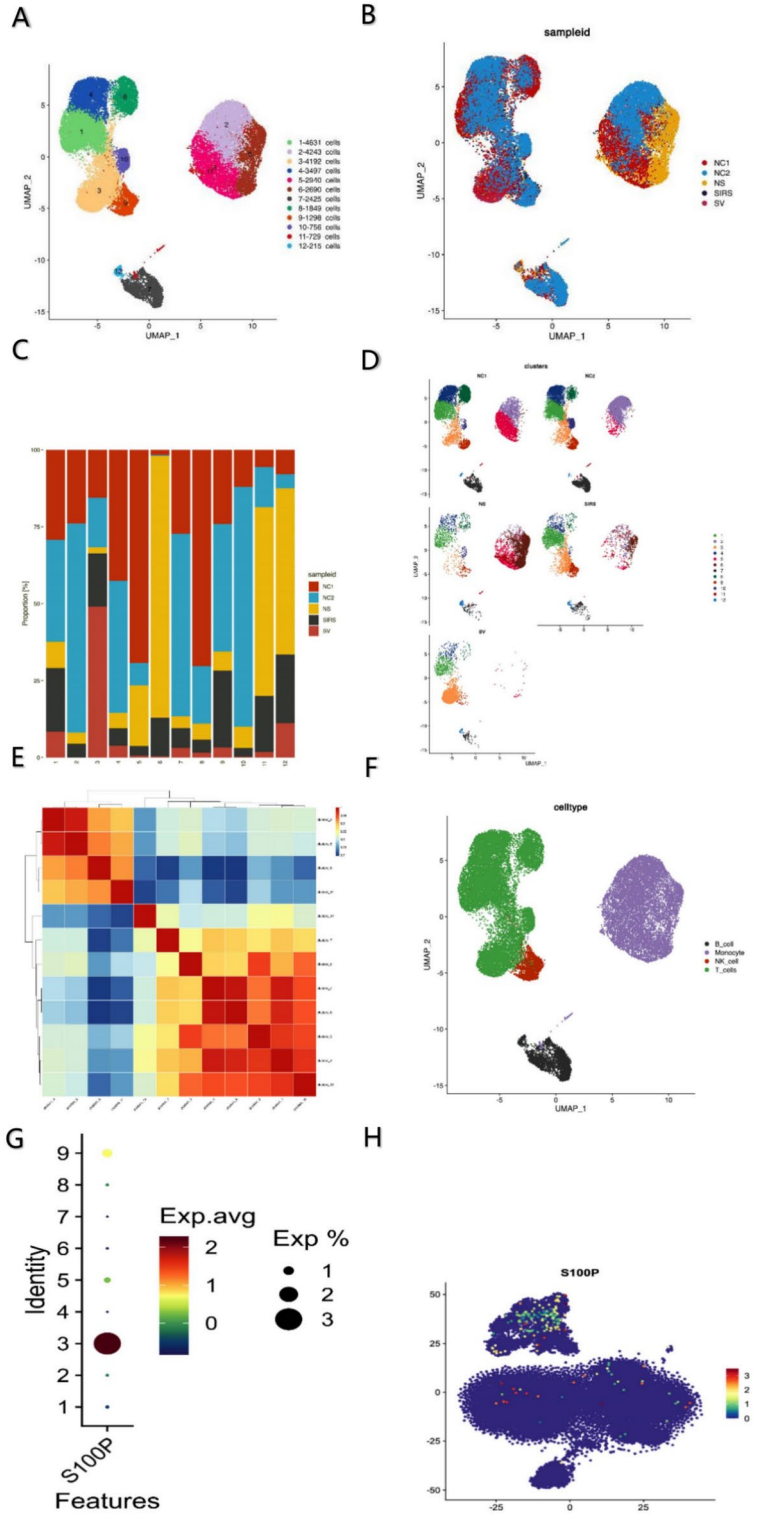

Fig. 10. Spatial distribution of core genes at the single-cell level. (**A**) The horizontal and vertical axes represent the first and second principal components, separately, after dimensionality decrement. (**B**) Cells from distinct sample sources are differentiated by distinct colors. (**C**) The proportion of each cell group is displayed on the basis of inter-sample cell distribution variations. (**D**) The lowered-dimensionality cell groups are distinguished by dissimilar colors. (**E**) A heatmap illustrates the degree of correlation between cell populations. (**F**) The UMAP plot shows the annotation results for cell types. (**G**–**H**) S100P expression and distribution in human peripheral blood mononuclear cells (PBMCs).

and is negatively correlated with patient survival rates[2]. Aside from that, mutations in B2M are associated with resistance to immune checkpoint inhibitor therapy, which may impact treatment efficacy in certain types of leukemia[41]. In AML, overexpression of B2M may accelerate myeloid proliferation by affecting the splicing of RUNX1. RUNX1, an essential transcriptional regulator, is frequently deregulated in leukemia and is often correlated with disease progression[42]. On top of that, the expression levels of B2M may interplay with mutations in other genes to influence the growth and differentiation of leukemic cells. B2M not only conducts a dramatic role in leukemia but is also considered a potential tumor suppressor gene in other cancers. As already revealed by associated research, mutations in B2M are correlated with tumor immune escape mechanisms, potentially affecting the antigen presentation function of major histocompatibility complex class I molecules[43]. This mechanism may also exist in hematologic malignancies such as leukemia, further affecting disease progression and treatment response.

S100A11, a calcium-binding protein belonging to the S100 protein family, has seen its role in multifarious cancers progressively elucidated in recent years. In leukemia, S100A11 is thought to impose conspicuous influence on disease progression and prognosis through multiple mechanisms. To begin with, in AML, S100A11 is considered a potential oncogenic factor and prognostic marker. As prior research demonstrates, its expression is tightly correlated with the tumor immune-suppressive microenvironment, potentially affecting the infiltration of tumor-associated macrophages (TAMs)[44]. Moreover, S100A11 exhibits correlations with immune-linked pathways, immune cells, and immune checkpoints in AML patients, which illustrates its underlying role in modulating the immune response against leukemia[45]. Aside from that, S100A11 may also influence the proliferation and apoptosis of leukemia cells by interacting with other proteins. For instance, the association of S100A11 with the TGF-β1/SMAD4 signaling pathway was studied in pancreatic cancer cells; although the specific mechanisms in leukemia remain unclear, similar signaling pathways may also conduct a role in leukemia[46]. At last, the role of S100A11 in leukemia may be associated with its localization within the cell cycle. In colorectal cancer cells, the nuclear accumulation of S100A11 during the G2/M phase, interacting with the cell cycle regulatory protein Cyclin B1, suggests a potential role in cell division, a mechanism that may also exist in leukemia cells[47].

As a large scaffold protein, AHNAK has validated its paramount role in a diverse array of diseases progressively unveiled in recent years. In leukemia, AHNAK may participate in disease progression and regulation through a diverse array of mechanisms. For instance, AHNAK might facilitate leukemia development by affecting cell proliferation and migration capabilities. In AML, AHNAK is bound up with epithelial-mesenchymal transition (EMT) of cells, a process crucial for tumor invasion and metastasis[48]. In addition, AHNAK may influence the pathological process of leukemia by modulating signaling pathways. Research demonstrates that AHNAK can accelerate tumor metastasis and invasion through the transforming growth factor-β (TGFβ) signaling pathway[49]. In certain types of leukemia, the expression levels of AHNAK are tightly linked with patient prognosis, which unveils its underlying as a biomarker for the diagnosis and treatment of leukemia[50].

S100A8, a calcium-binding protein, is regarded as a conspicuous biomarker in all manner of cancers. In AML, its high expression is bound up with chemoresistance and poor prognosis. As evidently demonstrated by relevant studies, S100A8 influences the apoptosis pathway, giving rise to resistance to etoposide and correlating with shorter overall survival[51]. Aside from that, S100A8 conducts a pivotal role within the bone marrow microenvironment. Bone marrow stromal cells can trigger the expression of S100A8 in AML cells by secreting interleukin-6 (IL-6) and activating the Jak/STAT3 signaling pathway. This intensified expression gives rise to the accumulation of a subpopulation of S100A8/A9 high-expressing cells, which exhibit higher resistance to chemotherapy drugs and BCL2 inhibitors[52]. S100A8 is also correlated with autophagy, which is a cellular process of degradation and recycling. S100A8 accelerates autophagy by directly interacting with the autophagy protein Beclin1, thereby strengthening cell survival and modulating chemoresistance in leukemia cells. Inhibition of S100A8 expression elevates the sensitivity of leukemia cells to chemotherapy and reduces autophagic activity[53].

ORM1 (Orosomucoid 1), an acute-phase reactant, was demonstrated in recent studies to conduct a conspicuous role in all manner of diseases, comprising cancer and thrombosis. The precise mechanisms by which ORM1 functions in leukemia remain unclear, yet existing research has unveiled its underlying impact on hematological disorders. Above all, the connection between ORM1 and thrombosis may offer insights into its role in leukemia. A study utilizing genome-wide association analysis (GWAS) revealed a correlation between the ORM1 gene and the variability in thrombin generation potential in plasma, which demonstrates that ORM1 might be involved in the pathogenesis and progression of leukemia by exerting certain influence on the coagulation process[54]. Moreover, ORM1 is implicated in the regulation of plasma free DNA levels, which function as a surrogate marker for neutrophil extracellular traps (NETs), conducting a paramount role in immune thrombosis[55]. Furthermore, studies on ORM1 in clear cell renal carcinoma reveal its impact on tumor progression through modulating apoptosis and cell proliferation. Despite the fact that this research primarily revolves around renal cancer, the mechanisms uncovered may also apply to leukemia. For instance, the expression levels of ORM1 correlate with changes in apoptosis-associated proteins, which demonstrates a potential analogous regulatory role of ORM1 in the survival and proliferation of leukemia cells[56]. Last but not least, investigations into ORM1's role in other diseases offer deep insights into its underlying involvement in leukemia. For example, in a rat model of septic stroke, ORM1 expression was strikingly upregulated, which exhibits its pivotal role in inflammatory responses[57]. Such inflammatory processes may be correlated with the pathophysiology of leukemia, as the development of leukemia often involves intricate immune and inflammatory reactions.

In exploring the potential mechanistic link between LYZ (lysozyme) and leukemia, multiple standpoints can be examined accordingly. First and foremost, LYZ, functioning as a secreted protein with antimicrobial properties, is typically expressed in monocytes and macrophages. Its prognostic significance in multifarious types of tumors was observed, particularly in hepatocellular carcinoma (HCC), where abnormal expression of LYZ is correlated with malignant tumor progression[58]. This aberrant expression may drive tumor progression

by affecting cellular metabolism and facilitating cell proliferation and migration. Furthermore, the specific mechanism of LYZ in leukemia remains unclear, yet relevant mechanisms identified in other studies can function as a reference. For instance, in AML, vitamin C, as a natural compound, has captured enormous attention on account of its anti-proliferative and pro-apoptotic activities. Vitamin C regulates TET enzymes in epigenetic modulation, a mechanism crucial in abnormal hematopoiesis and leukemogenesis[59]. Whether LYZ is involved in similar epigenetic regulatory mechanisms warrants further investigation. Furthermore, the intricate interplay between leukemic cells and the immune system may also implicate the function of LYZ. In chronic lymphocytic leukemia (CLL), dysregulated immune function is a remarkable issue, and novel targeted therapeutic strategies must take into account their impact on the immune milieu[60]. As a component of the immune system, LYZ's role in leukemia may be bound up with immune regulation.

TGFBI (Transforming Growth Factor Beta-Induced Protein) is recognized as a striking biomarker and a potential therapeutic target in a diverse array of cancers. In leukemia, the expression and function of TGFBI are likely tightly connected with disease progression and prognosis. As revealed by associated studies, the expression levels of TGFBI are positively correlated with the pathological grade of tumors, and in high-grade gliomas, TGFBI expression is noticeably higher than in normal brain tissue[61]. As this finding reveals, TGFBI may conduct a similar role in hematological malignancies such as leukemia. Moreover, the expression of TGFBI may be influenced by a variety of factors, comprising viral infections and exposure to environmental carcinogens. For instance, in the development of Burkitt's lymphoma, epigenetic modifications of TGFBI are believed to originate from the combined effects of EBV infection and aflatoxin B1 exposure[62]. This mechanism may also conduct a role in the pathogenesis of leukemia, which reveals that the expression of TGFBI in leukemia could be regulated by external environmental factors. Within the microenvironment of leukemia, TGFBI may accelerate tumor progression by affecting the formation of the bone marrow microenvironment. Correlated studies have arrived at a conclusion that TGFBI may collaborate with other members of the TGF-β family within the bone marrow microenvironment to create a pro-tumorigenic milieu, thereby facilitating the growth and spread of leukemia cells[63]. As evidenced by these significant findings, TGFBI not only functions within leukemia cells but also potentially influences disease progression by modulating its microenvironment.

S100P, a small calcium-binding protein, exhibits differential expression across all manner of tumor cell lines, thus exerting certain influence on tumor initiation, invasion, metastasis, and drug resistance. In leukemia, S100P expression is negatively correlated with clinical status and was observed in HL-60 and Jurat cell lines. Suppression of S100P expression triggers heightened leukemia cell proliferation and lessened chemosensitivity, while reinforcing S100P expression inhibits cell proliferation and ameliorates chemosensitivity. Apart from that, silencing of S100P strikingly accelerates autophagy, while its upregulation suppresses autophagy. Relevant studies have revealed that the p53/AMPK/mTOR pathway is bound up with S100P-mediated autophagy function. The knockdown of S100P expression results in an abatement in p53 and p-mTOR levels, along with an elevation in p-AMPK expression, thereby facilitating autophagy[64]. In oral squamous cell carcinoma (OSCC), S100P was identified as a key downstream effector of BCL10 in inhibiting OSCC progression. The mRNA and protein expression levels of S100P substantially declined in clones with silenced BCL10, while the transfection of an S100P expression plasmid restored the migration, invasion, proliferation, and tumor-forming abilities of shBCL10 transfectants. On top of that, BCL10 regulates S100P expression through signal transducer and activator of transcription 1 (STAT1) and activating transcription factor 4 (ATF4). Knockdown of BCL10 lowered S100P promoter activity but had no effect on the truncated STAT1/ATF4 S100P promoter[65]. In colorectal cancer (CRC), a positive feedback loop exists between S100P and Trx-1, which facilitates the EMT of CRC. As already demonstrated by relevant studies, knockdown of either Trx-1 or S100P inhibits EMT, while overexpression of Trx-1 or S100P reinforces EMT. Notably, S100A4 and AKT phosphorylation were identified as potential downstream targets of Trx-1 and S100P in CRC cells. Silencing of S100A4 or inhibition of AKT phosphorylation abolishes the EMT, migration, and invasion of CRC cells mediated by S100P or Trx-1[66].

LCN2 (Lipocalin-2) conducts an irreplaceable role in multifarious biological and pathological processes, which comprise cancer and inflammation. As already suggested by previous studies, LCN2 may be connected with the onset and progression of leukemia through multiple mechanisms. First and foremost, its role in regulating apoptosis could influence the pathological course of leukemia. Proteomic analysis has revealed noticeable changes in the expression of apoptosis-relevant proteins in mouse models lacking LCN2, which illustrates that LCN2 may regulate the survival and death of leukemia cells[67].Furthermore, LCN2 may influence the progression of leukemia through its interactions with the Wnt/β-catenin signaling pathway. As already illustrated by correlated studies, LCN2 can inhibit osteogenic differentiation induced by BMP9 by binding to LRP6, while accelerating adipogenesis. This mechanism may similarly operate in leukemia, thereby exerting certain influence on disease progression by modulating cellular differentiation and proliferation[68]. The role of LCN2 in inflammation may also be relevant to leukemia. Under both acute and chronic conditions, LCN2 may exhibit dual roles, which potentially suppresses inflammation following acute muscle ischemia while also being linked with chronic vascular damage. This dual functionality could conduct a paramount role in the inflammatory microenvironment of leukemia, thereby exerting certain influence on disease progression and patient prognosis[69].

In Fig. 2C, the differential proteins bound up with sepsis are associated with fatty acid metabolism, with protein palmitoylation serving as a crucial post-translational modification process in this regard. This process involves the covalent attachment of long-chain fatty acids, such as palmitic acid, to the cysteine residues of proteins via thioester bonds. Such modifications conduct a pivotal role in regulating protein trafficking, cellular localization, and stability. More importantly, they are involved in a variety of biological processes[70]. Among the differential proteins in this study, fatty acid-binding protein GSTO1conducts a noticeable role in multiple cellular processes, particularly in antioxidant defense and drug metabolism. In recent years, researchers have conducted in-depth studies on the function of GSTO1, uncovering its underlying roles in cancer and neurodegenerative

diseases[71]. The palmitoylation modification of GSTO1 may influence its intracellular localization and function, a modification commonly connected with protein membrane binding and signal transduction. To start with, the role of GSTO1 in cancer was extensively explored. Elevated expression of GSTO1 is associated with drug resistance in certain cancers, and inhibiting GSTO1 can elevate the efficacy of chemotherapy drugs[71]. Moreover, the inhibition of GSTO1 in breast cancer stem cells can activate the JNK-mediated apoptotic response, thereby reducing the migratory and invasive capabilities of cancer cells[72]. As evidently illustrated by the above research findings, GSTO1 may influence cancer progression by modulating cellular signaling pathways and apoptosis. Apart from that, the protective role of GSTO1 in neurodegenerative diseases has also garnered attention. Research suggests that GSTO1 can safeguard neuronal cells from oxidative stress damage by regulating the MAPK signaling pathway[73]. This protective effect may be linked with the antioxidant enzyme activity of GSTO1, which can neutralize reactive oxygen species within cells, thereby reducing cellular damage.

S100P belongs to the S100 family of calcium-binding proteins, which includes S100B, S100A6, S100A8/A9, and others. The family members a diverse array of biological functions, encompassing the modulation of protein phosphorylation levels, the regulation of enzymatic activity, the control of cellular growth, differentiation, and apoptosis, as well as active participation in maintaining intracellular calcium (Ca^{2+}) homeostasis, coagulation regulatory processes, and the elicitation of inflammatory immune responses.[74–76]. S100 family proteins are involved in the development of a diverse array of malignant diseases, degenerative diseases, and inflammatory diseases[77–79]. Associated studies have reached a conclusion that S100P overexpression can accelerate the metastasis of benign rat breast cell lines[80], and is further correlated with augmented single-cell migration and collective cell invasion processes[81,82]. Relevant literature suggests that S100P can be involved in multiple processes such as angiogenesis, immune evasion, and can inactivate p53[83,84]. As already demonstrated by relevant research, S100P is a new immunoreacted biomarker, and its prognostic significance has been discussed in an all-round manner[85]. Nevertheless, its role in sepsis and prediction of prognosis has not been systematically explored from multifarious aspects.

In Fig. 6C–D, the enrichment analysis results illustrate that genes differentially expressed in sepsis may be bound up with amyloidosis. Apart from that, studies have reached a conclusion that S100P conducts a striking role in multifarious neurological diseases, particularly in diabetic peripheral neuropathy (DPN) and neuropathic pain. Research shows that the serum concentration of S100P is tremendously elevated in patients with diabetic peripheral neuropathy, which reveals its remarkable potential as a biomarker for the early identification and treatment of diabetic neuropathy[86]. Moreover, S100P is bound up with the onset and progression of neuropathic pain. Associated studies on genes and pathways linked with neuropathic pain have revealed that S100P may be involved in the molecular mechanisms underlying neuropathic pain and could potentially serve as a novel target for future treatments of this condition[87]. In the context of diabetic peripheral neuropathy, S100P plays a role that transcends mere biomarker status. Emerging research indicates that it may also play a pivotal part in the neuropathological process by modulating immune responses and inflammatory activities. To be more specific, S100P may facilitate the onset and progression of neuropathy by affecting signaling pathways such as neuroactive ligand-receptor interactions, the complement and coagulation cascades, and cytokine-receptor interactions[87]. Furthermore, research has delved into the role of S100P in neuropathic pain. As illustrated by an in-depth analysis of the molecular mechanisms underlying neuropathic pain, S100P might influence the occurrence and maintenance of neuropathic pain by regulating interactions between neurons and non-neuronal cells. These findings offer groundbreaking insights for developing therapeutic strategies targeting S100P[88]. In other studies, research on biomarkers associated with diabetic neuropathy may explore the following directions: ① the impact of nerve stimulation on VGLUT2 expression and glutamate release; ② the role of APP family members in neurostimulation therapy; ③ the interaction between VGLUT2 and the APP family in diabetic neuropathy[89]. Such investigations could potentially uncover the molecular mechanisms underlying S100P's role in diabetic neuropathy and pave the way for new therapeutic strategies. The proteins in its family are involved in multifarious biological processes such as regulating apoptosis, proliferation, differentiation, migration, invasion, energy metabolism, calcium homeostasis, protein phosphorylation, antibacterial activity, and inflammation[87]. In inflammatory diseases and cancer, as associated studies have unveiled, in inflammatory bowel disease (IBD), S100P, along with other S100 proteins such as S100A8/A9 and S100A12, participates in the pathogenesis, activity, diagnosis, and therapeutic management of the disease. While most research has primarily focused on S100A8/A9, increasing evidence highlights the significance of S100P in IBD[88]. Abnormal expression of these proteins may be tightly linked with the pathophysiological processes of IBD, and further research could unveil their potential as diagnostic biomarkers. Aside from that, the role of S100P in tumors has garnered conspicuous attention. Members of the S100 protein family exhibit diverse functions in tumor development, particularly in the development of drug resistance. Abnormal expression of S100P may give rise to elevated resistance to therapy in tumors, thus, combinations of biological inhibitors or sensitizers targeting S100P could potentially elevate therapeutic responses[86]. An in-depth understanding of the molecular mechanisms of S100P in tumors can bring about the development of more effective strategies to overcome multidrug resistance.

We have identified S100P as being associated with pathological changes in renal fibrosis. As already suggested by correlated studies, selenium, an essential trace element, is crucial for human antioxidant defense, immune function, and thyroid hormone metabolism. In sepsis and kidney diseases, selenium's antioxidant properties may aid in alleviating inflammation and cellular damage. Apart from that, the gene LRRC19 is implicated in cell signaling and cell cycle regulation, and in the context of kidney diseases and sepsis, selenium-based adjuvant therapy might operate through the following mechanisms: ①Antioxidant action: Selenium could reinforce the activity of antioxidant enzymes, such as glutathione peroxidase, to lower oxidative stress and protect renal cells from damage; ② Anti-inflammatory action: Selenium might modulate inflammatory responses by imposing certain influence on the production of inflammatory cytokines and inflammatory signaling pathways, thereby alleviating renal inflammation; ③Influence on gene expression: Selenium could regulate cell cycle and cell death

by affecting the expression of genes like LRRC19, thereby affecting the progression and outcome of the diseases. Thus, we infer that in the adjunctive treatment of sepsis and selenium-based kidney diseases, differentially expressed genes may involve the following mechanisms: ① Regulation of inflammatory response: Differentially expressed genes may participate in the modulation of inflammatory responses, affecting the severity of sepsis and kidney diseases; ② Regulation of cell cycle: Differentially expressed genes may influence the progression of the cell cycle, thereby affecting cell proliferation and death, which conducts an irreplaceable role in disease development and treatment efficacy; ③ Regulation of antioxidant defense: Differentially expressed genes may be involved in the regulation of antioxidant enzyme activity and antioxidant responses, impacting the cell's resistance to oxidative stress[90]. Septicemia, as a complex systemic inflammatory response syndrome, involves intricate pathological mechanisms that encompass alterations in numerous signaling pathways and gene expression. Relevant studies have demonstrated that selenium, an essential trace element, conducts a pivotal role in modulating immune responses and mitigating oxidative stress. In the context of adjunctive therapy for septicemia and selenium-based kidney diseases, differentially expressed genes may influence disease progression and treatment efficacy through a diverse array of mechanisms. To start with, selenium deficiency could potentially result in lessened expression of sialoproteins in septic patients, thereby impairing the function of the antioxidant system. Selenium-containing proteins, such as glutathione peroxidase (GPX) and thioredoxin reductase (TXNRD), play crucial roles in maintaining cellular redox balance. As relevant studies suggest, in cases of selenium deficiency, the expression of these selenium-containing proteins noticeably declines, resulting in augmented oxidative stress, which may exacerbate the inflammatory response and tissue damage associated with sepsis[91,92]. In addition, selenium influences the course of sepsis by modulating the expression of genes associated with inflammation. It can regulate the production and release of inflammatory factors by affecting signaling pathways such as nuclear factor-κB (NF-κB).Research has illustrated that supplementation with selenium can lower the expression of inflammatory factors such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), thereby alleviating inflammatory responses[93,94]. On top of that, selenium may exert protective effects by exerting certain influence on the expression of genes associated with cell apoptosis. In septic and selenium-relevant kidney diseases, cell apoptosis is a crucial mechanism contributing to tissue damage. Selenium can protect tissues by modulating the expression of apoptosis-associated genes, such as Bcl-2 and the Caspase family, to inhibit cell apoptosis[95,96].

Nonetheless, in the learning of many bioinformatics approaches rooted in machine learning technology, there are particularly diverse analytical standpoints that can be adopted in future analytical research in the following modes: ① Regarding data collection and processing: unlike our data acquisition approaches, data on the expression and methylation of normal cells at all manner of differentiation stages can be collected from databases such as PCBC (Progenitor Cell Biology Consortium), Roadmap, and ENCODE; furthermore, we sequence and perform mass spectrometry through blood, utilizing the One-Class Logistic Regression (OCLR) machine learning method to train data on stem cells and cells at diverse differentiation levels, extracting cellular expression and methylation features to construct a stemness model; ② In terms of data analysis, we use websites and software to calculate the degree of association between genes and diseases, while they calculate the stem cell index rooted in the previously obtained stemness model, computing the mRNAsi and mDNAsi stem cell indices from data in TCGA. These indices are specified to range between 0 and 1, with values closer to 1 revealing lower cell differentiation and stronger stem cell characteristics. ③ In exploring how disease development associates with relevant genes, we employ meta-analysis, ROC curves, and survival rates, which are derived by integrating mRNAsi and mDNAsi with clinical data, gene expression, and DNA methylation features from TCGA tumor samples to characterize how stem cell indices correlate with tumor progression and biological processes. ④ Apart from that, the study analyzes the immune microenvironment of tumors to dig into its correlation with cancer cell stemness. ⑤ For identifying and validating key genes, we utilize PPI protein network mapping to examine interactions among key genes and conduct enrichment analysis to annotate their functions and pathways. ⑥ Regarding the validation of expression levels of key genes, our team employs Q-PCR to verify distinctions in expression between sepsis and normal groups, extending this validation across pan-cancer studies and confirming gene expression levels and survival probabilities associated with pathological stages. As a consequence, by comparing machine learning-based bioinformatics analysis approaches, it is possible to apply and practice the discovery of expression characteristics of cellular biomarkers specific to sepsis, thereby offering novel viewpoints for the treatment of sepsis[97,98].

Our research centers around starting from preliminary sequencing data, by employing bioinformatics approaches to probe deep into S100P as a potential key target for diagnosing sepsis. Through in vitro cell experiments, we have verified its differential expression in sepsis and healthy controls, which demonstrates that this gene conducts a pivotal role in the occurrence and development of sepsis. Nonetheless, the specific mechanism of its action in sepsis is still unclear, and we will fix attention on exploring this aspect in the later stage.

Conclusion

Through bioinformatics analysis and in vitro experiments, discrepancies in expression levels between sepsis and NC were identified accordingly. We screened and validated one gene, which revealed reinforced expression in sepsis, illustrating its significant potential as a candidate core target.

Availability of data and materials

We intend to share individual deidentified participant data. Peripheral bloodRNAsequencing(RNA sequencing) data from 19 patients with sepsis and 10 H ealthy people areavailable 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 svalid forever.

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Author contributions

SYZ, LHL AND HYC designed the study. LHL 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.

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Declarations

Competing interests

Authors state no competing interests.

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

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 ofSouthwest Medical University (Ethical Approval No. ky2018029). The Registration NumberSouthwest Medical University (Ethical Approval No. ky2018029). The Registration Number was ChiCTR1900021261.was ChiCTR1900021261.

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

Additional information
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