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ORIGINAL RESEARCH

Cuproptosis-Related Genes as Prognostic Biomarkers for Sepsis: Insights into Immune Function and Personalized Immunotherapy

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Background: This study aimed to discover diagnostic and prognostic biomarkers for sepsis immunotherapy through analyzing the novel cellular death process, cuproptosis.

Methods: We used transcriptome data from sepsis patients to identify key cuproptosis-related genes (CuRGs). We created a predictive model and used the CIBERSORT algorithm to observe the link between these genes and the septic immune microenvironment. We segregated sepsis patients into three subgroups, comparing immune function, immune cell infiltration, and differential analysis. Single-cell sequencing and real-time quantitative PCR were used to view the regulatory effect of CuRGs on the immune microenvironment and compare the mRNA levels of these genes in sepsis patients and healthy controls. We established a sepsis forecast model adapted to heart rate, body temperature, white blood cell count, and cuproptosis key genes. This was followed by a drug sensitivity analysis of cuproptosis key genes.

Results: Our results filtered three key genes (LIAS, PDHB, PDHA1) that impact sepsis prognosis. We noticed that the high-risk group had poorer immune cell function and lesser immune cell infiltration. We also discovered a significant connection between CuRGs and immune cell infiltration in sepsis. Through consensus clustering, sepsis patients were classified into three subgroups. The best immune functionality and prognosis was observed in subgroup B. Single-cell sequencing exposed that the key genes manage the immune microenvironment by affecting T cell activation. The qPCR results highlighted substantial mRNA level reduction of the three key genes in the SP compared to the HC. The prediction model, which combines CuRGs and traditional diagnostic indicators, performed better in accuracy than the other markers. The drug sensitivity analysis listed bisphenol A as highly sensitive to all the key genes.

Conclusion: Our study suggests these CuRGs may offer substantial potential for sepsis prognosis prediction and personalized immunotherapy.

Keywords: sepsis, cuproptosis, prognosis, immune cell infiltration, nomogram

Introduction

Sepsis, a life-threatening organ dysfunction, arises from an abnormal response to infection within the host body.[1] It affects approximately 49 million people worldwide each year and accounts for around 11 million fatalities.[2] The early symptoms of sepsis are relatively vague and can be easily confused with other diseases. Some clinical scoring systems, such as SOFA and qSOFA, cannot accurately reflect patients' individual differences. Despite recent progress in sepsis diagnosis and treatment, both the incidence and death rates remain high. Over 250 sepsis biomarkers, including procalcitonin, C-reactive protein, cytokines, among others, have been discovered.[3–7] Early diagnosis and prognosis assessment for early intervention before organ dysfunction is critical to improving survival rate of sepsis. Due to their poor sensitivity and specificity, the existing biological markers cannot be used for sepsis prognosis prediction.[8,9]

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Therefore, there is a shortage of clinically-relevant, prognostic biomarkers. The identification and validation of reliable sepsis biomarkers hold the key to guiding clinical decisions as well as refining diagnostic and treatment approaches.

Cuproptosis, a newly identified form of cell death, is reliant upon the accumulation of copper ions within cells, thereby leading to cell death via copper ion carriers.[10] Distinct from known cell death regulation mechanisms such as apoptosis, necrosis, and ferroptosis, this non-apoptotic pathway engages copper, existing as Cu^{+} and Cu^{2+} within organisms, in the direct regulation of both physiological and pathological cellular processes. Cuproptosis is related to mitochondrial dysfunction. The elevation of intracellular copper ion concentrations triggers the aggregation of lipoic acid-modified proteins like lipoylated dihydrolipoyl transacetylase (Lip-DLAT) and lipoylated dihydrolipoamide succinyltransferase (Lip-DLST) within the tricarboxylic acid cycle of mitochondria, under the mediation of key protein Ferredoxin 1 (FDX1). Subsequently, this downregulates iron-sulfur (Fe-S) cluster proteins such as FDX1, lipoic acid synthetase (LIAS), and succinate dehydrogenase B (SDHB), instigating protein toxicity stress, and resulting in cell death.[11,12] The mitochondria serve as the hub of cell metabolism regulation, and studies have shown that mitochondrial dysfunction is closely related to the progression of sepsis. Research has found a pronounced fragmentation of mitochondria in septic endothelial cells, associated with decrease in mitochondrial membrane potential and increase in reactive oxygen generation.[13] Moreover, therapeutic interventions aimed at mitigating mitochondrial function have promising applications for improving the prognosis of sepsis.[14] However, the precise role of cuproptosis in sepsis remains indefinite at present.

Dysregulation of the immune system plays a pivotal role in sepsis progression. At the onset, sepsis elicits an overwhelming discharge of pro-inflammatory mediators proficient at eradicating pathogens, but also culpable for inflicting immune-related damage.[15] Immune cells including macrophages, neutrophils, dendritic cells, and regulatory T cells (Tregs) orchestrate and harmonize the inflammatory response via secreting anti-inflammatory cytokines. Excessive inflammation potentially triggers cell pyroptosis, creates neutrophil extracellular traps (NETosis), and instigates widespread cellular death.[16] The subsequent phase of sepsis is marked by immune suppression featuring diminished quantity and function of macrophages, deterred antigen presentation ability, and compromised lymphocyte proliferation activity.[17,18] Natural Killer (NK) cells also play a dual role in the progression of sepsis. On one hand, they can prevent the spread of infection by eliminating infected cells. On the other hand, an excessive NK cell response could lead to a systemic inflammatory response, accelerating the progression of sepsis.[19,20] Concurrently, the emission of inhibitory cytokines reinforces immune suppression. This scenario creates an environment conducive for secondary infections which largely contribute to heightened patient mortality rates. Yet, the regulatory impact of cuproptosis on the immune microenvironment during sepsis is still surrounded by uncertainty.

Hence, our intention is to build a new model for cuproptosis-related genes (CuRGs) associated with sepsis. This model will be used to evaluate the prognosis of sepsis and further understand the impact of CuRGs on immune cell infiltration in sepsis. Our objective is to uncover more details about the potential molecular immune procedures involved in sepsis progression and explore innovative interventions and therapeutic strategies for sepsis.

Materials and Methods

Data Collection and Differential Analysis

The GSE65682 dataset, encompassing healthy and sepsis samples, was gleaned from the Gene Expression Omnibus (GEO) database. The transcriptome dataset underwent normalization through the use of the "sva" script to mitigate the batch effect. Subsequent to the eradication of sepsis samples devoid of a 28-day survival record, a collection of 42 healthy samples along with 479 sepsis samples were incorporated into the prognosis analysis (The baseline and clinical characteristics of septic patients from GSE65682 was in Supplementary Table 1). The differential gene expression analysis was conducted with parameters of $|\text{fold change}| \geq 2$ and $p < 0.05$.

Construction of CuRGs Risk Model for Sepsis

The prognostic value of nine differentially expressed CuRGs (DE-CuRGs) for sepsis was assessed using a univariate Cox analysis. A multivariate Cox analysis was then implemented to derive a formula for evaluating the CuRGs risk score of

each sepsis sample: CuRGs risk score = LIAS x (−2.64) + PDHA1 x (−3.10) + PDHB x (−2.82). The "caret" package was deployed to categorize the sepsis samples into training and testing cohorts in a 1:1 ratio, with the CuRGs risk score of each sample calculated accordingly. Leveraging the optimal survival cutoff, the sepsis samples were then segmented into low- and high-risk groups.

Generation of CuRGs Molecular Subgroups for Sepsis

The "ConsensusClusterPlus" tool was utilized to categorize sepsis patients in the GSE65682 dataset into various CuRGs molecular subgroups, with an optimal K range of 2–9. Subsequently, according to the consensus matrix, it was discovered that when K=3, the degree of mixing in the consensus matrix is the lowest. Therefore, it was decided to partition the samples into three subgroups: A, B, and C. A principal component analysis was subsequently executed to elucidate the distribution pattern of sepsis within these CuRGs molecular subgroups, employing the "ggplot2" script for this purpose. To estimate the 28-day survival outcome for the sepsis samples within the CuRGs subgroup, the "survival" script was engaged, linking it with the correspondingly available clinical survival information for each sepsis sample.

Immune Infiltration Analysis

In order to investigate the immune infiltration of healthy control and sepsis samples, the CIBERSORT algorithm was applied using the "CIBERSORT R script v1.03" to determine the proportion of immune cells present. The association analysis between the immune cells and CuRGs was executed via the "ggplot2" R package. The immune score was evaluated by utilizing the "estimate" R package, while the analysis of the immunological function score was conducted with the R package "GSVA".

Function Enrichment Analysis

Setting the selection threshold at $p < 0.05$ enabled the calculation of differentially expressed genes (DEGs) in the CuRGs subgroups, achieved through the deployment of the "limma" script. The "clusterProfiler" script was employed to facilitate the enrichment of these DEGs into gene ontology (GO) terms. Furthermore, to assess the Kyoto Encyclopedia of Genes and Genomes (KEGG) terms related to sepsis in the CuRGs subgroups, the "GSVA" script was implemented.

Single-Cell Sequencing Analysis

In this study, we analyzed the scRNA-seq dataset GSE167363, acquired from the GEO database, which comprised data from five sepsis patients and two healthy controls.[21] Dimensionality reduction and clustering of the dataset utilized functions from Seurat version 3.1.2. Gene expression was normalized and scaled using the NormalizeData and ScaleData functions in Seurat. This was followed by the identification of the top 2000 variable genes using the FindVariableFeatures function to carry out Principal Component Analysis (PCA). The first 20 principal components were then implemented to categorize cells into distinct clusters via the FindClusters function. For a two-dimensional visualization of the cellular distribution, we applied the Uniform Manifold Approximation and Projection (UMAP) algorithm. Expression profiles for each cell type were visualized using DoHeatmap, a heatmap-generating function from Seurat. To infer the putative functions of the DEGs, GO and KEGG analyses were performed using the clusterProfiler R package, version 3.16.1. Pathways significantly enriched with DEGs were identified, with an emphasis on those paths with an adjusted p-value (p_adj) of less than 0.05. These analyses included Gene Ontology gene sets across the molecular function (MF), biological process (BP), and cellular component (CC) categories.

Establishment of Nomogram and Independent Prognostic Analysis

In consideration of the clinical variables and the 28-day survival outcome, an evaluation was conducted to verify the independence of the CuRGs risk score, utilizing both univariate and multivariate Cox analyses. The "survivalROC" script was employed to assess the area under the curve (AUC) over periods of 7, 14, and 28 days.

Population Recruitment

We conducted a recruitment of 30 patients diagnosed with sepsis in accordance with the Sepsis-3 criteria, a reference for sepsis and septic shock, from Daping Hospital, Army Medical University. The specifics details of inclusion and exclusion criteria for septic patients can be found in Supplementary Table 2. In addition, 15 healthy individuals, matched by age, voluntarily participated from the State Key Laboratory of Trauma, Burns, and Combined Injury. All participants extended a written informed consent before their participation in the study. Within 24 hours of admission, blood samples (10mL) were collected from septic patients, while samples from the healthy controls were obtained during their recruitment.

Quantitative Real-Time RT-PCR (qRT-PCR)

The qRT-PCR method refers to previous research.[5] The relative gene expression levels were determined using the efficiency-corrected $2^{-\Delta\Delta CT}$ method, with β-actin serving as an internal control.

Statistical Analysis

R software (version 4.1.2) was utilized for all statistical analyses. Any P-value less than 0.05 was deemed to indicate a statistically significant difference.

Results

Differential Expression Analysis of CuRGs in Healthy and Sepsis Groups

Initially, differential gene analysis was conducted on the transcriptome of blood samples from sepsis patients using the limma package, with a screening criterion of $p<0.05$. The volcano plot Results revealed differential gene upregulation and downregulation in sepsis patients when compared to the healthy control group (Figure 1A). Additionally, a Venn diagram was utilized to identify 9 differential CuRGs in sepsis, shedding light on the role of CuRGs (Figure 1B). The PPI network diagram depicted evident interactions involving 10 CuRGs (Figure 1C). Furthermore, the results of the differential expression analysis demonstrated significant downregulation of FDX1, LIAS, LIPT1, DLAT, PDHA1, PDHB, and GLS, while DLD and MTF1 were significantly up-expressed in the sepsis group (Figure 1D).

The Prognostic Characteristics of CuRGs in Sepsis

In the subsequent study, we investigated the prognostic characteristics of CuRGs in sepsis. Based on univariate Cox analysis, we evaluated the prognostic significance of 10 CuRGs and the network results revealed that three CuRGs, including LIAS, PDHH1, and PDHB, were associated with the 28-day survival outcome of sepsis. Additionally, we observed a significant negative correlation between DLAT, MTF1, LIPT1, and FDX1 (Figure 2A). The results of multivariate Cox analysis indicated that three CuRGs were independent prognostic factors for sepsis, among which LIAS and PDHB were identified as favorable factors, while PDHA1 was a risk factor (Figure 2B).

We calculated the coefficients and expression levels of three independent prognostic factors using multifactor Cox analysis. Using these results, we computed the CuRGs score for each sepsis sample and stratified the samples into subgroups based on the median CuRGs score. Figure 2C–E revealed a significant elevation of PDHA1 expression in the high CuRGs score group, while LIAS and PDHB were expressed at higher levels in the low CuRG score group. Unsupervised PCA analysis demonstrated two distinct distribution patterns of sepsis samples within the CuRGs score subgroups (Figure 2F). Clinical survival outcomes showed that the 28-day prognosis for the low CuRGs score group was superior to that of the high CuRGs score group (Figure 2G). Time-dependent ROC curve analysis demonstrated AUC of 0.733, 0.652, and 0.662 for 7, 14, and 28 days, respectively (Figure 2H). These findings suggest that constructing a CuRGs score can effectively stratify sepsis samples into different CuRGs score subgroups, which may have prognostic value for 28-day survival outcomes.

Validation of the CuRGs Prognostic Model

In order to assess the independence and reliability of CuRGs scores in predicting 28-day clinical outcomes of sepsis samples, we employed the "caret" script to divide 479 sepsis samples from GSE65862 into a 1:1 ratio. Subsequently, 240 samples were

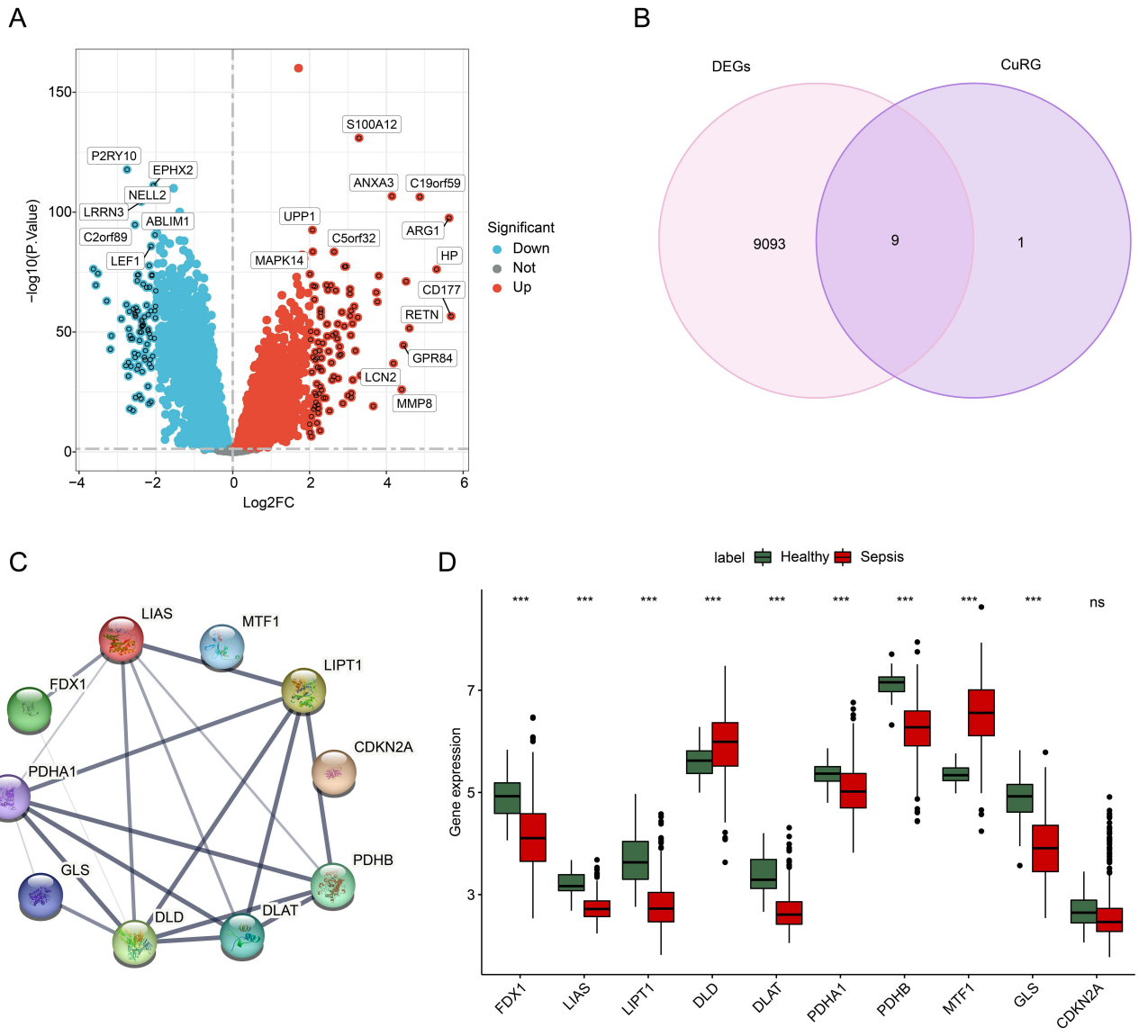

Figure 1 Difference analysis of CuRGs between healthy and sepsis groups. (**A**) Difference analysis of healthy and sepsis groups in GSE65682. The cutoff for selecting DEGs is set at P<0.05. (**B**) Identification of DE-CuRGs. (**C**) PPI network displays the relationship between 10 CuRGs. (**D**) Expression profile of 10 CuRGs in the healthy and sepsis groups. ***P<0.001; ns: P>0.05.

assigned to the training set while 239 samples were assigned to the validation set. Based on the median value of CuRGs scores, we categorized the sepsis samples from both independent cohorts into low and high CuRGs subgroups (Figure 3A and B). Consistent with the CuRGs prognostic model results for the entire cohort, we observed that LIAS and PDHB were significantly overexpressed in the low CuRGs score subgroup while PDHA1 was significantly overexpressed in the high CuRGs score subgroup. In the training set, the time-dependent ROC curves for 7-, 14-, and 28 days showed AUC values of 0.731, 0.662, and 0.694, respectively. In the validation set, the time-dependent ROC curves for 7-, 14-, and 28 days showed AUC values of 0.732, 0.641, and 0.628 (Figure 3C–F). Finally, the 28-day clinical survival outcomes revealed that the prognosis of the low CuRGs subgroup was significantly better than that of the high CuRGs subgroup in both independent cohorts (Figure 3G and H).

Relationship Between CuRGs Prognosis Model and Immune Microenvironment in Sepsis

The immune microenvironment in sepsis patients is closely related to patient prognosis, therefore we further analyzed the relationship between cuproptosis-related key genes and sepsis immune microenvironment. First, we analyzed the

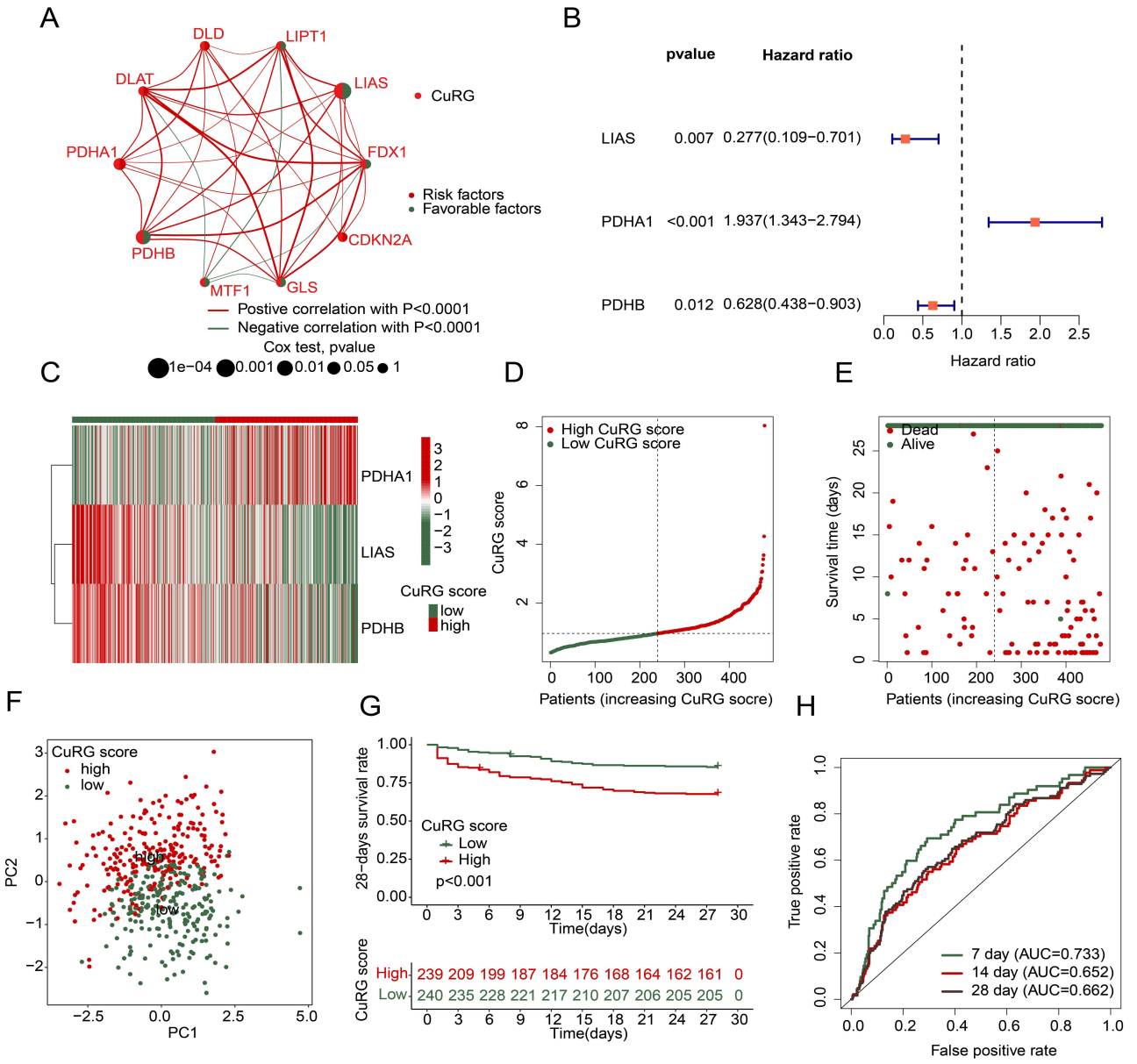

Figure 2 Developing a prognostic model for predicting the outcome of sepsis related to CuRGs. (**A**) The network showed the correlation and prognostic value of CuRGs. (**B**) Multivariate Cox analysis. (**C**) A cluster heatmap illustrating the gene expression level of PDH1, LIAS, and PDHB. (**D** and **E**) Risk subgroup classification and analysis of clinical survival outcomes at 28 days. (**F**) Principal Component Analysis (PCA) plot displaying the distribution of sepsis in different risk subgroups. (**G**) A comparison of the survival curves between the high-risk and low-risk groups. (**H**) ROC analysis of 7-, 14-, and 28 days.

composition of immune cells in septic patients through the Cibersort algorithm (Figure 4A). Then, through the analysis of immune cell function, we found significant differences in immune cell function between CuRGs high-risk and low-risk groups. Compared to the low-risk group, the high-risk group had poorer immune function, including cytolytic activity, HLA, inflammation promoting, MHC class I, para inflammation, T cell co-inhibition, T cell co-stimulation, TIL, type I IFN response, and type II IFN response (Figure 4B). At the same time, the immune score of the high-risk group was lower than that of the low-risk group, further indicating that CuRGs inhibits immune function in sepsis patients (Figure 4C). In addition, we also compared the immune cell infiltration between the two groups. The results showed that the proportions of B cells naïve, B cells memory, T cells CD8, T cells CD4 naïve, T cells gamma delta, NK cells resting, NK cells activated, dendritic cells resting, and dendritic cells activated were significantly lower in the high-risk group compared to the low-risk group, while the proportions of macrophages M0, NK cells activated, and eosinophils

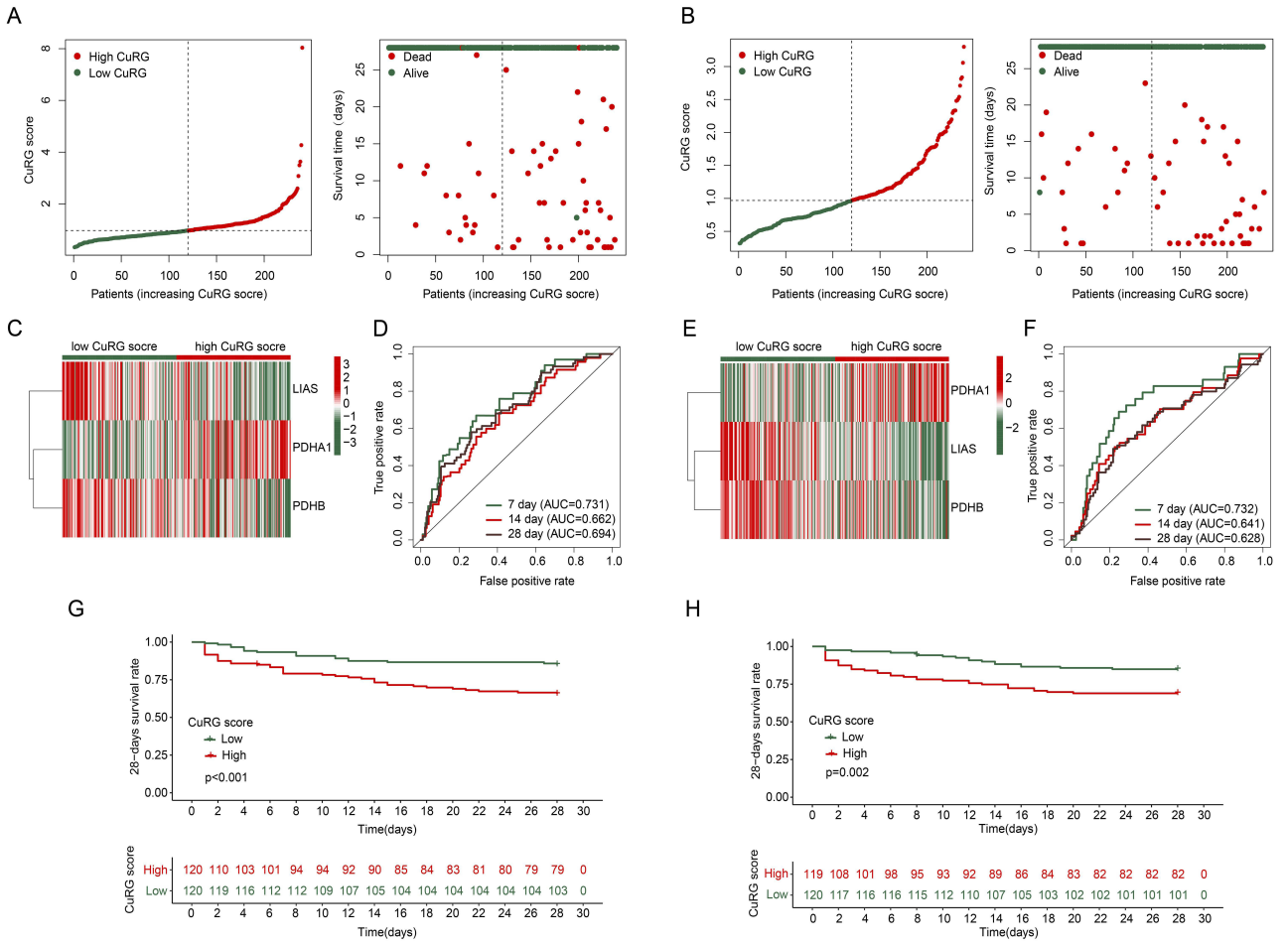

Figure 3 Validation of the CuRGs prognostic model in the training and validation sets. (**A** and **B**) Stratification of high and low CuRG samples. (**C**) Expression profiling analysis of three independent prognostic factors in the training set. (**D**) Time-dependent ROC curve analysis in the training set. (**E**) Expression profiling analysis of three independent prognostic factors in the validation set. (**F**) Time-dependent ROC curve analysis in the validation set. (**G** and **H**) Evaluation of the 28-day clinical survival outcomes of sepsis samples in the training and validation sets.

were significantly higher in the high-risk group (Figure 4D). Correlation analysis also showed a positive correlation between CuRG score and macrophages M0, NK cells activated, and eosinophils, and a negative correlation with B cells memory, T cells CD8, and T cells CD4 naïve, which was consistent with the immune infiltration results (Figure 4E). The correlation analysis between cuproptosis-related key genes and immune cells showed that the expression of PDHA1 was positively correlated with macrophages M0 and eosinophils, and negatively correlated with neutrophils. The expression of LIAS was positively correlated with T cells CD8 and negatively correlated with dendritic cells activated. The expression of PDHB was positively correlated with T cells CD4 memory and monocytes, and negatively correlated with dendritic cells activated (Figure 4F). The above results indicate a correlation between CuRG key genes and immune microenvironment of sepsis.

Consensus Clustering Subtypes Analysis of Sepsis

In the subsequent study, we further analyzed the molecular subtype characteristics of sepsis samples based on three independent prognostic factors (CuRG key genes). According to the expression profiles of the three CuRG key genes, we obtained three subgroups of sepsis samples, namely CuRGs subgroup A with 135 samples, CuRGs subgroup B with 214 samples, and CuRGs subgroup C with 130 samples (Figure 5A). Prognostic results showed that CuRGs subgroup B had better 28-day clinical survival outcomes compared to CuRGs subgroups A and C, while the prognosis outcomes of CuRGs subgroups A and C were similar (Figure 5B). The unsupervised PCA plot clearly differentiated the three CuRGs

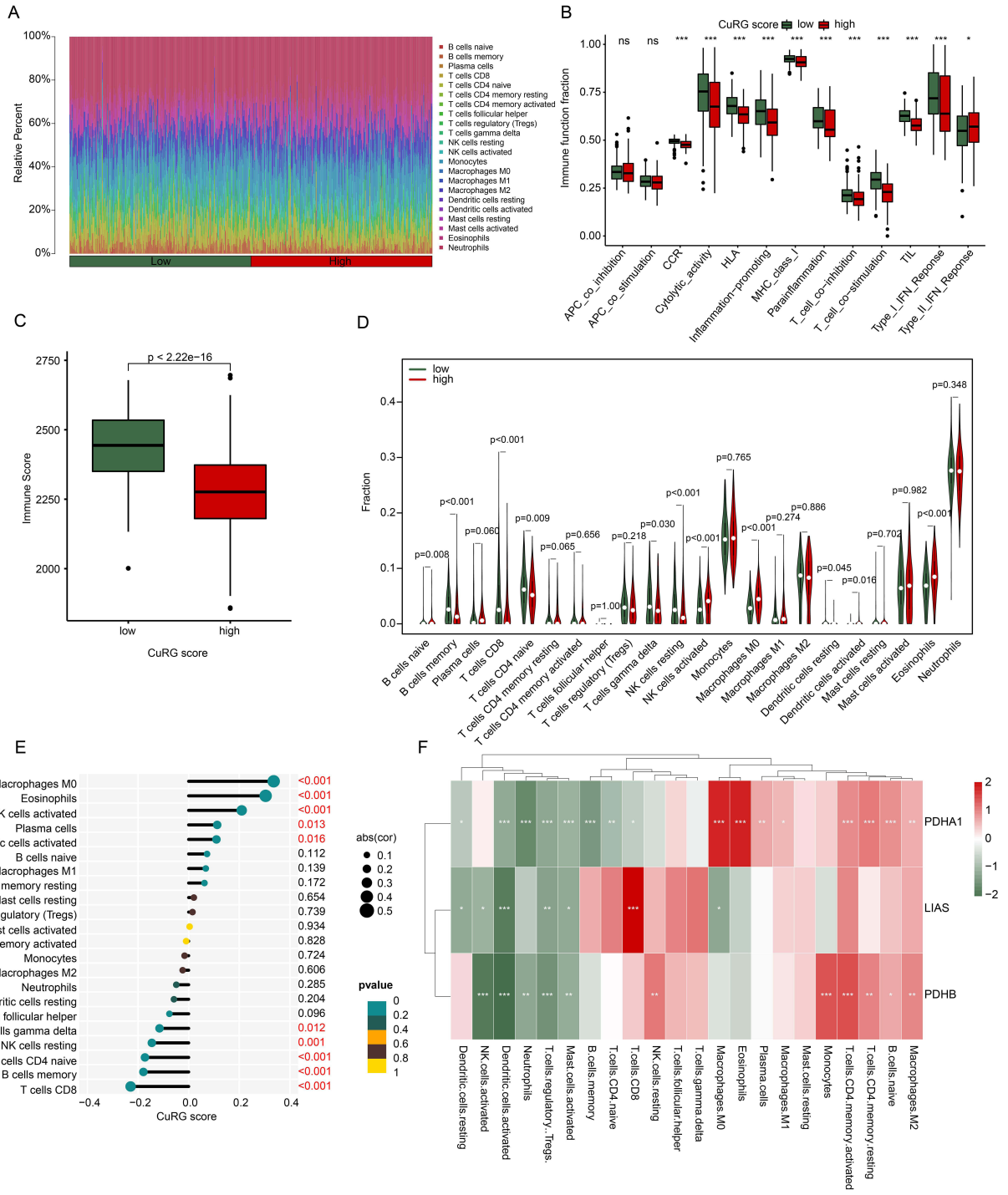

Figure 4 Association of CuRGs risk score and immune infiltration. (**A**) Immune function infiltration landscape and of sepsis in CuRGs risk subgroups. (**B**) Immune function analysis between the low- and the high-risk groups. (**C**) Immune Score between the low- and the high-risk groups. (**D**) Relationship between CuRG risk score and 23 immune cells. (**E**) Relationship between CuRG risk score and immune cells. (**F**) Correlation analysis of 3 prognostic factors and immune infiltration. *P<0.05; **P<0.01; ***P<0.001; ns: P>0.05.

subgroups into three distinct distribution patterns (Figure 5C). Figure 5D demonstrates the potential connection between gene expression profiles of the 10 CuRGs and clinical features of the sepsis samples, suggesting a lower expression of cuproptosis in CuRGs subgroup C. It is noteworthy that the CuRG score of CuRGs subgroup B, which had a better 28-day clinical prognosis outcome compared to CuRGs subgroups A and C, was the lowest (Figure 5E and F).

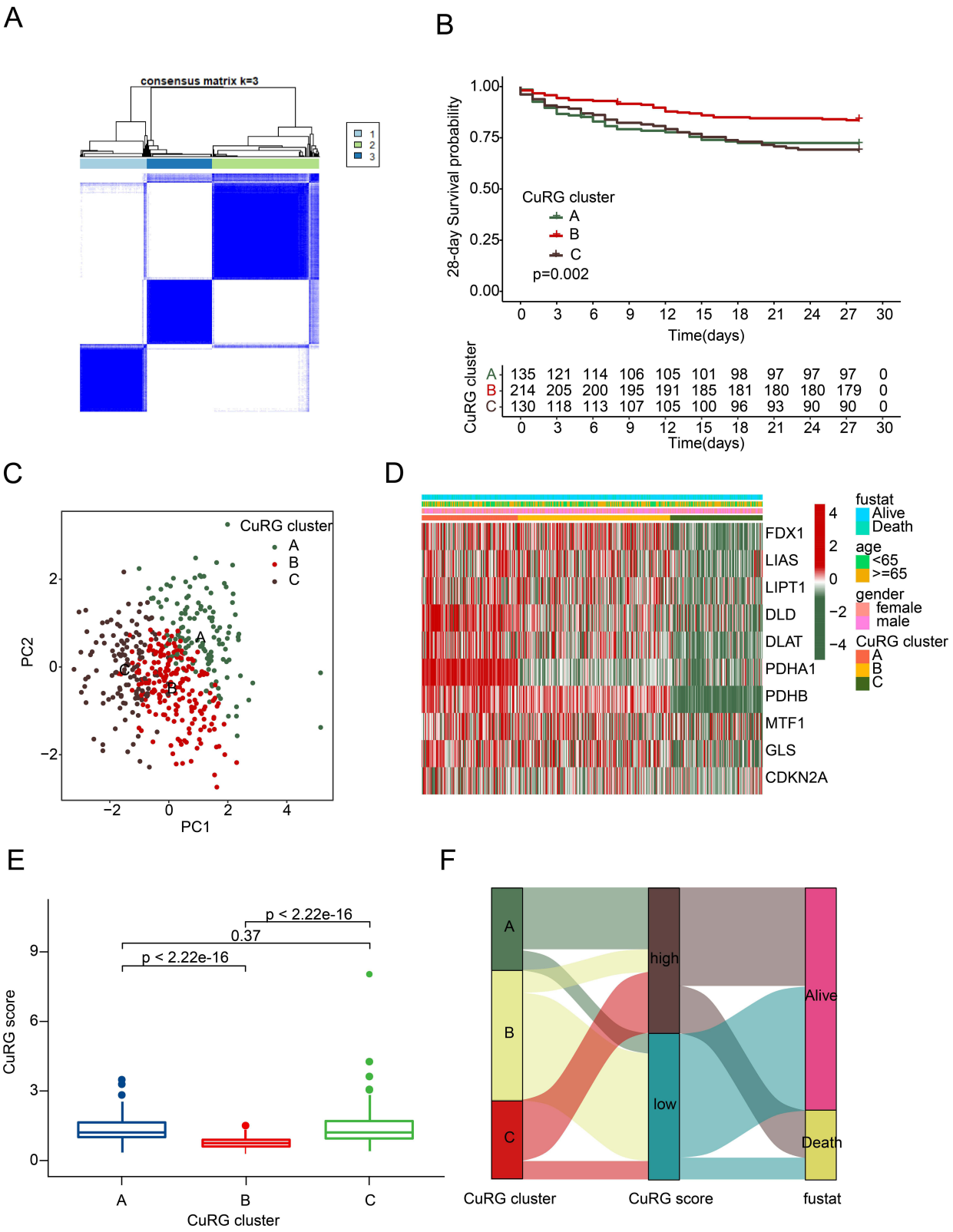

Figure 5 Molecular subgroup and clinical prognosis analysis of CuRGs for sepsis. (**A**) Consensus clustering analysis. (**B**) 28 days clinical survival outcome of sepsis in cluster subgroups. (**C**) PCA analysis of cluster A, cluster B, and cluster C based on DE-CuRGs. (**D**) Relationship of DE-CuRGs expression and clinical features in cluster A, cluster B and cluster C for sepsis. (**E**) CuRG score value of cluster A, cluster B, and cluster C subgroups. (**F**) Relationship between risk score, cluster, CuRG score, and clinical survival status.

Immunological Analysis and Differential Gene Enrichment Analysis of CuRGs

Molecular Subtypes given the certain relevance of CuRGs to the sepsis immune microenvironment, we further compared the immune functions and immune infiltration of the three CuRGs molecular subtypes. The results show that the Immune Score of the three CuRG molecular subtypes is different, among which the CuRGs subgroup B with the best prognosis has the highest Immune Score (Figure 6A). The results of the immune function analysis found that the cytolytic activity, HLA, inflammation promoting, MHC class I, para inflammation, T cell co-stimulation, and TIL of CuRGs subgroup B are significantly higher than those of CuRGs subgroup A and C (Figure 6B). The results of the immune infiltration analysis showed that the proportions of B cells memory, T cells CD8, T cells CD4 naïve, and NK cells resting in CuRGs subgroup B are significantly higher than those of CuRGs subgroup A and C. Meanwhile, the CuRGs subgroup C with the worst prognosis has the highest proportion of Tregs, NK

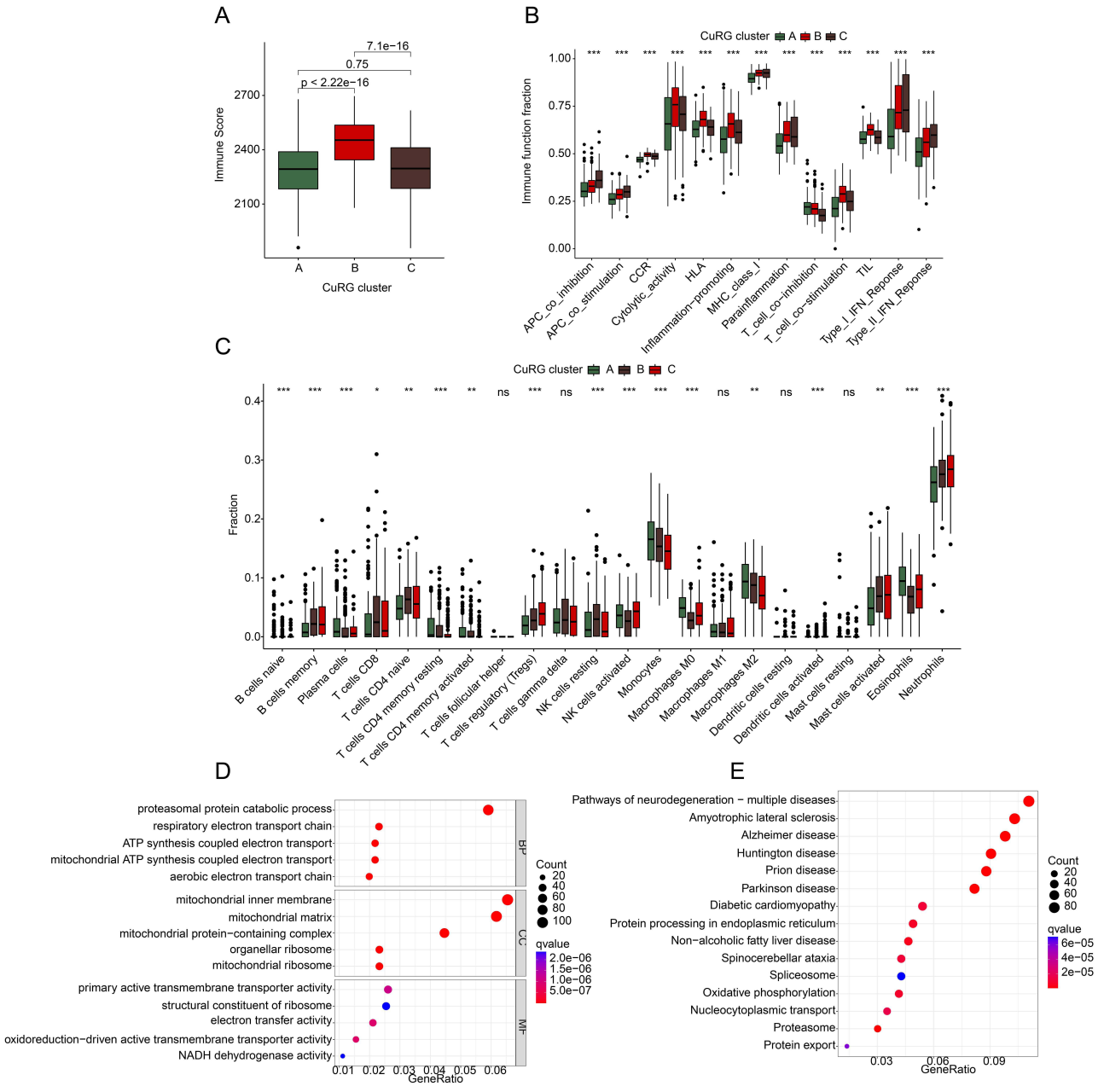

Figure 6 Immune analysis and differential enrichment analysis of CuRGs molecular subtypes in sepsis. (**A**) Immune scores of CuRGs molecular subtypes in sepsis. (**B**) Functional analysis of immune functions in CuRGs molecular subtypes in sepsis. (**C**) Infiltration analysis of immune cells in CuRGs molecular subtypes in sepsis. Differential genes of CuRGs molecular subtypes in sepsis were subjected to (**D**) GO analysis and (**E**) KEGG enrichment analysis. *P<0.05; **P<0.01; ***P<0.001; ns: P>0.05.

cells activated, Mast cells resting, and Neutrophils (Figure 6C). These results indicate that the immune microenvironments of various CuRGs molecular subtypes differ and are related to prognosis, suggesting that personalized immune therapy related to cuproptosis may be helpful for the prognosis of sepsis. We further carried out differential analysis of different subtypes and carried out GO analysis and KEGG enrichment analysis of differential genes. The results show that the differential genes are mainly involved in proteasomal protein catabolic process, respiratory electron transport chain, and ATP synthesis coupled electron transport and other mitochondrial function-related biological processes, while the KEGG analysis results show that pathways related to cognition are significantly enriched (Figure 6D and E).

CuRGs Affect the Immune Microenvironment of Sepsis by Regulating T Cells

Given the notable link between CuRGs and immune cell infiltration in sepsis, we conducted an in-depth analysis to elucidate how CuRGs modulate the immune microenvironment via single-cell sequencing. Initially, we assessed the expression levels of critical CuRGs across different cellular subsets of peripheral blood mononuclear cells (PBMCs). Our findings indicated a peak expression of these genes in T cells (Figure 7A). Subsequently, we categorized the T cell cohort

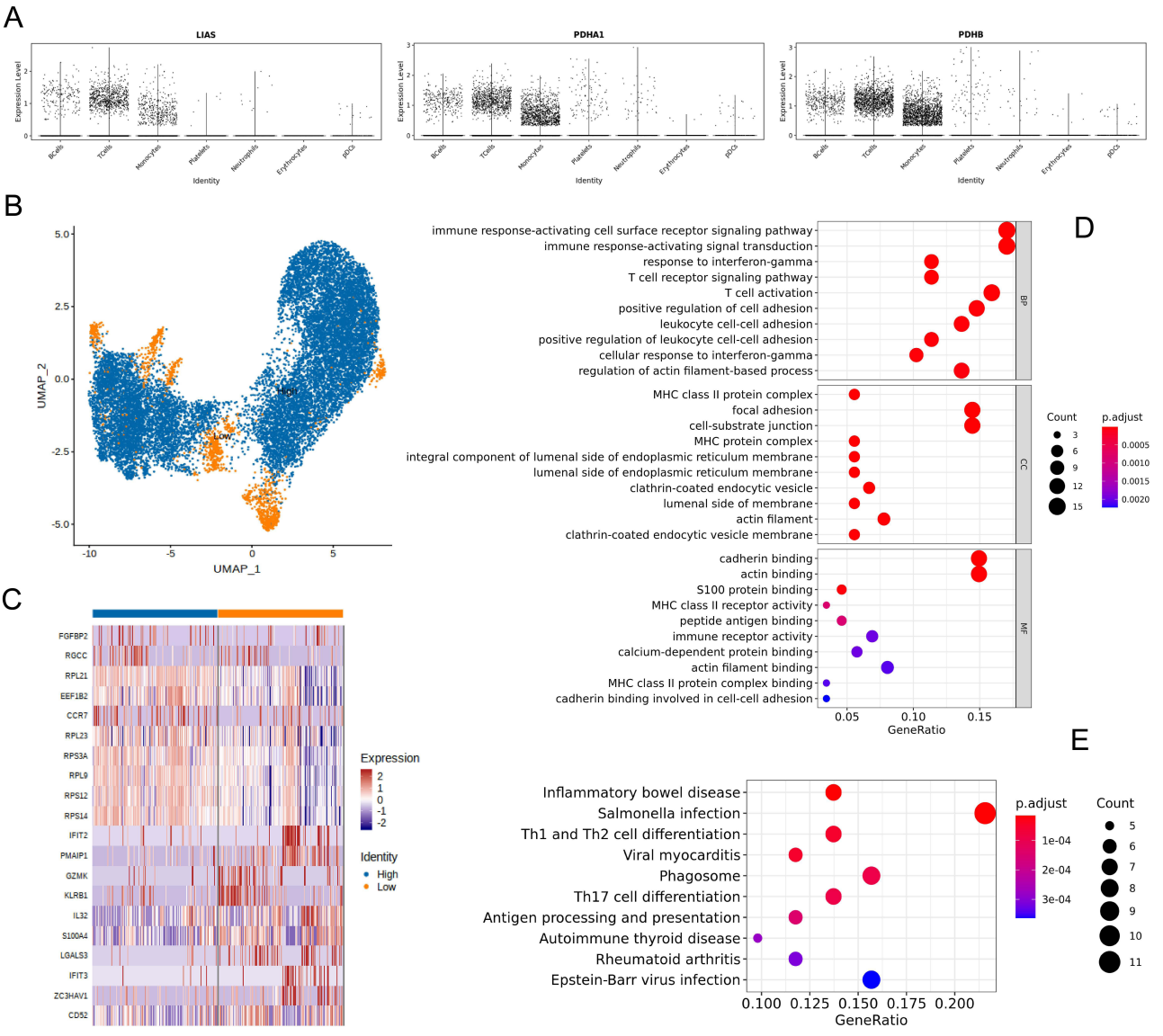

Figure 7 Key genes of CuRGs in single-cell sequencing analysis of PBMCs. (**A**) Expression levels of key genes in major groups of PBMCs cells. (**B**) UMAP plots showing T cell clusters with high and low expression of key genes. (**C**) Heatmap of differential genes in T cell clusters with high and low expression of key genes. (**D**) GO enrichment analysis and (**E**) KEGG pathway enrichment for T cell clusters with high and low expression of key genes.

into two subgroups, characterized by high and low CuRG expression based on three pivotal genes, and executed a comparative analysis (Figure 7B and C). Enrichment investigation of the genes that differed between groups indicated a predominant association with biological processes such as immune response-activating cell surface receptor signaling, T cell receptor signaling, and T cell activation pathways. Moreover, these genes played a role in the differentiation pathways of Th1 and Th2 cells, along with Th17 cells (Figure 7D and E). This evidence infers that CuRGs could modulate the immune microenvironment in sepsis by impacting T cell differentiation, activation of T cell surface receptors, and activation of T cells.

The CuRGs-Related Nomogram Can Predict the Prognosis of Sepsis

In order to evaluate the prognostic significance of CuRGs risk score in sepsis. Univariate and multivariate Cox regression analysis were performed to determine if CuRGs risk score was an independent prognostic factor for sepsis. The results indicated that the CuRGs risk score was a significant and independent prognostic indicator for sepsis in both univariate analysis ($p < 0.001$, HR = 1.394 (1.199–1.621)) and multivariate Cox analysis ($p < 0.001$, HR = 1.393 (1.194–1.626)) (Figure 8A and B). Additionally, a nomogram was developed using the CuRGs risk score and other clinical characteristics of sepsis patients to predict the clinical survival probability at 7, 14, and 28 days (Figure 8C). Lastly, ROC curve

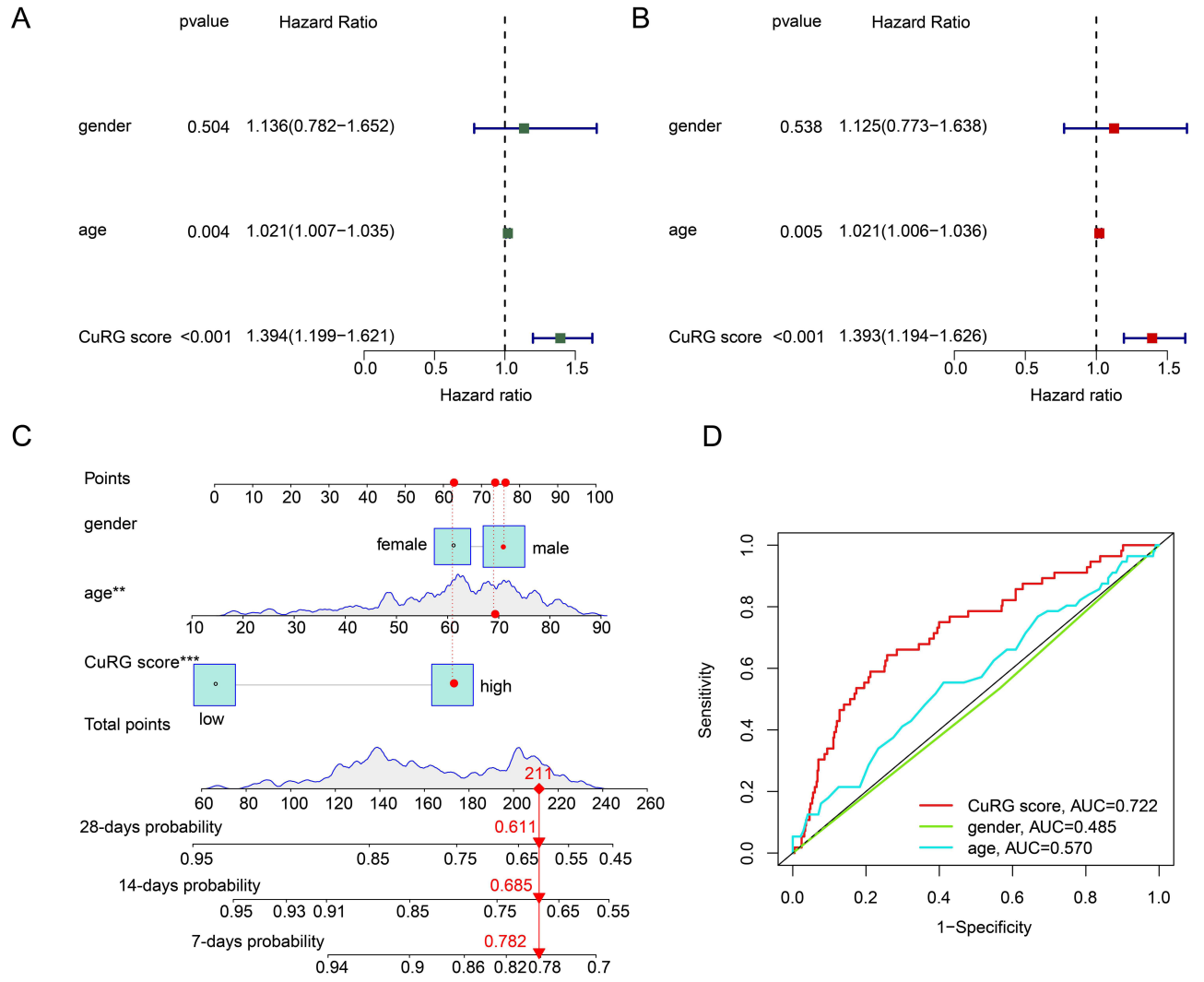

Figure 8 Independent prognostic analysis of CuRGs in sepsis. (**A**) Univariate and (**B**) Multivariate analysis revealed that CuRGs score is an independent prognostic factor for sepsis. (**C**) A prognostic nomogram model was constructed based on the CuRGs key genes. (**D**) ROC curve of the prognostic nomogram.

analysis revealed that the CuRGs risk score exhibited superior sensitivity and specificity when compared to other clinical indicators (Figure 8D).

Validating and Drug Sensitivity Analysis of Cuproptosis Key Genes in the Clinical Data

To elucidate the diagnostic and prognostic value of these key genes in sepsis, 30 sepsis patients and 15 healthy volunteers were recruited (clinical information provided in Supplementary Table 3). Sepsis patients exhibited higher heart rate, temperature, and white blood cell count compared to healthy volunteers (Supplementary Table 3). qPCR results demonstrated significantly lower mRNA expression of CuRG key genes in sepsis compared to the healthy control group, which was consistent with the bioinformatics analysis (Figure 9A). Moreover, we constructed a sepsis prediction Nomogram model (Figure 9B) by incorporating routine diagnostic indicators of sepsis and CuRG key genes. ROC curve analysis demonstrated that the AUC value of the sepsis copper death prediction nomogram model was significantly higher compared to other indicators (Figure 9C). Additionally, we collected blood routine data from sepsis patients and healthy volunteers and observed lower proportions of lymphocytes and basophils in the sepsis group compared to the healthy control group, and higher proportions of neutrophils (Supplementary Table 4). The correlation analysis demonstrated a positive correlation between the expression of the key gene PDHA1 and neutrophils, and negative correlations with lymphocytes, basophils, and eosinophils, further supporting the regulatory role of CuRG key genes in the immune microenvironment of sepsis (Figure 9D). Additionally, we performed drug sensitivity analysis on CuRG key genes, conducting a screening for agonists targeting key genes. A Venn diagram was utilized to identify bisphenol A, a compound capable of simultaneously activating three key targets. Subsequently, molecular docking was conducted between the compound and the protein targets (Figure 9E–H). Molecular docking analysis revealed that bisphenol A could form hydrogen bond interactions with the SER352 and PHE143 sites of LIAS, the THR87 and ALA88 sites of PDHA1, and the ASN172 site of PDHB (Figure 9F–H).

Discussion

In our study, we examined transcriptomic data from sepsis patients, using the GEO database to identify key genes (PDHB, PDHA1, LIAS) associated with the prognosis of sepsis, which was followed by the construction of a prognostic risk model. The scope of the research expanded to investigate the link between these critical genes, often connected to overall mortality, and the immune microenvironment in sepsis. Through consensus clustering analysis, we divided sepsis patients into three subgroups associated with mortality. Subgroup B was identified as the cohort with the most favorable prognosis and strong immune function. To delve into potential molecular mechanisms, we conducted enrichment analysis. Blood samples from healthy individuals and septic patients were used for qPCR validation to verify the expression levels of these genes. These gene expressions, combined with traditional indicators, were used to create a diagnostic model for sepsis. Further, drug sensitivity testing identified bisphenol A as a compound that can potentially influence all three key genes, suggesting its potential in prognosis of sepsis.

This study establishes a significant correlation between sepsis prognosis and the expression of key genes - PDHB, PDHA1, and LIAS - involved in cuproptosis. Both PDHB and PDHA1, primarily located in mitochondria, function as catalytic subunits of the Pyruvate Dehydrogenase (PDH). This fundamental enzyme catalyzes the oxidation and dehydrogenation of pyruvate to acetyl CoA.[22] Earlier research suggests that sepsis leads to PDH inactivity, impacting peripheral blood mononuclear cells, skeletal muscle cells, and vascular endothelial cells.[23,24] This inactivity primarily ensues from PDHA1 hyperacetylation.[25] According to a study by Kang et al, excessive acetylation followed by PDHA1 inactivation in renal tubular cells is a key mechanism behind sepsis-induced kidney injury.[25] PDH plays a crucial role in the tricarboxylic acid (TCA) cycle, facilitating the merging of acetyl CoA, its catalysis product, with oxaloacetate to produce citrate in the TCA cycle.[26] Recent studies suggest that citrate may convert into itaconic acid, a metabolite inhibiting succinate dehydrogenase-mediated oxidation and promoting anti-inflammatory transcription factors.[27] Hence, it appears modifications in PDHB and PDHA1 significantly influence sepsis development.

The gene LIAS plays a critical role, as it encodes for the mitochondrial lipoic acid pathway.[28] α-lipoic acid functions as a vital cofactor for the Pyruvate Dehydrogenase Complex (PDC) and α-Ketoglutarate Dehydrogenase Complex, both essential for energy production.[29] Lipoic acid and its reduced form, dihydrolipoic acid (DHLA), are potent micronutrients

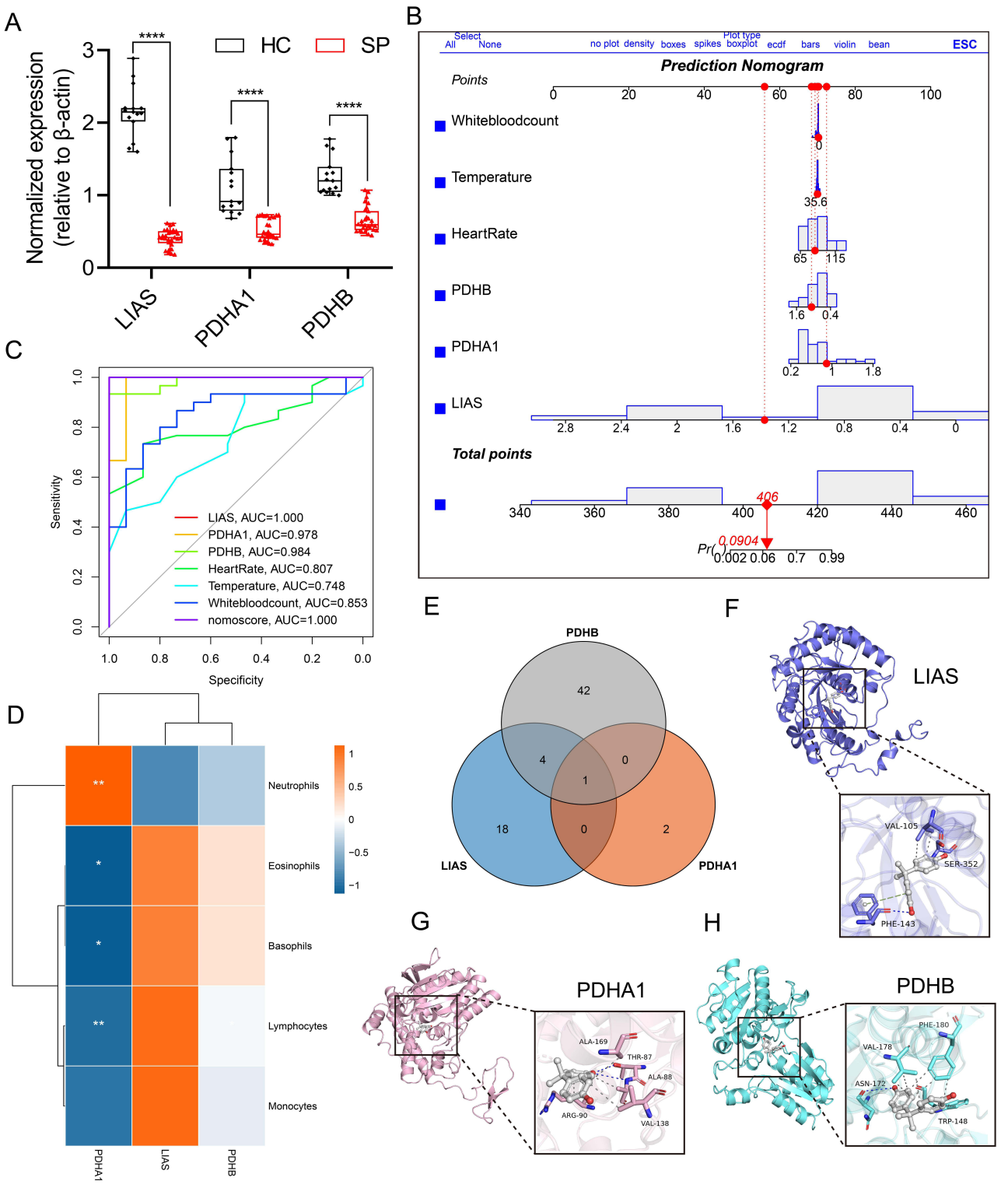

Figure 9 Construction of Prediction Nomogram Model and Drug Sensitivity of CuRGs in Sepsis. (**A**) Normalized gene expression of the three key genes between healthy controls (HC) and sepsis patients (SP) using qPCR. (**B**) Construction of a prediction nomogram model based on multivariate indicators. (**C**) ROC curve of the multivariate indicators. (**D**) Pearson's correlation analysis between the proportion of immune cells in blood routine and the three hub genes. (**E**) Molecular docking analysis of bisphenol A with LIAS (**F**), PDHA1 (**G**), and PDHB (**H**). *P<0.05; **P<0.01; ****P<0.0001.

with a range of pharmacological and antioxidant properties.[30] Clinical findings suggest that lipoic acid has potential as a therapeutic agent in sepsis treatment.[31] Studies reveal that LIAS-deficient mice infused with LPS display a heightened prevalence of early plasma pro-inflammatory cytokines, such as TNF-α and NF-κB. This can prompt severe inflammatory reactions and tissue damage.[32] On the contrary, another research found that LIAS-overexpressing mice exhibit increased lung tissue resistance against oxidative stress and inflammation.[28] Consequently, LIAS could emerge as a significant prognostic marker for sepsis.

This investigation uncovers that genes related to cuproptosis modulate the immune microenvironment of sepsis, with the high-risk group demonstrating diminished immune efficacy and lessened immune cell infiltration. It is observed that the immune microenvironment significantly differs across cuproptosis scored subtypes amongst sepsis patients. Interestingly, CuRGs primarily interact with Macrophages, Eosinophils, and activated NK cells, hinting towards a potential role of cuproptosis in controlling the influence of these immune cells within the sepsis immune microenvironment. Macrophages feature prominently amongst innate immune cells with their apoptosis and pyroptosis found to be quite critical in shaping the immune environment of sepsis. Macrophage pyroptosis is chiefly correlated with the activation of the NLRP3/ASC/caspase-1 inflammasome. This leads to caspase-1 cleavage of pro-inflammatory cytokine precursors—pro-IL-1β and pro-IL-18, formation of cell membrane pores, cell swelling, and lysis—resulting in macrophage pyroptosis.[33] Unchecked macrophage apoptosis might also foster immune suppression, potentially linked to increased HMGB1, PD-L1/PD-1 expression, and insufficient autophagy.[34] It has been discovered that HMGB1 stimulates macrophage apoptosis in a dose and time-regulated manner by binding and activating RAGE, subsequently triggering caspase-3 which induces macrophage apoptosis.[34] In response to LPS treatment, macrophages display mitochondrial dysfunction, hampered mitochondrial autophagy, and heightened mitochondrial-dependent apoptosis.[35] The investigation into whether macrophages experience cuproptosis and the specific processes involved is ongoing. It has also been noted that a drop in eosinophils coincides with sepsis. Furthermore, a steady decrease in eosinophil count within the first 48 hours of admission can predict mortality and admission rates in sepsis patients.[36] In patients with ARDS, bronchial eosinophil activity markers see a surge correlated with the intensity of ARDS.[36]

NK cells play an important role in both innate and adaptive immunity, hence, the quantity and function of NK cells might be potential therapeutic targets in the regulation of sepsis. Research on sepsis has found that neutralizing IL-10 enhances the ability of NK cells to secrete IFN-γ, increasing the survival rate of mice with septic shock.[19] However, other studies have shown that over-activation of NK cells can result in tissue organ damage. Guo et al found that knocking out the IL-15 gene in mice to inhibit NK cell function significantly improved the survival rate of mice with sepsis.[37] This implies that for patients with hyperactive NK cells, therapeutic interventions that moderately inhibit their functionalities can be selected. Though NK cells belong to the innate immune system, newest research found that they possess the ability to generate memory cells, exerting memory immune responses similar to adaptive immune cells. Stimuli such as hapten, viruses, and cytokines can all prompt the body to produce memory NK cells,[38,39] especially post-viral infection, the cytotoxic capacity and ability to produce IFN-γ of memory NK cells significantly increase,[40] which certainly plays a crucial role in immune regulation of sepsis.

While this study offers certain insights, it also has some limitations. It shows a correlation between CuRGs and prognosis, and their regulation of the sepsis immune microenvironment. Nonetheless, the exact mechanisms are still undefined, signaling the need for in-depth future research. Developing new sepsis intervention measures for CuRGs also has important significance. In addition, a comparison of clinical information between high-risk and low-risk groups reveals that gender might be a confounding factor. The relationship between gender and cuproptosis needs further investigation. We corroborated our bioinformatics analysis by developing a novel diagnostic model, using samples from sepsis patients. However, our sample size was limited, emphasizing the need for broader clinical studies going forward. Finally, additional drug validation for bisphenol A is required to evaluate its impact.

Conclusions

In conclusion, our machine learning analysis identified three hub genes (LIAS, PDHB, and PDHA1) related to cuproptosis. These genes have the potential to serve as prognostic biological markers in sepsis. Our analysis of immune

infiltration revealed that the identified hub genes could have crucial roles in the immune response during sepsis. The consensus analysis offers a theoretical foundation for personalized immunotherapy in the treatment of sepsis.

Data Sharing Statement

The dataset presented in this study can be found in online repositories. The names of the repository and accession numbers can be found in the article.

Ethical Statement

This research received approval from the Ethics Committee of the Research Institute of Surgery, Army Medical University (2021-179) and was registered by the Chinese Clinical Trial Registry (ChiCTR2200055772). The procedures employed in this study adhere to the tenets of the Declaration of Helsinki. All procedures were performed under the approval of the Ethics Committee after patients provided informed consent.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Srzić I, Nesek Adam V, Tunjić Pejak D. Sepsis definition: what's new in the treatment guidelines. *Acta Clin Croat*. 2022;61:67–72. doi:10.20471/acc.2022.61.s1.11
2. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*. 2016;315:801–810. doi:10.1001/jama.2016.0287
3. Pierrakos C, Velissaris D, Bisdorff M, Marshall JC, Vincent JL. Biomarkers of sepsis: time for a reappraisal. *Crit Care*. 2020;24:287. doi:10.1186/s13054-020-02993-5
4. Shu Q, She H, Chen X, Zhong L, Zhu J, Fang L. Identification and experimental validation of mitochondria-related genes biomarkers associated with immune infiltration for sepsis. *Front Immunol*. 2023;14:1184126. doi:10.3389/fimmu.2023.1184126
5. She H, Tan L, Yang R, et al. Identification of featured necroptosis-related genes and imbalanced immune infiltration in sepsis via machine learning. *Front Genet*. 2023;14:1158029. doi:10.3389/fgene.2023.1158029
6. She H, Tan L, Zhou Y, et al. The landscape of featured metabolism-related genes and imbalanced immune cell subsets in sepsis. *Front Genet*. 2022;13:821275. doi:10.3389/fgene.2022.821275
7. She H, Tan L, Wang Y, et al. Integrative single-cell RNA sequencing and metabolomics decipher the imbalanced lipid-metabolism in maladaptive immune responses during sepsis. *Front Immunol*. 2023;14:1181697. doi:10.3389/fimmu.2023.1181697
8. Oberhoffer M, Vogelsang H, Russwurm S, Hartung T, Reinhart K. Outcome prediction by traditional and new markers of inflammation in patients with sepsis. *Clin Chem Lab Med*. 1999;37:363–368. doi:10.1515/cclm.1999.060
9. Phua J, Koay ES, Lee KH. Lactate, procalcitonin, and amino-terminal pro-B-type natriuretic peptide versus cytokine measurements and clinical severity scores for prognostication in septic shock. *Shock*. 2008;29:328–333. doi:10.1097/SHK.0b013e318150716b
10. Chen L, Min J, Wang F. Copper homeostasis and cuproptosis in health and disease. *Signal Transduct Target Ther*. 2022;7:378. doi:10.1038/s41392-022-01229-y
11. Tang D, Chen X, Kroemer G. Cuproptosis: a copper-triggered modality of mitochondrial cell death. *Cell Res*. 2022;32:417–418. doi:10.1038/s41422-022-00653-7
12. Li SR, Bu LL, Cai L. Cuproptosis: lipoylated TCA cycle proteins-mediated novel cell death pathway. *Signal Transduct Target Ther*. 2022;7:158. doi:10.1038/s41392-022-01014-x

13. She H, Hu Y, Zhou Y, et al. Protective effects of dexmedetomidine on sepsis-induced vascular leakage by alleviating ferroptosis via regulating metabolic reprogramming. *J Inflamm Res*. 2021;14:6765–6782. doi:10.2147/jir.S340420
14. Zhang H, Feng YW, Yao YM. Potential therapy strategy: targeting mitochondrial dysfunction in sepsis. *Mil Med Res*. 2018;5:41. doi:10.1186/s40779-018-0187-0
15. van der Poll T, Shankar-Hari M, Wiersinga WJ. The immunology of sepsis. *Immunity*. 2021;54:2450–2464. doi:10.1016/j.immuni.2021.10.012
16. Rubio I, Osuchowski MF, Shankar-Hari M, et al. Current gaps in sepsis immunology: new opportunities for translational research. *Lancet Infect Dis*. 2019;19:e422–e436. doi:10.1016/s1473-3099(19)30567-5
17. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nat Rev Immunol*. 2013;13:862–874. doi:10.1038/nri3552
18. Wiersinga WJ, van der Poll T. Immunopathophysiology of human sepsis. *EBioMedicine*. 2022;86:104363. doi:10.1016/j.ebiom.2022.104363
19. Hiraki S, Ono S, Kinoshita M, et al. Neutralization of IL-10 restores the downregulation of IL-18 receptor on natural killer cells and interferon-γ production in septic mice, thus leading to an improved survival. *Shock*. 2012;37:177–182. doi:10.1097/SHK.0b013e31823f18ad
20. Kim EY, Ner-Gaon H, Varon J, et al. Post-sepsis immunosuppression depends on NKT cell regulation of mTOR/IFN-γ in NK cells. *J Clin Invest*. 2020;130:3238–3252. doi:10.1172/jci128075
21. Qiu X, Li J, Bonenfant J, et al. Dynamic changes in human single-cell transcriptional signatures during fatal sepsis. *J Leukoc Biol*. 2021;110:1253–1268. doi:10.1002/jlb.5ma0721-825r
22. Park S, Jeon JH, Min BK, et al. Role of the pyruvate dehydrogenase complex in metabolic remodeling: differential pyruvate dehydrogenase complex functions in metabolism. *Diabetes Metab J*. 2018;42:270–281. doi:10.4093/dmj.2018.0101
23. Vary TC. Sepsis-induced alterations in pyruvate dehydrogenase complex activity in rat skeletal muscle: effects on plasma lactate. *Shock*. 1996;6:89–94. doi:10.1097/00024382-199608000-00002
24. Nuzzo E, Berg KM, Andersen LW, et al. Pyruvate dehydrogenase activity is decreased in the peripheral blood mononuclear cells of patients with sepsis. A prospective observational trial. *Ann Am Thorac Soc*. 2015;12:1662–1666. doi:10.1513/AnnalsATS.201505-267BC
25. An S, Yao Y, Hu H, et al. PDHA1 hyperacetylation-mediated lactate overproduction promotes sepsis-induced acute kidney injury via Fis1 lactylation. *Cell Death Dis*. 2023;14:457. doi:10.1038/s41419-023-05952-4
26. Bhandary S, Aguan K. Pyruvate dehydrogenase complex deficiency and its relationship with epilepsy frequency--An overview. *Epilepsy Res*. 2015;116:40–52. doi:10.1016/j.eplepsyres.2015.07.002
27. Lampropoulou V, Sergushichev A, Bambouskova M, et al. Itaconate links inhibition of succinate dehydrogenase with macrophage metabolic remodeling and regulation of inflammation. *Cell Metab*. 2016;24:158–166. doi:10.1016/j.cmet.2016.06.004
28. Xu G, Zhao Y, Tao Y, et al. Lias overexpression alleviates pulmonary injury induced by fine particulate matter in mice. *Environ Geochem Health*. 2023;45:6585–6603. doi:10.1007/s10653-023-01651-3
29. Packer L, Witt EH, Tritschler HJ. alpha-Lipoic acid as a biological antioxidant. *Free Radic Biol Med*. 1995;19:227–250.
30. Bast A, Haenen GR. Lipoic acid: a multifunctional antioxidant. *Biofactors*. 2003;17:207–213. doi:10.1002/biof.5520170120
31. Ziegler D, Nowak H, Kempler P, Vargha P, Low PA. Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: a meta-analysis. *Diabet Med*. 2004;21:114–121. doi:10.1111/j.1464-5491.2004.01109.x
32. Yi X, Kim K, Yuan W, et al. Mice with heterozygous deficiency of lipoic acid synthase have an increased sensitivity to lipopolysaccharide-induced tissue injury. *J Leukoc Biol*. 2009;85:146–153. doi:10.1189/jlb.0308161
33. Wang T, Zhong H, Zhang W, et al. STAT5a induces endotoxin tolerance by alleviating pyroptosis in kupffer cells. *Mol Immunol*. 2020;122:28–37. doi:10.1016/j.molimm.2020.03.016
34. Zhu XM, Yao YM, Liang HP, et al. Effect of high mobility group box-1 protein on apoptosis of peritoneal macrophages. *Arch Biochem Biophys*. 2009;492:54–61. doi:10.1016/j.abb.2009.09.016
35. Wang Y, Mao X, Chen H, et al. Dexmedetomidine alleviates LPS-induced apoptosis and inflammation in macrophages by eliminating damaged mitochondria via PINK1 mediated mitophagy. *Int Immunopharmacol*. 2019;73:471–481. doi:10.1016/j.intimp.2019.05.027
36. Al Duhailib Z, Farooqi M, Piticaru J, Alhazzani W, Nair P. The role of eosinophils in sepsis and acute respiratory distress syndrome: a scoping review. *Can J Anaesth*. 2021;68:715–726. doi:10.1007/s12630-021-01920-8
37. Guo Y, Luan L, Patil NK, et al. IL-15 enables septic shock by maintaining NK cell integrity and function. *J Immunol*. 2017;198:1320–1333. doi:10.4049/jimmunol.1601486
38. Paust S, Gill HS, Wang BZ, et al. Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigen-specific memory of haptens and viruses. *Nat Immunol*. 2010;11:1127–1135. doi:10.1038/ni.1953
39. Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. *Nature*. 2009;457:557–561. doi:10.1038/nature07665
40. Piersma SJ, Bangru S, Yoon J, et al. NK cell expansion requires HuR and mediates control of solid tumors and long-term virus infection. *J Exp Med*. 2023;220. doi:10.1084/jem.20231154