Articles

Machine Learning and Experimental Validation Ubiquitination-Related Key Genes in Sepsis: The Perspective of Chinese-Western Pharmaceutical Therapeutic Strategies

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Abstract

Objective: Sepsis, a global health challenge, is characterized by a systemic inflammatory response syndrome (SIRS) triggered by infection, resulting in a complex interplay between inflammation and immune suppression. Recent research highlights ubiquitination, a critical post-translational modification, as pivotal in maintaining immune equilibrium and cellular stress responses by modulating protein stability and activity in inflammatory pathways. Despite these insights, significant knowledge gaps persist regarding the specific ubiquitination regulatory network in sepsis, including the spatiotemporal expression of key ubiquitin ligases and deubiquitinases and their link to organ dysfunction progression. These gaps hinder the development of precision therapies targeting the ubiquitination regulatory network. This study, integrating traditional Chinese and Western medicine, aims to elucidate ubiquitination's role in sepsis immune-metabolic reprogramming and perform high-throughput screening of traditional Chinese and small-molecule drugs targeting sepsis-related ubiquitinated proteins. The goal is to identify precise intervention targets and potential treatments, facilitating personalized strategies based on ubiquitination molecular profiling for sepsis patients. Methods: This study systematically integrated four independent sepsis transcriptomic datasets from the GEO database (GSE32707, GSE54514, GSE69528, and GSE137340), comprising a total of 423 clinical samples (110 healthy controls and 313 sepsis patients), to establish a multi-cohort analytic framework for ubiquitination-related genes (UbRGs). Differential expression analysis between sepsis and control samples was performed using the limma package in R, with thresholds set at $|\log_2 FC| > 0.2$ and adjusted $P < 0.05$. The resulting differentially expressed genes (DEGs) were intersected with 1,838 UbRGs retrieved from the GeneCards database via Venn analysis to identify sepsis-associated differentially expressed UbRGs. Multi-level functional annotation was conducted using GO/KEGG pathway enrichment analysis and gene set enrichment analysis (GSEA). Three machine learning algorithms—LASSO regression, random forest (RF), and XGBoost—were jointly applied to pinpoint key signature genes. CIBERSORT was used for immune infiltration analysis to quantify peripheral blood immune cell subpopulations, and the area under the ROC curve was employed to exploratorily assess the internal discriminative ability of candidate genes, aiding in the selection of optimal diagnostic markers. The expression levels of the candidate signature genes were preliminarily validated by RT-qPCR in LPS-induced THP-1 and RAW264.7 macrophage cell lines as in vitro sepsis models. Finally, potential drugs (traditional Chinese medicine monomers and small-molecule compounds) targeting UbRGs were screened using COREMINE, HERB, TCMSP, and other databases, and their binding affinities were assessed by molecular docking. Results: This study identified 448 differentially expressed Ubiquitin-Related Genes (UbRGs) associated with sepsis by analyzing multiple sepsis cohorts. (1) Functional annotation revealed significant enrichment of UbRGs in biological processes including protein ubiquitination, NF-κB signaling pathway, and apoptosis. (2) Through LASSO regression, random forest (RF), and XGBoost with cross-validation, 11 key signature genes (e.g., UBE2C, KLHL3, GAPDH) were identified. Among these, 7 genes (including E4F1 and UBE2C) showed moderate or higher discriminative ability (AUC > 0.7) for distinguishing sepsis from control samples within the current merged dataset. (3) Immune infiltration analysis indicated a significant increase in mast cells and B cells in sepsis, with a moderate negative correlation between M macrophages and resting CD4+ T cells ($r = -0.55$, $P < 0.05$), and activated dendritic cells ($r = -0.54$, $P < 0.05$). The high-expression sepsis group was enriched in pathways such as NF-κB signaling, apoptosis, and ubiquitin-mediated proteolysis, suggesting a potential role in modulating immune cell functions in sepsis progression. (4) Single-gene functional mechanism analysis highlighted that immune infiltration analysis of 4 characteristic DE-UbRGs (FBXO7, SIAH1, SUMO2, UBE2C) correlated significantly with various immune cells and pathways related to cellular stress response, energy metabolism, and immune activation, emphasizing their importance in inflammatory responses and cell homeostasis. (5) RT-qPCR confirmed decreased mRNA levels of FBXO7, SIAH1, and SUMO2, and increased mRNA level of UBE2C in an in vitro sepsis cell model (THP-1) ($P < 0.05$). (6) Small-molecule drugs like Sirolimus and Temozolomide exhibited strong binding affinity with the 4 characteristic DE-UbRGs, and TCMs associated with these UbRGs and their active ingredients also demonstrated favorable binding abilities. Conclusion: Using machine learning, this study screened for ubiquitination-related key genes closely linked to sepsis pathology, including inflammation and immune microenvironment changes, and also preliminarily identified potential TCM monomers and small-molecule drugs for targeted intervention. This work lays the groundwork for personalized treatment strategies based on ubiquitination molecular subtyping with translational promise, though additional independent validation and prospective investigations are still needed.

Keywords: TNBC; chemoresistance; bibliometric analysis; drug resistance mechanism

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Introduction

Sepsis is a life-threatening organ dysfunction syndrome caused by a dysregulated host response to infection. Its core pathological mechanisms include immune imbalance and organ damage. It can occur at any age, with high incidence and mortality rates. Approximately 48.9 million new cases of sepsis and 11 million sepsis-related deaths occur annually, making it a major global public health concern[1-4]. In China, recent surveys have shown that there are 4.8 to 6.1 million hospitalized sepsis cases annually, and the standardized sepsis-related mortality rate is 66.7 per 100,000 people[5,6]. However, early diagnosis and precise treatment of sepsis still face significant challenges. In recent years, the rise of molecularly targeted drugs has provided a new direction for sepsis treatment. Researchers have developed various molecularly targeted drugs aimed at specific inflammatory mediators or immune pathways[7-11]. By precisely intervening in key inflammatory pathways, the immune homeostasis of the body can be restored. However, most of the existing targeted drugs focus on a single pathway, and the molecular mechanisms of sepsis have not been fully elucidated. In particular, the relevant immune mechanisms are extremely complex, so their clinical application and effectiveness still have significant limitations. Failure to precisely regulate the excessive inflammatory response and immunosuppressive state in sepsis patients will be unfavorable for treatment outcomes[12]. This indicates the importance of developing diagnostic biomarkers for sepsis to achieve individualized and precise treatment. With the development of high-throughput sequencing technology[13], in the face of the current challenges in molecular targeted therapy for sepsis, mining key regulatory genes and signaling pathways of sepsis through bioinformatics methods has become an important strategy for exploring its pathogenesis and potential therapeutic targets.

Ubiquitination is a process in which ubiquitin molecules specifically modify target proteins under the action of enzymes (such as ubiquitin-activating enzymes, ubiquitin-conjugating enzymes, ubiquitin ligases, and deubiquitinating enzymes). It participates in almost all life activities by regulating protein localization, metabolism, function, and degradation[14]. Ubiquitination plays a crucial role in the development of various inflammatory diseases and tumorigenesis[15]. The ubiquitination system plays an important role in the pathological process of sepsis by regulating inflammatory signaling pathways, cell death, etc.[16-18]. For example, E3 ligases regulate the activation of the NLRP3 inflammasome by catalyzing the ubiquitination of the NLRP3 inflammasome or other components of the inflammasomes, inducing apoptosis of immune cells, and thus exacerbating the development of sepsis[19,20]. In contrast, YTHDF1 promotes NLRP3 ubiquitination by upregulating WWP1, thereby inhibiting caspase-1-dependent pyroptosis and alleviating sepsis[21]. In recent years, based on the core role of ubiquitination in sepsis, potential molecular targets have been provided for the intervention and treatment of sepsis. Researchers have developed various targeted treatment strategies for sepsis, such as natural compound regulation, proteasome inhibitors, and nanotechnology delivery systems. For instance, Gramine improves sepsis-induced myocardial dysfunction by binding to NF-κB p105 and inhibiting its ubiquitination[22]. The ubiquitin-activating enzyme E1 inhibitor PYR-41 can reduce lung injury in septic mice[23]. Intravenous administration of Skp2 mRNA-encapsulated lipid nanoparticles (LNP) can inhibit ferroptosis in lung epithelium and alleviate lung injury in septic mice[24]. Although these strategies show potential in experimental studies, their clinical application still has a long way to go. Meanwhile, there is currently a lack of reliable indicators to evaluate the changes in the ubiquitination status in patients with sepsis, making it impossible to accurately identify patient subgroups that may benefit from specific targeted therapies. The research on ubiquitination-related genes (UbRGs) in sepsis, their dynamic regulatory networks, and their clinical translation value urgently need in-depth exploration.

Due to the current lack of approved specific drugs for sepsis, many researchers have also turned their attention to Traditional Chinese Medicines (TCMs) research. TCM has a long-standing history in preventing and treating various diseases. Xuebijing (XBJ) is a TCM compound injection approved by the State Food and Drug Administration (SFDA) of China for the clinical treatment of sepsis. Studies have confirmed that XBJ injection is effective in treating sepsis by integrating and regulating multiple pathways such as immunity, metabolism, and coagulation through multiple components and multiple targets[25]. It can also effectively treat disseminated intravascular coagulation (DIC) caused by severe sepsis[26] and significantly reduce the mortality rate of sepsis patients[27]. Not only TCM compounds but also various small-molecule compounds isolated from TCM have shown potential in treating sepsis. Research indicates that many small-molecule compounds provide precise targeted intervention and treatment for sepsis by regulating multiple signaling pathways, such as NF-κB, TLR4, MAPK, NLRP3, AMPK, and PI3KAKT[28]. For example, Berbamine (BA, BBM) dihydrochloride, isolated from TCMs like Berberis, Coptis chinensis, and Cortex Phellodendri, plays a protective role in

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sepsis by targeting Notch1 to regulate the inflammatory response of macrophages, inhibiting inflammation and promoting the degradation of Notch1, significantly improving the survival rate of septic mice and reducing lung inflammation[29]. Moreover, small-molecule compounds can also affect the pathological process of sepsis by regulating the ubiquitination pathway. For instance, the small-molecule PSSM0332 dissociates the CRL4A DCAF8 E3 ligase complex, reduces the ubiquitination of NcoR1, and inhibits the inflammatory response in a mouse model of sepsis-induced myocardial dysfunction[30]. Therefore, TCM and small-molecule drugs have significant value in the treatment of sepsis. Exploring the synergistic treatment of TCM, its active ingredients, and small-molecule drugs, evaluating the biological network mechanism between drugs and diseases through a multi-component, multi-target regulatory network, especially focusing on ubiquitination-related targets, and achieving precise targeted treatment according to the disease phenotypes and genotypes of patients can more effectively promote patient recovery and safeguard patient life and health, providing new potential for sepsis treatment.

However, how to integrate the ubiquitination regulatory network with changes in the immune microenvironment remains a challenge in the study of the molecular mechanism of sepsis. Traditional studies often screen differentially expressed genes based on a single dataset, but batch effects and technical heterogeneity may lead to result bias[31]. Integrative analysis of multi-cohort data can improve the reliability of gene screening. In addition, machine learning algorithms (e.g., LASSO regression, Random Forest) have shown unique advantages in feature gene screening and disease prediction model construction, capable of identifying key molecules with biological significance and diagnostic potential from high-dimensional data[32-34]. Recent studies have indicated that the immune cell infiltration patterns (e.g., macrophage polarization, T-cell exhaustion) in sepsis patients are closely associated with disease progression [35-37]. However, there is still a lack of systematic evidence on how UbRGs affect the outcome of sepsis by regulating immune cell functions.

Against the above background, we explored the differentially expressed ubiquitination-related genes and their regulatory pathways in sepsis by integrating multi-omics data. Three machine learning algorithms, namely LASSO regression, random forest (RF), and XGBoost[38], were employed to screen key feature genes. The diagnostic efficacy and immune regulatory role of candidate genes were evaluated by combining CIBERSORT immune infiltration analysis and ROC curves. The expression of candidate genes was verified in a sepsis macrophage model through RT-qPCR. Finally, small molecule drugs and TCMs that affect sepsis ubiquitination targets were screened and verified by molecular docking, aiming to provide new evidence for diagnostic markers and targeted therapeutic strategies based on the ubiquitination regulatory network.

Materials and Methods

Data Sources

All datasets used in this study were obtained from the GEO database, including GSE32707 (GPL10558, Illumina HumanHT-12 V4.0), GSE54514 (GPL6947, Illumina HumanHT-12 V3.0), GSE69528 (GPL10558, Illumina HumanHT-12 V4.0), and GSE137340 (GPL10558, Illumina HumanHT-12 V4.0) ─ four datasets in total, all of which are microarray gene expression profiles derived from Homo sapiens. Probe-to-gene mapping for each platform was performed using the respective official annotation files (the Soft files corresponding to each GPL). Specifically, GSE32707 includes 34 normal samples and 58 sepsis samples; GSE54514 comprises 36 normal samples and 127 sepsis-associated ARDS samples; GSE69528 contains 28 normal samples and 83 sepsis samples; and GSE137340 includes 12 normal samples and 45 sepsis samples. All sepsis cases met the corresponding diagnostic criteria for sepsis, and the control groups consisted of healthy individuals or postoperative non-infected patients. The rationale for merging these four cohorts was threefold: (1) all samples were transcriptomic data derived from whole blood or peripheral blood mononuclear cells (PBMCs); (2) principal component analysis (PCA) after batch correction did not reveal significant batch separation; and (3) as an exploratory bioinformatics study, combining multiple independent datasets increases sample size and enhances statistical power. Nevertheless, it should be noted that heterogeneity exists across cohorts in terms of infection source, disease severity, and sampling time. Both normal samples and sepsis samples from the 4 datasets were included in the subsequent analysis. All data used in the study were public data, so ethical approval was not required.

Ubiquitination modification, as one of the most prevalent forms of post-translational modifications, mediates functions such as the regulation of protein homeostasis in organisms. In recent years, it has attracted increasing attention. The GeneCards database provides comprehensive information about human genes. We obtained UbRGs from the GeneCards database, retaining only protein-coding genes and those with a relevance score > 5.

Identification of differentially expressed genes

To investigate the potential mechanisms, biological characteristics, and pathways of differentially expressed genes (DEGs) in sepsis, we first removed batch effects across the four GEO datasets using the ComBat function from the sva package, with dataset source and platform as covariates. The batch-corrected datasets were then merged, and subsequently annotated using the limma package. After data preprocessing, we obtained a list of DEGs between sepsis and normal samples. DEGs were identified using the following criteria: adjusted *P*-value (adj.*P*.Val) < 0.05 and $|\log_2 FC| \geq 0.2$, where $\log_2 FC > 0.2$ denoted upregulated genes and $\log_2 FC < -0.2$ denoted downregulated genes. *P*-values were adjusted using the Benjamini−Hochberg method. Finally, we took the intersection of these DEGs (adj.*P*.Val < 0.05 and $|\log_2 FC| \geq 0.2$) with the ubiquitination-related genes (UbRGs) via Venn diagram analysis to obtain the differentially expressed UbRGs. Volcano plots and heatmaps were generated using the ggplot2 and pheatmap packages in R, respectively.

GO, KEGG, and GSEA enrichment analyses of differentially expressed genes

Gene Ontology (GO) analysis can analyze gene products in terms of functions, involved biological pathways, and cellular localization, and thus is widely used in functional enrichment studies of a large number of genes. The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a widely used database that contains information on genomes, biological pathways, diseases,

and chemical substances. Gene Set Enrichment Analysis (GSEA) evaluates the distribution of predefined gene sets in a gene list ranked by phenotypic relevance to measure the contribution of genes to phenotypes. The ClusterProfiler R package was used for gene annotation and enrichment analysis of differentially expressed UbRGs. A *P*-value < 0.05 and a false discovery rate (FDR) (q-value) < 0.05 were considered statistically significant. The Benjamini-Hochberg method was used to correct *P*-values.

Lasso regression analysis

Read gene expression data and gene list files using R language, and then filter out the expression data of genes included in the list according to the gene list file. Use the glmnet package to perform Least absolute shrinkage and selection operator (LASSO) regression analysis, with gene expression values as predictor variables and sample groups as response variables. Use the cv.glmnet function to conduct cross-validation (CV) on the model, plot the CV graph, determine the optimal regularization parameter to select the optimal model, and then identify genes with non-zero coefficients, i.e., the selected characteristic genes, based on the coefficients of Lasso regression. Use the glm function to construct an initial logistic regression model for the screened characteristic genes. By checking the *P*-values of the model coefficients, select genes with significance ($P < 0.05$) as new model variables. Subsequently, based on the significant genes, construct a new logistic regression model and use the stepwise method for variable selection. Extract the coefficients, summary statistics, and confidence intervals of the new model, and calculate the Odds Ratio, 95% confidence interval, and *P*-value for each gene. Finally, use the trained final model to predict the samples and calculate the risk scores. LASSO regression employed 10-fold cross-validation and used the λ.min value ─ corresponding to the minimum cross-validation error ─ as the optimal regularization parameter for model selection.

RF Random Forest Regression

Random Forest (RF) is an ensemble learning method based on decision trees. It consists of multiple decision trees, each of which is trained on a randomly sampled dataset during the training process. In the training process, n sample data are drawn from the training set with replacement to form a new training set. For each training set, a part of variables are randomly selected for training to form a single decision tree model. When making classification predictions, all sub-models output their prediction results for the same prediction data. Finally, in the form of voting, the classification category with the most votes is output as the final output result of the model. Random forest employed MeanDecreaseAccuracy as the importance metric and retained the top 20 genes.

XGBoost Extreme Gradient Boosting

Extreme Gradient Boosting (XGBoost) is an algorithm based on the Gradient Boosting Decision Tree (GBDT). Its core idea is to iteratively train decision trees, where each tree is designed to correct the errors of the previous one. The XGBoost model was tuned via random search over a predefined hyperparameter space (including max_depth, eta, gamma, subsample, and colsample_bytree), with the objective of maximizing the AUC on the validation set.

We took the intersection of the genes selected by the three machine learning algorithms as the key genes, and visualized the results of the three algorithms using a Venn diagram.

Single-gene Gene Set Enrichment Analysis (GSEA)

Gene Set Enrichment Analysis (GSEA) evaluates the distribution of predefined gene sets in a gene list sorted by phenotypic relevance to measure the contribution of genes to phenotypes. First, we divided the characteristic UbRGs screened by three machine learning models into a high-expression group and a low-expression group according to their expression levels. Next, we used clusterProfiler to perform functional enrichment assessment on the high-expression and low-expression groups of different genes. The *P*-values were corrected using the Benjamini-Hochberg method, and *P*.adj < 0.05, |NES| > 1 indicated significant enrichment.

Immune infiltration analysis

The CIBERSORT algorithm is an analytical tool for estimating immune cell infiltration. It employs linear support vector regression to separate the transcriptome matrix and determine the composition and quantity of immune cells in the mixed cell population. We used the CIBERSORT package and grouped the four integrated gene sets into the normal group and the sepsis group, and filtered out the data with an immune cell enrichment value greater than zero. Then we obtained and presented the precise findings regarding the immune cell infiltration abundance matrix. The content of immune cells in the samples was represented by box plots. The interrelationships among immune cells were established using the Spearman algorithm, and the correlation heatmap was generated using the ggplot2 package.

Receiver Operating Characteristic (ROC) Curve

The receiver operating characteristic (ROC) curve is a comprehensive indicator reflecting the continuous variables of sensitivity and specificity, which uses the synthesis method to represent the relationship between sensitivity and specificity. The area under the ROC curve (AUC) typically ranges from 0.5 to 1. An AUC > 0.7 indicates a good predictor, and an AUC approaching 1 represents better predictive efficiency. We used the pROC package to construct the ROC curves of UbRGs and calculated the AUC to evaluate the diagnostic efficacy of characteristic UbRGs.

RT-qPCR

In this study, the human monocyte cell line THP-1 and the mouse macrophage cell line RAW264.7 were used for in vitro experimental verification. RPMI-1640 medium and DMEM medium were selected to culture THP-1 and RAW264.7 respectively. The cells were seeded in 6-well plates (at a density of 1×106cells/mL). During the logarithmic growth phase of the cells, lipopolysaccharide (LPS) at a concentration of 1 μg/mL was added for 24 hours to establish an in vitro septic cell model, while an equal volume of PBS was added to the control group. Each experimental condition was performed with three independent biological replicates (using different batches of cells), and each biological replicate contained three technical replicates. Total cellular RNA was extracted using TRIzol reagent, and the RNA was reverse-transcribed into cDNA with the PrimeScript RT kit. To determine whether GAPDH remains

stable as a reference gene under LPS challenge, we collected LPS-treated cells (n=3) and measured the Ct value changes of GAPDH using RT-qPCR. With GAPDH as the internal reference gene, the SYBR Green qPCR kit was used for RT-qPCR to detect the mRNA expression levels of FBXO7, SIAH1, SUMO2, and UBE2C. The 2^(-ΔΔCt) method was used to calculate the relative gene expression, and the t-test was used to compare the differences between groups. The primer sequences for human genes FBXO7, SIAH1, SUMO2, UBE2C, and the reference gene GAPDH are as follows: FBXO7 forward 5′-CCCTCCTCGATTCAGAGCATTC-3′ and reverse 5′-GCATGCTAGTCTGATTGGAGC-3′; SIAH1 forward 5′-CAGGACCCTACCCAGTGAATC-3′ and reverse 5′-CTGCGCTGTCCTCCAATGT-3′; SUMO2 forward 5′-AGGATGGTTCTGTGGTGCAG-3′ and reverse 5′-TCCATTTCCAACTGTCGTTCAC-3′; UBE2C forward 5′-GATGACCCTCATGGCAGTGG-3′ and reverse 5′-CCACACAAGGGGCTTGCTA-3′; GAPDH forward 5′-TGACATCAAGAAGGTGGTGAAGCAG-3′ and reverse 5′-GTGTCGCTGTTGAAGTCAGAGGAG-3′. For mouse (Mus musculus) genes Fbxo7, Siah1, Sumo2, Ube2c, and the reference gene Gapdh, the primer sequences are: Fbxo7 forward 5′-CCTCCCCGATACAGAGCATT-3′ and reverse 5′-TCACTGTGGGTGCGTTGTAA-3′; Siah1 forward 5′-TCTCCGCCCACAGAGATGAG-3′ and reverse 5′-TTGTTGGATGCAGTTGTGCC-3′; Sumo2 forward 5′-TTCTTTCATTTCCCTGTCCCCAT-3′ and reverse 5′-TCAACCAGAACAGAACATCGGT-3′; Ube2c forward 5′-AGCCGGCACCGTATATGAAG-3′ and reverse 5′-TCACTGTGGGTGCGTTGTAA-3′; Gapdh forward 5′-GGGGTCCCAGCTTAGGTTC-3′ and reverse 5′-CCAATACGGCCAAATCCGTT-3′.

Screening of Small-Molecule Drugs, TCMs and Bioactive Components

Four characteristic DE-UbRGs (FBXO7, SIAH1, SUMO2, UBE2C) were retrieved from the COREMINE database (https://www.coremine.com/medical). Small molecule drugs and TCMs that may map to sepsis-related ubiquitination characteristic genes were screened with $P < 0.05$ as the criterion. The components of TCMs were mainly obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) database (https://old.tcmsp-e.com/tcmsp.php), the HERB database (http://herb.ac.cn/), and literature queries, and were screened by oral bioavailability (OB ≥ 30%), drug-likeness (DL ≥ 0.18), and SwissADME (http://www.swissadme.ch/).

Molecular docking

The standardized information of core targets was obtained from the Uniprot database (https://www.uniprot.org), and the 3D structure files (usually in .pdb format) of target proteins were downloaded from the PDB (Protein Data Bank) database (https://www.rcsb.org/). The structure files (.sdf format) of small-molecule drugs and active components of TCMs were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). After preprocessing the structures of target proteins, small-molecule drugs, and active components of TCMs , they were uploaded to the CB-Dock2 system [39,40] (https://cadd.labshare.cn/cb-dock2/php/blinddock.php) respectively. Molecular docking was performed based on AutoDock Vina to obtain the binding results of ligand molecules and receptor proteins. The "Vina score" (kcal/mol) represents the score of the complex obtained by performing molecular docking of the receptor and ligand with the corresponding pocket parameters using the Vina program. A lower value indicates a higher affinity between the receptor and the ligand.

Statistical analysis

Data processing and analysis were performed using R software (version 4.1.0). Continuous variables were expressed as mean ± standard deviation. The Wilcoxon rank-sum test was used to compare the differences between the two groups. Unless otherwise specified, Spearman's correlation analysis was used to calculate the correlation coefficients between different components. $P < 0.05$ was considered statistically significant.

Results

Flowchart

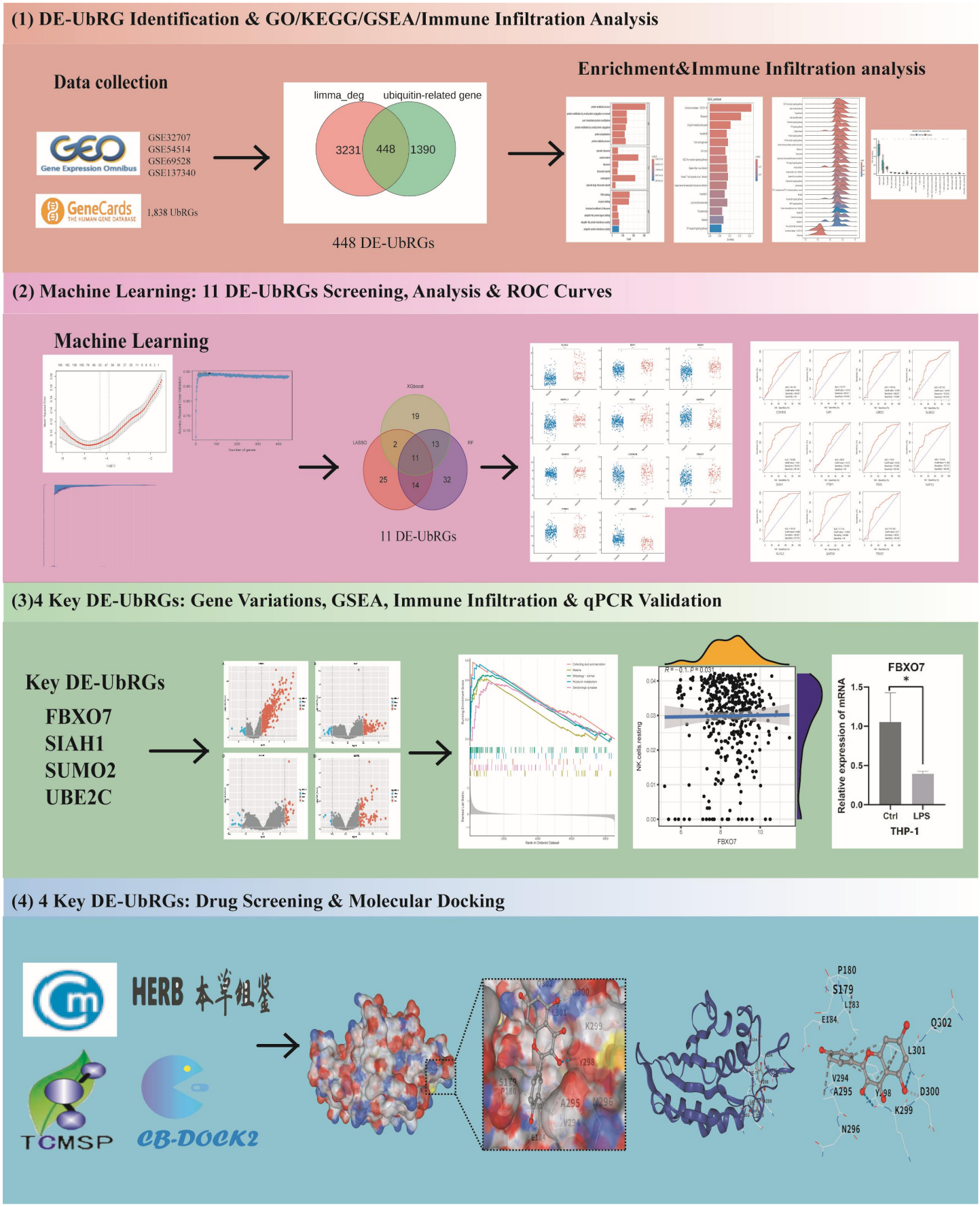


Figure 1. A graphic abstract of this study.

Identification of sepsis-related DE-UbRGs

The research workflow is illustrated in Fig. 1. This study analyzed four publicly available gene expression datasets (GSE32707, GSE54514, GSE69528, and GSE137340) to identify differentially expressed genes (DEGs) associated with sepsis. Principal component analysis (PCA) was performed using R to normalize the datasets and mitigate batch effects, ensuring consistent data distributions across samples (Fig. 2A-B). Differential expression analysis using the limma package identified 3,679 DEGs, including 2,023 downregulated and 1,656 upregulated genes (Fig. 2C-D, $P < 0.05$). These DEGs were then intersected with 1,838 ubiquitination-related genes (UbRGs) from the GeneCards database, revealing 448 different-ially expressed ubiquitination-related genes (DE-UbRGs) associated with sepsis, as presented in a Venn diagram (Fig. 2E).

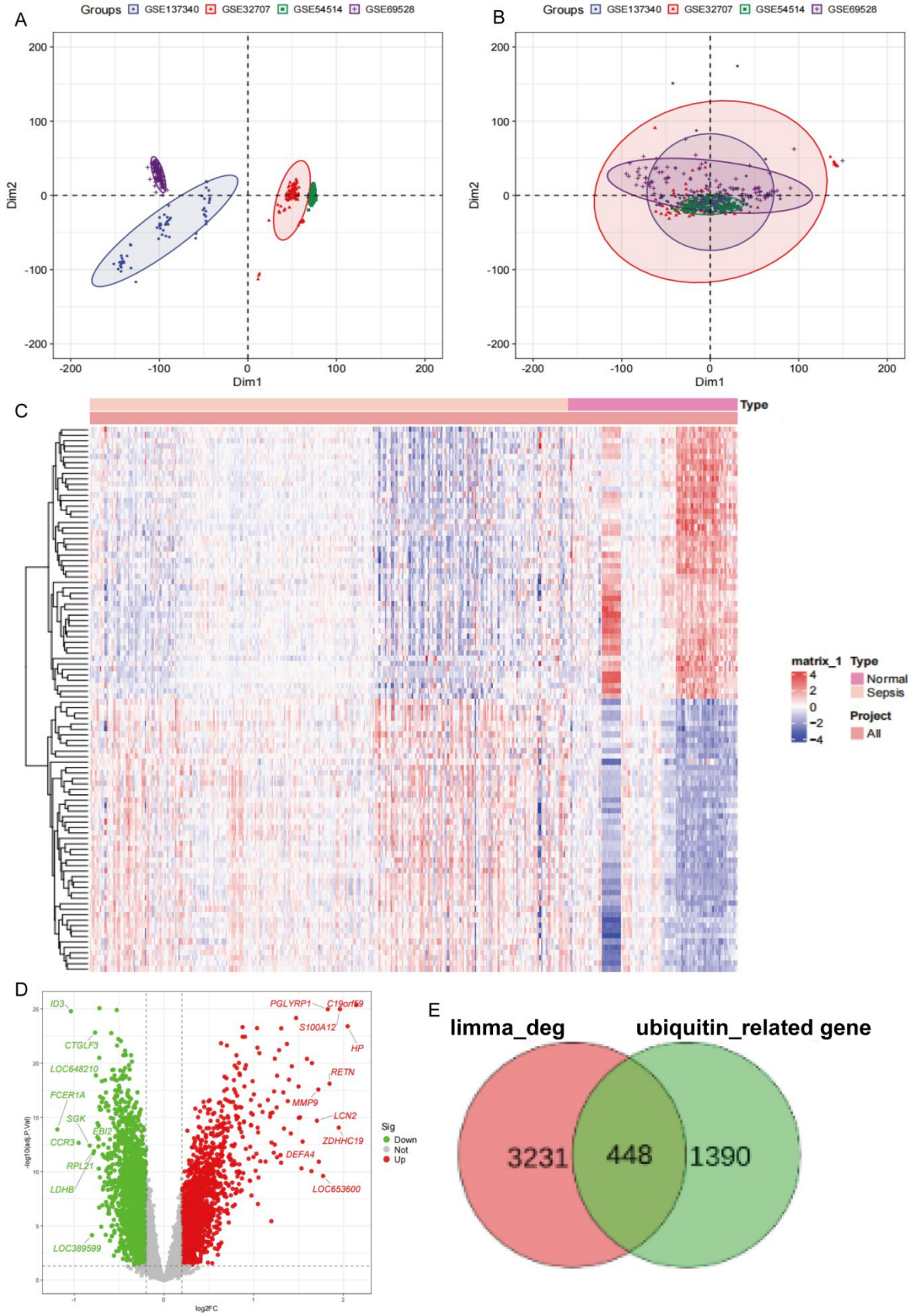


Figure 2. Identification of differentially expressed UbRGs. (A-B): Standardization of the dataset; (C) Heatmap of differentially expressed genes in sepsis; (D) Volcano plot of differentially expressed genes in sepsis; (E) Venn diagram of the intersection between DEGs and UbRGs.

Enrichment analysis and immune infiltration analysis of DE-UbRGs

GO functional analysis and KEGG pathway analysis were performed on these 448 differentially expressed UbRGs to explore their biological processes (BP), molecular functions (MF), cellular components (CC), and biological pathways in sepsis. In terms of BP, they were mainly enriched in protein metabolic process, protein modification by small protein conjugation or removal, and post-translational protein modification. In CC, they were mainly concentrated in cytosolic ribosome, nuclear lumen, and ribosome. In MF, they were mainly enriched in RNA binding, enzyme binding, and structural constituent of ribosome (Fig. 3A). In the KEGG pathways, they were mainly enriched in Coronavirus disease-COVID-19, ribosome, and ubiquitin mediated proteolysis (Fig. 3 B). Meanwhile, the results of GSEA analysis indicated that the 448 UbRGs were mainly enriched in 30 pathways, and the results were presented in the ridge plot of GSEA analysis (Fig. 3C). At the same time, it was shown that the 448 UbRGs were positively enriched in 4 most significantly up-regulated pathways, namely Chemokine signaling pathway, NF-kappa B signaling pathway, TNF signaling pathway, Toll-like receptor signaling pathway, etc., suggesting that these pathways might tend to be up-regulated or activated when UbRGs were highly expressed; while they were significantly negatively enriched in Coronavirus disease-COVID-19 and Ribosome, suggesting that when UbRGs were highly expressed, these two pathways might tend to be down-regulated or inhibited (Fig.3 C-D), and the differences were statistically significant. Subsequently, the CIBERSORT package was used to analyze the immune cell infiltration levels in the sepsis group and the normal group. The abundance of immune cell infiltration was estimated to obtain the relative proportions of infiltrating immune cells in the sepsis group and the normal group. The results showed that the proportions of each immune cell in the normal group and the disease group were different (Fig. 3E). As shown in the figure (Fig. 3F, $P < 0.05$), the infiltration levels of B cells memory, B cells naïve, Mast cells activated, and Mast cells resting in the sepsis group were higher than those in the normal group, while the infiltration levels of Monocytes, Eosinophils, and Plasma cells were relatively lower, and the differences were statistically significant. Meanwhile, the Spearman correlation coefficient analysis of the immune cell infiltration levels in the two groups found that M0 macrophages had a moderately strong negative correlation with resting CD4+ T cells (r = -0.55), and M0 macrophages had a moderately strong negative correlation with activated dendritic cells (r = -0.54) (Fig. 3G).

Screening of sepsis-related characteristic DE-UbRGs by machine learning models and their differential analysis

To further screen for characteristic genes, we performed Lasso regression analysis and identified 52 characteristic genes (Fig. 4 A). Through RF random forest regression, 70 characteristic genes were obtained (Fig. 4 B). Additionally, 45 characteristic genes were identified via XGBoost analysis(Fig. 4 C). Subsequently, we took the intersection of the three sets of genes and obtained 11 characteristic DE-UbRGs for sepsis. According to UniProt and Gene Ontology annotations, the 11 hub genes were functionally classified as follows: ubiquitin ligases (FBXO7, SIAH1, E4F1, UBE2C), ubiquitin-like modifier (SUMO2), substrates (GAPDH, PTBP1, CDKN1B), substrate-specific adaptor protein (KLHL3), histone chaperone (NAP1L1), and peroxisomal protein import receptor (PEX5). The results are presented in a Venn diagram (Fig. 4 D). To further analyze whether there were significant differences in the expression of these 11 sepsis-related DE-UbRGs between the normal group and the sepsis group, we used the ggplot package in R language to analyze the expression differences of these genes. As shown in the figure, there were significant differences in the expression of these 11 characteristic DE-UbRGs between the normal and disease groups ($P < 0.05$). The expression levels of GAPDH and UBE2C were higher in the sepsis group than in the normal group (Fig. 4 I, N). In contrast, the expression levels of KLHL3, E4F1, SIAH1, NAP1L1, PEX5, SUMO2, CDKN1B, FBXO7, and PTBP1 were lower in the sepsis group (Fig. 4 E-H, J-M, O). To understand the correlation between the expression of these 11 characteristic DE-UbRGs and sepsis, we used the pROC package to draw ROC curves. As shown in the figure, the AUC values were as follows: CDKN1B AUC = 0.651; E4F1 AUC = 0.757; UBE2C AUC = 0.726; SUMO2 AUC = 0.673; SIAH1 AUC = 0.762; PTBP1 AUC = 0.585; PEX5 AUC = 0.704; NAP1L1 AUC = 0.746; KLHL3 AUC = 0.791; GAPDH AUC = 0.712; FBXO7 AUC = 0.673 (Fig. 4 P-Z). These 11 characteristic DE-UbRGs demonstrated moderate potential for discriminating sepsis from control samples in the present merged dataset. Among them, E4F1, UBE2C, SIAH1, PEX5, NAP1L1, KLHL3, and GAPDH show a moderate correlation (AUC > 0.7).

Gene differences and GSEA analysis between high- and low-expression groups of key DE-UbRGs

Based on the *P*-value and correlation magnitude, we screened 4 key DE-UbRGs (FBXO7, SIAH1, SUMO2, and UBE2C) for validation. To explore the gene differences between the high-expression and low-expression groups, we used the ggplot package to analyze the DEGs of the 4 characteristic UbRGs between the high- and low-expression groups, with the criteria of $P.adj < 0.05$, $|logFC| > 0.2$. The results showed that there were 383 differential genes between the high-and low-expression groups of FBXO7, including 22 down-regulated genes and 361 up-regulated genes (Fig. 5 A). There were 129 differential genes between the high-and low-expression groups of SIAH1, including 23 down-regulated genes and 106 up-regulated genes (Fig. 5 D). There were 40 differential genes between the high-and low-expression groups of SUMO2, including 12 down-regulated genes and 28 up-regulated genes (Fig. 5 G). There were 101 differential genes between the high-and low-expression groups of UBE2C, including 9 down-regulated genes and 92 up-regulated genes (Fig.5 J). Meanwhile, we also conducted GSEA analysis to explore the

functions and regulated signaling pathways of the 4 characteristic DE-UbRGs (*P*.adj < 0.05and |NES| > 1). The results indicated that when FBXO7 was highly expressed, it was significantly enriched in Mitophagy-animal, etc. while when it was lowly expressed, it was significantly enriched in Diabetic cardiomyopathy, etc. (Fig. 5 B-C). When SIAH1 was highly expressed, it was significantly enriched in Mitophagy-animal, etc., and when it was lowly expressed, it was significantly enriched in Primary immunodeficiency, etc. (Fig.5 E-F). When SUMO2 was highly expressed, it was significantly enriched in Ribosome, etc., and when it was lowly expressed, it was significantly enriched in Taste transduction (Fig. 5 H-I), etc. When UBE2C was highly expressed, it was significantly enriched in Parkinson disease, etc., and when it was lowly expressed, it was significantly enriched in Th1 and Th2 cell differentiation, etc. (Fig. 5 K-L).

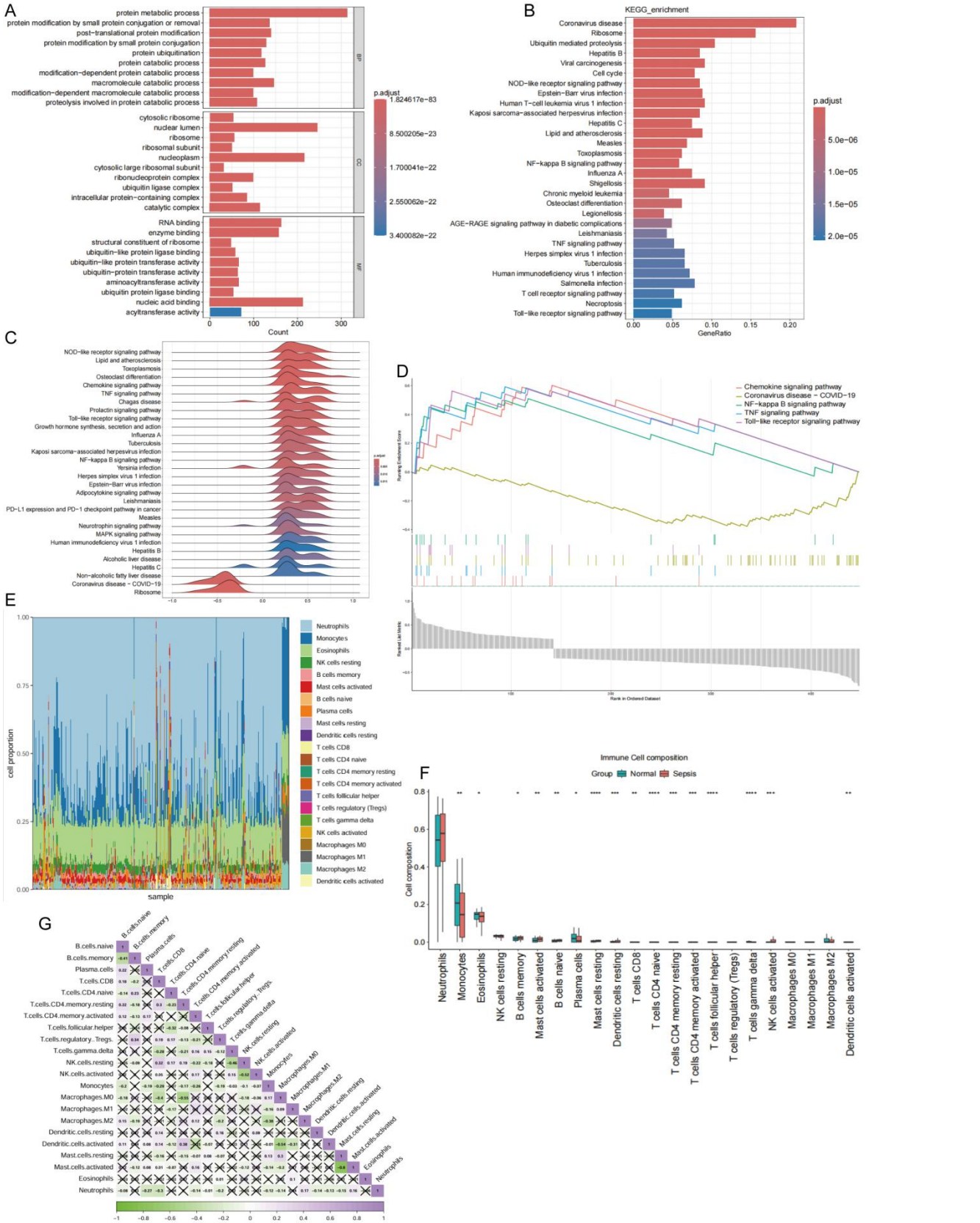


Figure 3. Enrichment analysis and immune infiltration analysis of DE-UbRGs. (A) GO functional analysis; (B) KEGG pathway analysis; (C-D) GSEA analysis of DE-UbRGs; (E-G) Immune infiltration analysis of DE-UbRGs

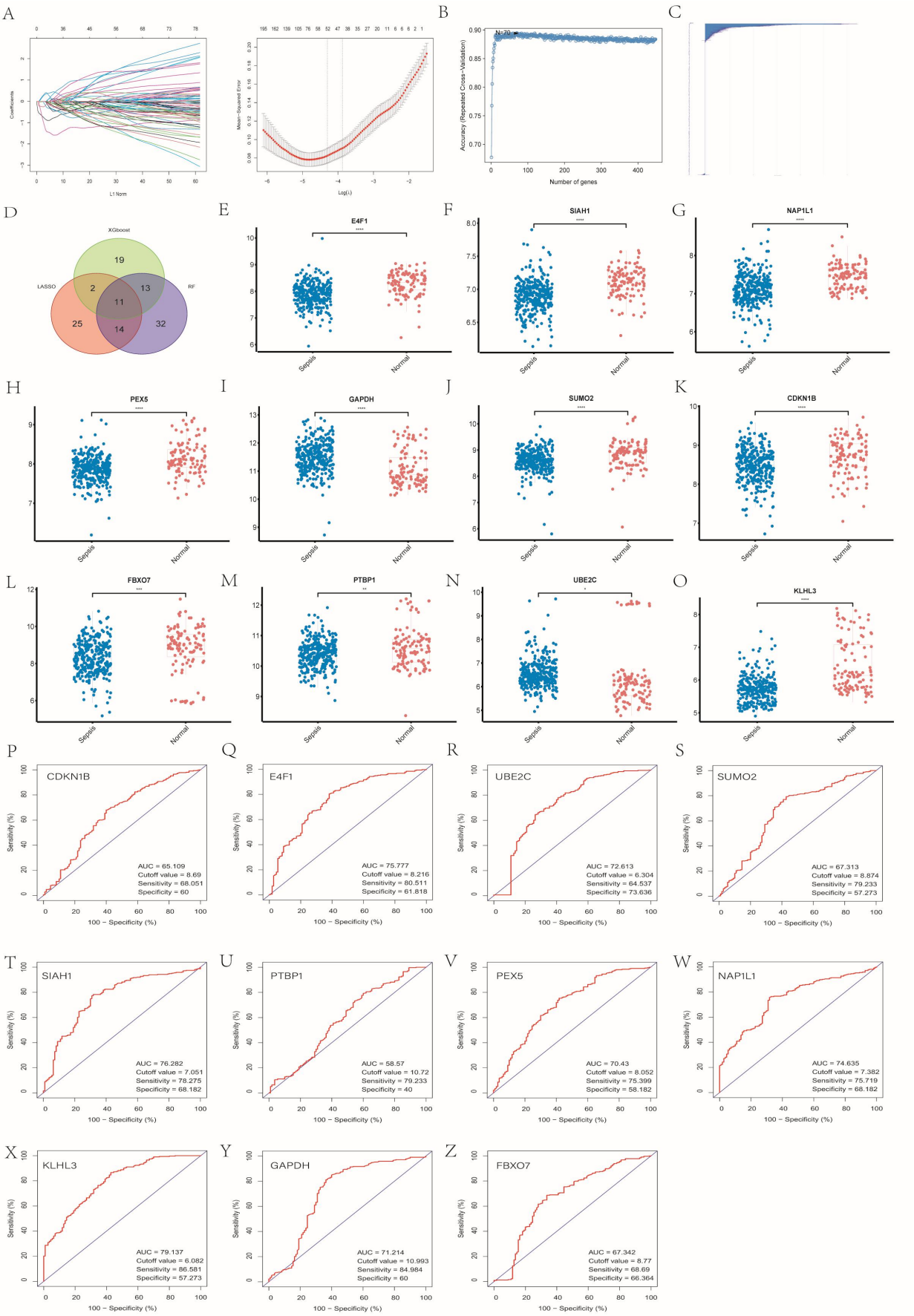


Figure 4. Screening of sepsis-related characteristic differentially expressed ubiquitination-related genes (DE-UbRGs) by machine learning models and differential analysis. (A) Lasso regression analysis identified 52 sepsis-related genes. (B) Random forest regression selected 70 sepsis-related genes. (C) XGBoost analysis identified 45 characteristic genes. (D) The intersection of these machine learning methods revealed 11 distinct DE-UbRGs. (E-O) Expression differences of these 11 DE-UbRGs were compared between normal and disease groups. (P-Z) Correlation analysis was conducted between the 11 DE-UbRGs and sepsis.

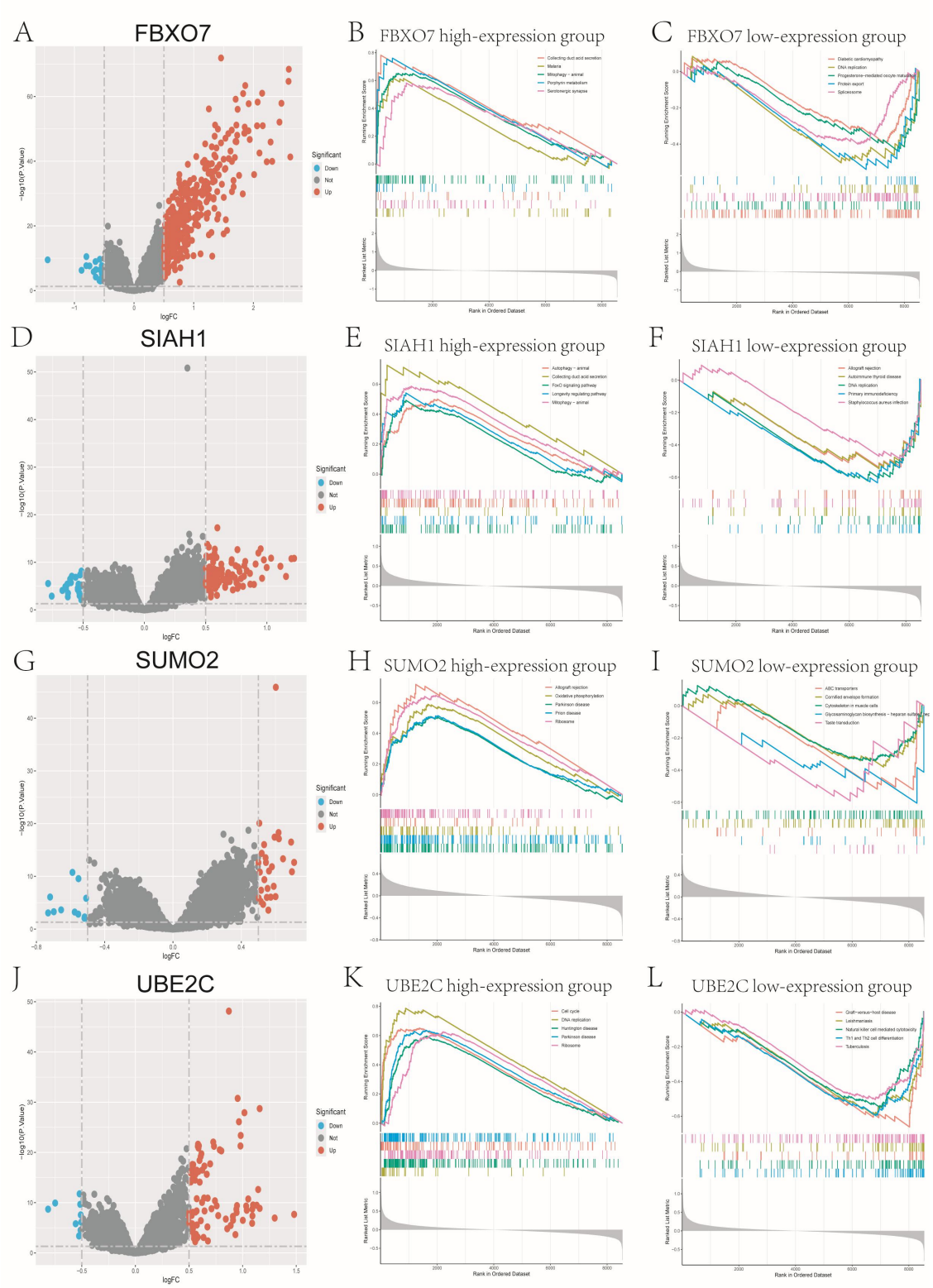


Figure 5. Differential gene analysis and GSEA analysis between the high-expression group and low-expression group of DE-UbRGs. (A) Differential gene analysis between high- and low-expression groups of FBXO7; (B-C) GSEA analysis of FBXO7; (D) Differential gene analysis between high- and low-expression groups of SIAH1; (E-F) GSEA analysis of SIAH1; (G) Differential gene analysis between high- and low-expression groups of SMUO2; (H-I) GSEA analysis of SMUO2; (J) Differential gene analysis between high- and low-expression groups of UBE2C; (K-L) GSEA analysis of UBE2C.

Correlation analysis between key genes and immune cells

To investigate the immune cell infiltration status of these four key DE-UbRGs, we employed the CIBERSORT package to estimate the immune cell abundances of the genes and used the ggplot package to visualize the data. The results showed that: FBXO7 was positively correlated with naïve B cells (r = 0.15, *P* = 0.0028), memory B cells (r = 0.19, *P* = 6.5e-05), resting mast cells (r = 0.11, *P* = 0.021), activated mast cells (r = 0.13, *P* = 0.0077), and neutrophils (r = 0.25, *P* = 1.1e-07); and negatively correlated with plasma cells (r = -0.16, *P* = 0.0011), activated CD4+ memory T cells (r = -0.13, *P* = 0.0078), γδ T cells (r = -0.17, *P* = 0.00048), resting NK cells (r = -0.1, *P* = 0.031), monocytes (r = -0.14, *P* = 0.0036), M0 macrophages (r = -0.29, *P* = 1.9e-09), M1 macrophages (r = -0.27, *P* = 2.3e-08), M2 macrophages (r = -0.28, *P* = 8.2e-09), and activated dendritic cells (r = -0.14, *P* = 0.0031) (Fig. 6).

SIAH1 was positively correlated with plasma cells (r = 0.2, *P* = 2.2e-05), resting NK cells (r = 0.21, *P* = 2.1e-05), monocytes (r = 0.22, *P* = 3.6e-06), and eosinophils (r = 0.21, *P* = 2.1e-05); and negatively correlated with naive B cells (r = -0.18, *P* = 0.00015), memory B cells (r = -0.14, *P* = 0.0031), activated CD4+ memory T cells (r = -0.22, *P* = 3e-06), regulatory T cells (r = -0.14, *P* = 0.0048), activated NK cells (r = -0.27, *P* = 2.2e-08), resting dendritic cells (r = -0.23, *P* = 1e-06), activated dendritic cells (r = -0.18, *P* = 0.00013), resting mast cells (r = -0.21, *P* = 2e-05), and activated mast cells (r = -0.19, *P* = 5.8e-05) (Fig. S1).

SUMO2 was positively correlated with plasma cells (r = 0.24, *P* = 3.9e-07), γδ T cells (r = 0.18, *P* = 0.00019), resting NK cells (r = 0.23, *P* = 1.8e-06), monocytes (r = 0.25, *P* = 1.9e-07), M0 macrophages (r = 0.26, *P* = 1e-07), M1 macrophages (r = 0.12, *P* = 0.013), M2 macrophages (r = 0.24, *P* = 7.1e-07), and eosinophils (r = 0.22, *P* = 6.5e-06); and negatively correlated with naive B cells (r = -0.26, *P* = 3.4e-08), memory B cells (r = -0.26, *P* = 4.2e-08), activated NK cells (r = -0.19, *P* = 0.00011), resting dendritic cells (r = -0.19, *P* = 7.7e-05), resting mast cells (r = -0.26, *P* = 3.7e-08), activated mast cells (r = -0.27, *P* = 1.2e-08), and neutrophils (r = -0.22, *P* = 6.8e-06) (Fig. S2).

UBE2C was positively correlated with CD4+ memory T cell activation (r = 0.2, *P* = 5.1e-05), NK cell activation (r = 0.13, *P* = 0.008), M0 macrophages (r = 0.21, *P* = 1.7e-05), M1 macrophages (r = 0.29, *P* = 9.1e-10), M2 macrophages (r = 0.19, *P* = 8.7e-05), resting dendritic cells (r = 0.14, *P* = 0.0038), and activated dendritic cells (r = 0.17, *P* = 0.00063); and negatively correlated with CD8+ T cells (r = -0.13, *P* = 0.0057), naive CD4+ T cells (r = -0.2, *P* = 5.1e-05), follicular helper T cells (r = -0.17, *P* = 0.00037), and neutrophils (r = -0.15, *P* = 0.0022) (Fig. S3).

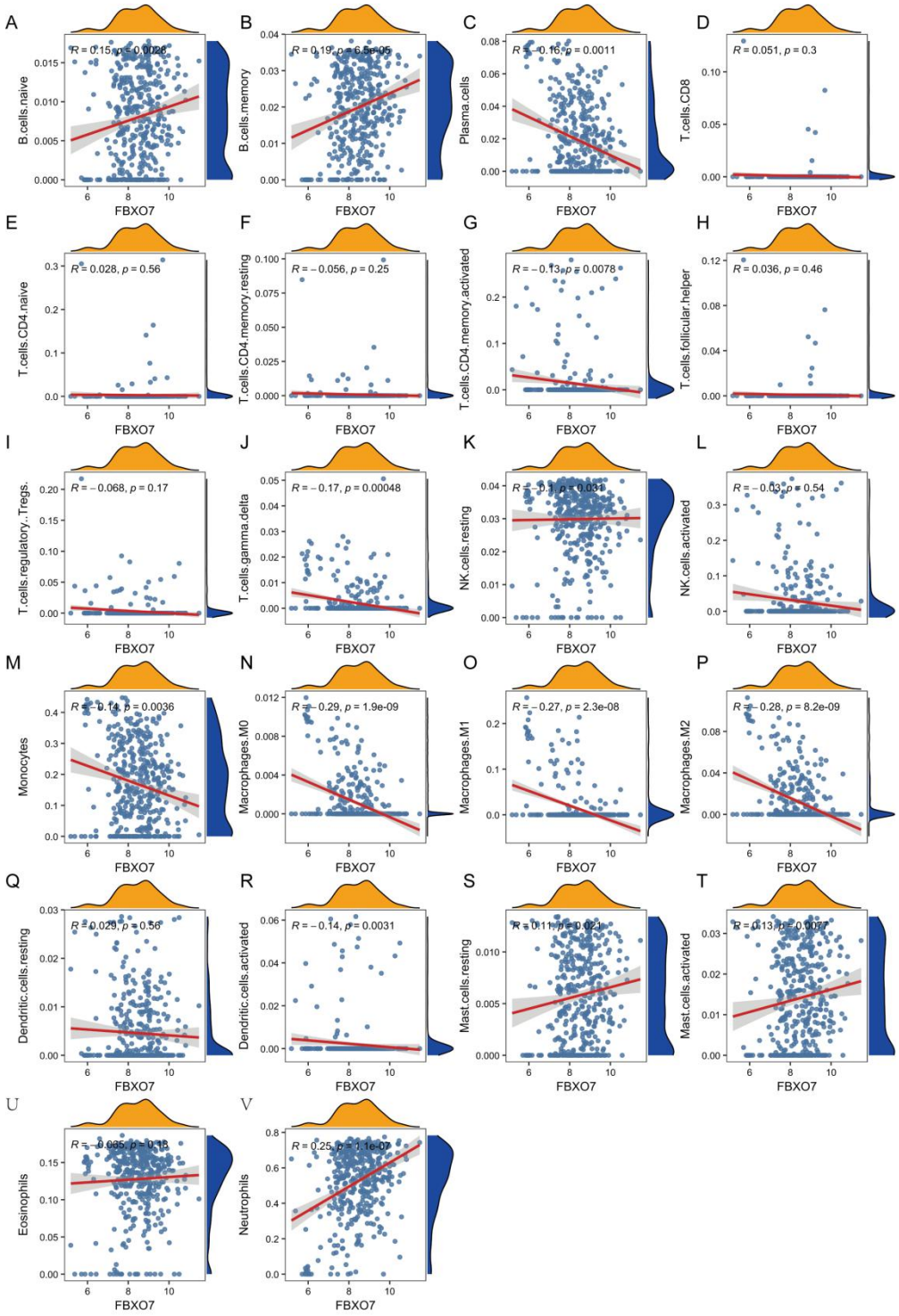


Figure 6. Correlation analysis between FBXO7 and immune cells (A) naïve B cells, (B) memory B cells, (C) plasma cells, (D)

CD8+ T cells, (E) naive CD4+ T cells, (F) resting CD4+ memory T cells, (G) activated CD4+ memory T cells, (H) follicular helper T cells, (I) regulatory T cells, (J) γδ T cells, (K) resting NK cells, (L) activated NK cells, (M) monocytes, (N) M0 macrophages, (O) M1 macrophages, (P) M2 macrophages, (Q) resting dendritic cells, (R) activated dendritic cells, (S) resting mast cells, (T) resting mast cells, (U) eosinophils, (V) neutrophils.

Validation of the expression of key DE-UbRGs genes in the sepsis macrophage model

The results of RT-qPCR are shown in Fig 7. In THP-1 cells, the mRNA expression levels of FBXO7, SIAH1, and SUMO2 were significantly decreased in the LPS intervention group (P = 0.0386, 0.0007, 0.008), while the mRNA expression level of UBE2C was significantly increased (P = 0.0109), all of which were consistent with the results of bioinformatics analysis. Nevertheless, RT-qPCR merely reflects mRNA abundance changes and does not permit direct inference of changes in protein expression or ubiquitination activity. In RAW264.7 cells, after 24 hours of LPS intervention, the mRNA expression levels of SIAH1, SUMO2, and UBE2C were significantly decreased (P = 0.0079, 0.0028, 0.0021), and there was no significant change in the mRNA expression level of FBXO7 (P = 0.442).

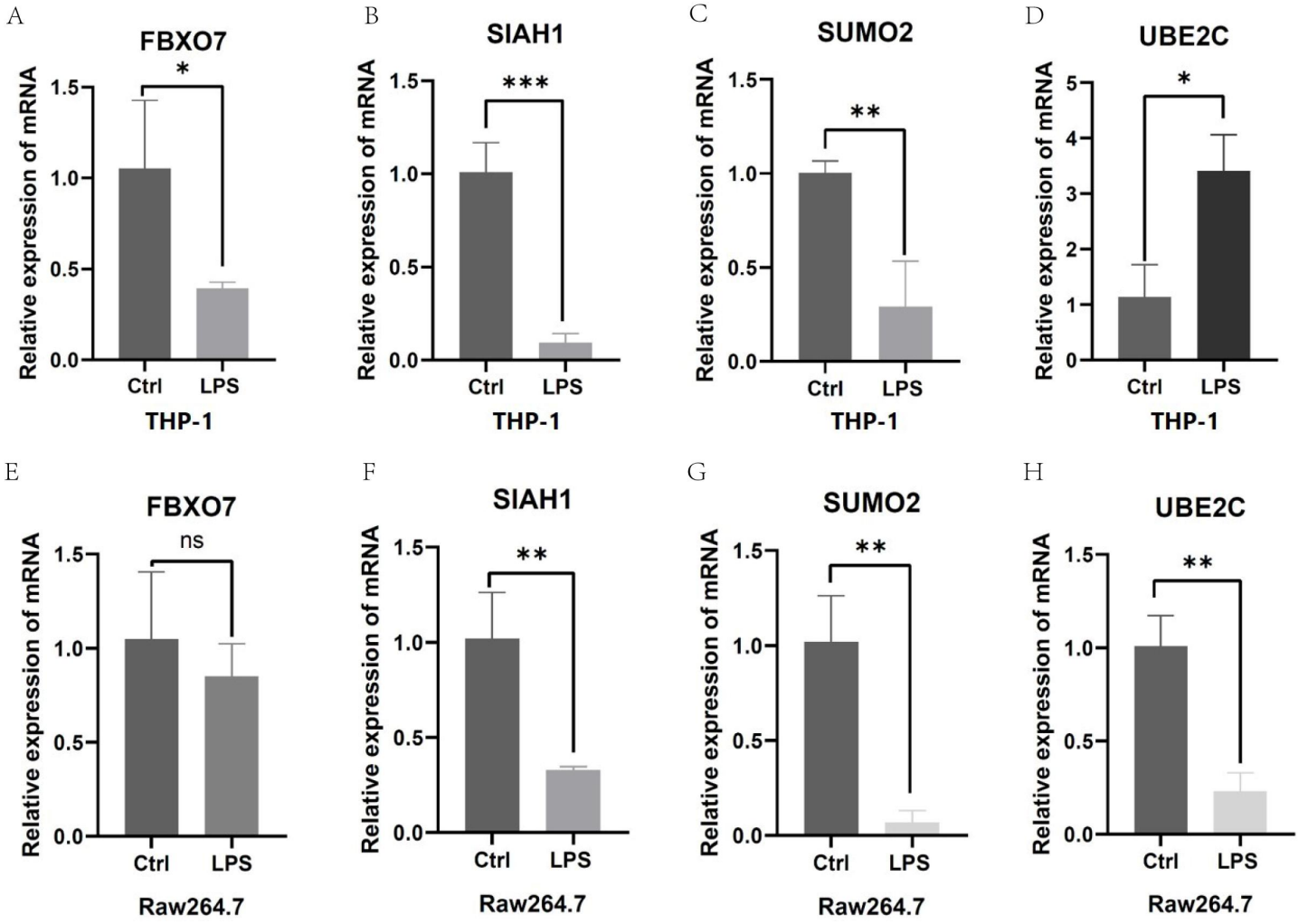


Figure 7. Results of RT-qPCR. (A-D) mRNA expression levels of FBXO7, SIAH1, SUMO2 and UBE2C in THP-1 cells; (E-H) mRNA expression levels of FBXO7, SIAH1, SUMO2 and UBE2C in RAW 264.7 cells.

Potential small-molecule drugs and molecular docking of characteristic DE-UbRGs

Based on the Coremine Medical database, eight potential small-molecule drugs that commonly map to the four sepsis-characteristic DE-UbRGs (FBXO7, SIAH1, SUMO2, UBE2C) were screened (P < 0.05). These include nutritionally essential amino acid (Lysine), herbicide (alachlor), neuromodulator (p-Chloroamphetamine), natural moisturizing factor (Pyrrolidonecarboxylic Acid), protein synthesis inhibitor (Cycloheximide), mTOR inhibitor (Sirolimus), DNA alkylating agent (Temozolomide), and platinum derivative (Iproplatin). These compounds may participate in sepsis pathology by regulating ubiquitination processes. CB-DOCK2 molecular docking showed that all drugs exhibited negative Vina scores (kcal/mol) with the targets, indicating binding activity. Among them, Sirolimus showed the highest binding activity with all four targets, with the optimal binding energy to UBE2C (−8.0 kcal/mol).

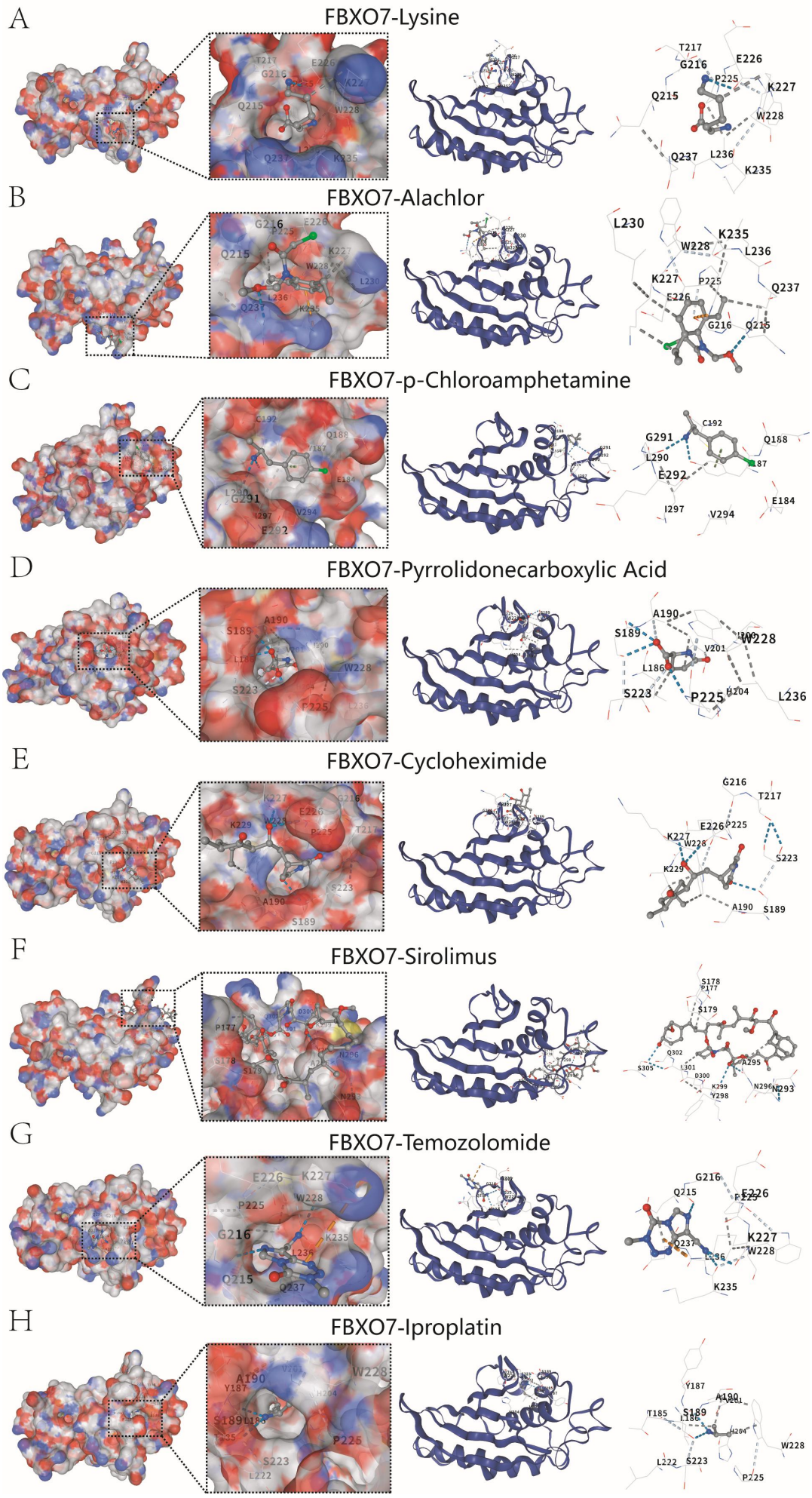


Figure 8. Molecular docking visualization of FBXO7 small-molecule drugs. Overall binding mode of ligand within protein pocket, enlarged binding pocket view with interaction details and interaction diagram. Dashed line coding: Light blue & dark blue= H-bonds,

gray = hydrophobic, yellow = ionic, orange = cation-π, green = π-π stacking. (A) Lysine, alachlor; (B)p-Chloroamphetamine; (C)Pyrrolidonecarboxylic Acid; (D)Cycloheximide; (E) Sirolimus; (F)Temozolomide; (G)Iproplatin.

Screening of potential TCMs and active ingredients and molecular docking of key DE-UbRGs

The four characteristic DE-UbRGs, FBXO7, SIAH1, SUMO2, and UBE2C, were imported into the Coremine database with a screening criterion of $P < 0.05$. Potential mapping relationships were identified: FBXO7 with Oenanthe javanica (water dropwort), SIAH1 with Areca catechu (betel nut/Areca peel), SUMO2 with Brassica rapa (turnip), and UBE2C with Fructus Arctii (great burdock achene). The active compound groups of Oenanthe javanica are flavonoids and coumarins; Areca catechu contains proanthocyanidins and catechins; Brassica rapa includes flavonoids and liquiritin-type compounds; Fructus Arctii comprises lignans, among others. These may regulate inflammatory signal transduction in sepsis by interfering with ubiquitination modification and inhibiting the ubiquitination-mediated activation of the NLRP3 inflammasome. However, the specific biological associations require further experimental validation. Through searches in the TCMSP and HERB databases and literature review, combined with oral bioavailability (OB ≥ 30%) and drug-likeness (DL ≥ 0.18) criteria, and further screening via the SwissADME platform, five potential active components were selected from each of the four traditional Chinese medicines (20 in total). These compounds exhibit relatively favorable oral absorption properties and drug-like potential (Supplementary Table 1), suggesting possible in vivo biological activity, although their specific plasma concentrations and tissue distribution characteristics need confirmation by further pharmacokinetic studies. Molecular docking of the four DE-UbRGs with their corresponding active components from the traditional Chinese medicines was performed using CB-DOCK2. The results showed that all candidate components had Vina scores below -5 kcal/mol (Supplementary Table 1), indicating stable binding potential with the target proteins. Notably, SIAH1 with Procyanidin B1 exhibited the lowest binding energy (-9.3 kcal/mol), while UBE2C with beta-carotene (-7.5 kcal/mol), FBXO7 with Kaempferol (-6.5 kcal/mol), and SUMO2 with Liquiritin (-6.5 kcal/mol) also demonstrated relatively strong binding tendencies. Detailed docking conformations and interaction relationships are presented in Fig. 9 and Figs. S8–S10.

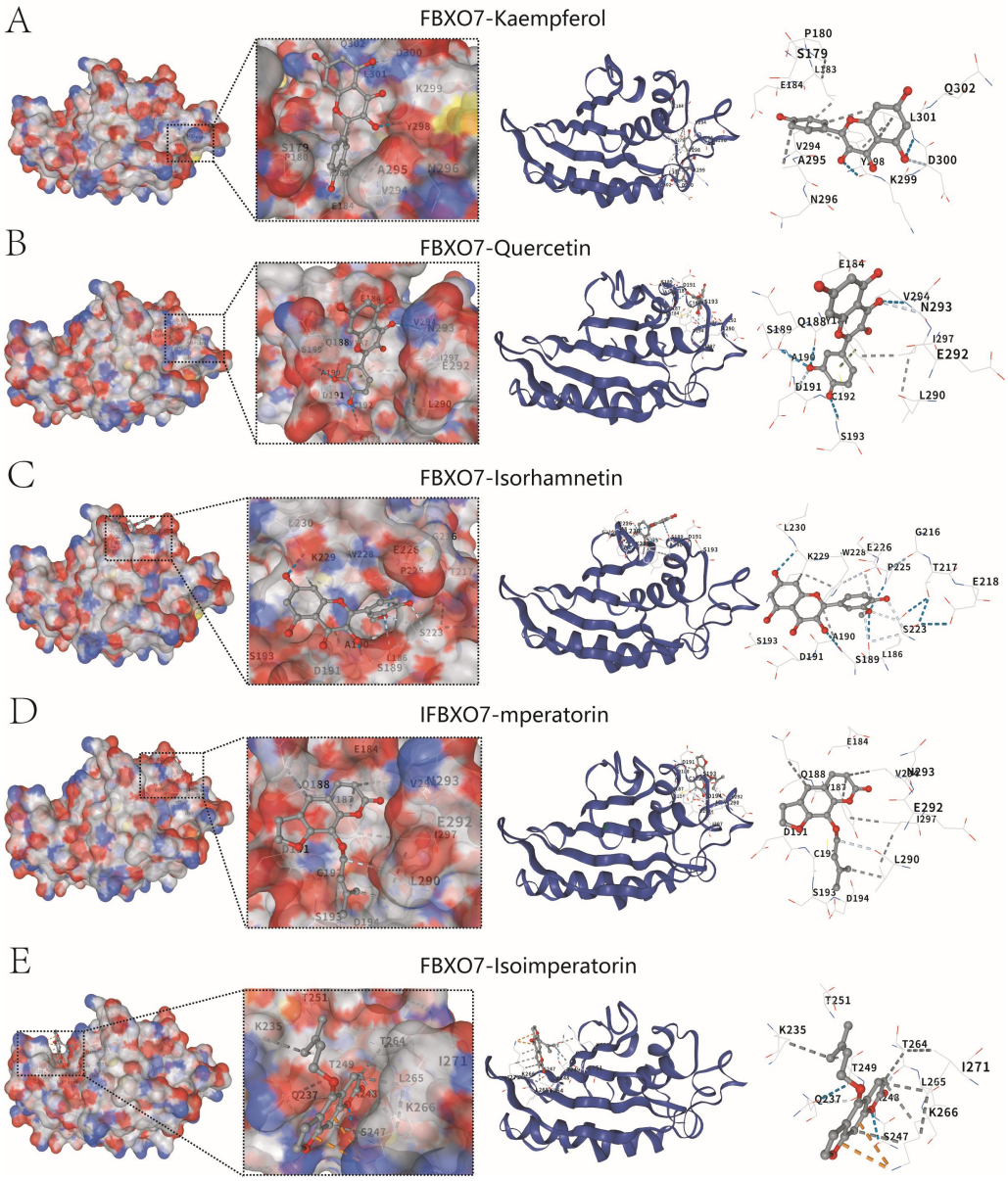


Figure 9. Molecular docking visualization of active components of TCM with FBXO7. (A) Kaempferol, (B)Quercetin, (C) Isorhamnetin, (D) Imperatorin, (E) Isoimperatorin. Overall binding mode of ligand within protein pocket, enlarged binding pocket view with interaction details and interaction diagram. Dashed line coding: Light blue & dark blue= H-bonds, gray = hydrophobic, yellow = ionic, orange = cation-π, green = π-π stacking.

Discussion

In this study, four datasets (GSE32707, GSE54514, GSE69528, GSE137340) from the GEO database were standardized and analyzed, and 3,679 differentially expressed genes (DEGs) in sepsis were identified. Among them, 1,656 were upregulated and 2,023 were downregulated in the sepsis group. This extensive change in gene expression highlights the complex molecular landscape of sepsis. A total of 1,838 UbRGs were screened from the GeneCards database, and by intersecting with sepsis DEGs, 448 sepsis-related UbRGs were obtained. Functional enrichment analysis of these UbRGs indicated that they are involved in various biological processes, molecular functions, and cellular components, suggesting that ubiquitination may be a key mechanism regulating the immune response and inflammatory pathways during sepsis. Pathway analysis showed that these UbRGs were significantly enriched in 15 key pathways, such as the NF-κB signaling pathway, apoptosis, and ubiquitin-mediated proteolysis, which is consistent with recent research findings. For example, tumor necrosis factor receptor-associated factor 6 (TRAF6), with ubiquitin E3 ligase activity, activates the NF-κB signaling pathway through K63-site ubiquitination, while K48-site polyubiquitination negatively regulates the signal feedback between RANKL and IFN-γ[41]. In contrast, the deubiquitinase, TNF α -induced protein 3 (TNFAIP3), terminates NF-κB and MAP kinase signaling by removing K-63-linked polyubiquitin chains from TRAF2, TRAF6, and NEMO/IKKγ, thereby inhibiting tissue inflammation[42]. TNFAIP3 was found to exert an anti-inflammatory effect in the retinal vasculature by inhibiting NF-κB activation[43]. Downregulation of TNFAIP3 expression may lead to excessive activation of NF-κB signaling, thereby exacerbating the systemic inflammatory response[44].

Using machine learning models (LASSO, XGBoost, and RF), we identified 11 characteristic ubiquitination-related genes (UbRGs) and evaluated their discriminative ability between normal and sepsis groups based on differential expression analysis and ROC curves within the current merged dataset. The statistically significant expression differences of these genes between the normal and sepsis groups suggested their potential as candidate biomarkers. Subsequently, we focused on four characteristic DE-UbRGs (FBXO7, SIAH1, SUMO2, and UBE2C). FBXO7, SIAH1, and SUMO2 were significantly downregulated in the sepsis group ($P < 0.05$), while UBE2C was significantly upregulated ($P < 0.05$). In subsequent experiments using LPS-treated THP-1 cells, RT-qPCR was performed to preliminarily validate the transcriptional expression of these four UbRGs, which showed trends consistent with the bioinformatics results, thereby providing initial experimental support for their candidacy as biomarkers. Nevertheless, it should be noted that RT-qPCR only reflects mRNA abundance and does not directly demonstrate changes at the protein level or ubiquitination activity. However, the functional differences of FBXO7 between THP-1 cells and RAW264.7 cells may be mainly attributed to three aspects: first, inherent species differences, namely distinct gene regulation and signaling pathway dynamics between human and mouse; second, differences in cell origin and differentiation status: THP-1 is an induced immature human monocyte line, whereas RAW264.7 is a terminally differentiated mouse macrophage line; third, the multifunctional nature of FBXO7 itself and its cell-type dependence. Therefore, these differences reflect the species specificity and cellular context dependence of FBXO7 function, and caution should be exercised when extrapolating the results to other species or cell models. We conducted immune infiltration analysis and GSEA analysis on the 4 UbRGs, indicating that they may play important roles in the inflammatory response and the maintenance of cellular homeostasis.

The pathological process of sepsis is closely associated with the dynamic changes in the infiltration pattern of immune cells. Recent studies have suggested that ubiquitination genes are involved in the pathological process of sepsis by mediating protein degradation and regulating signal transduction pathways, thereby influencing the function and activity of immune cells[45]. FBXO7 (F-box protein 7), a key component of the SKP1-CUL1-F-box (SCF) E3 ligase complex, can specifically recognize various substrate proteins and regulate the stability and function of target proteins through the ubiquitin-proteasome system (UPS). FBXO7 participates in the regulation of proteasome complexes, mitophagy, the cell cycle, cell proliferation, and germ cell differentiation[46]. In this study, the immune infiltration analysis of FBXO7 showed a significant correlation with multiple immune cells. Positive correlations were observed with naive B cells, memory B cells, two mast cell states, and neutrophils, especially the strongest correlation with neutrophils. There were more negatively correlated cell types, including plasma cells, several T cell types, NK cells, all macrophage subtypes, etc., with the strongest negative correlation with M0 macrophages. These results imply that FBXO7 may inhibit the function of myeloid immune cells (such as macrophages and monocytes) while promoting the activities of certain B cells and neutrophils. Considering that the excessive activation of macrophages in sepsis exacerbates inflammation, the low expression of FBXO7 may weaken the inhibition of macrophages, that is, promote myeloid cell infiltration, leading to uncontrolled inflammation. In the GSEA analysis, the positively correlated pathways were concentrated in cellular stress response and metabolic regulation, such as acid secretion, mitophagy, malaria-related pathways, etc. These may reflect the adaptive mechanisms of cells under sepsis stress. The negatively correlated pathways were mainly related to energy metabolism and immune activation, such as oxidative phosphorylation, RIG-I signaling, proteasome, etc. This indicates that when FBXO7 is lowly expressed, cellular energy production is hindered, and the antiviral response ability decreases, which is consistent with the characteristics of the immune paralysis stage of sepsis. It may inhibit metabolic pathways and drive disease deterioration, making it a research target with translational value, but the dynamic changes in the course of human sepsis still need to be verified. Fbxo7 negatively regulates NF-κB signaling through the ubiquitination of TRAF2 and cIAP1[47]. FBXO7 mediates the proteasomal degradation of UXT-V2, resulting in the inhibition of the NF-κB signaling pathway[48]. Additionally, FBOX7 plays a role in mitophagy[49], and the chemical inhibition of FBXO7 reduces inflammation and confers neuroprotection by stabilizing the mitochondrial kinase PINK1[50]. Overall, FBXO7 can inhibit the inflammatory response through

the NF-κB signaling pathway and maintain mitochondrial homeostasis to suppress excessive inflammation. The mechanism research and targeted therapy of FBXO7 still face challenges, but it has shown significant clinical translational potential. A study has shown that ginsenoside Rg1 treats sepsis-induced acute lung injury by regulating FBXO3 (a homologous protein of FBXO7), providing direct evidence for targeting F-box proteins. This study found that Rg1 reduces the stability of FBXO3 in an m6A-dependent manner, thereby activating the PGC-1α/Nrf2 signaling pathway and alleviating oxidative stress injury[51]. Considering the structural and functional similarities between FBXO7 and FBXO3, this study provides strong theoretical support for targeting FBXO7 in the treatment of sepsis. The main challenges in targeted therapy of FBXO7 include differences in tissue-specific functions, sepsis heterogeneity, and drug delivery obstacles. Future research should focus on verifying the regulatory mechanism of FBXO7 in sepsis, optimizing the characteristics of lead compounds, and developing personalized treatment strategies. Combining the strategies of repurposing old drugs and combination therapy, targeting FBXO7 is expected to provide new treatment options for this complex syndrome of sepsis, compensating for the limitations of the current mainly supportive treatment.

SIAH1 (Seven in Absentia Homolog 1) is a RING-type E3 ubiquitin ligase that participates in the regulation of key biological processes such as cell apoptosis, autophagy, metabolic stress response, and tumorigenesis by mediating the ubiquitination and degradation of specific substrate proteins. Its function is significantly "environment-dependent". Previous studies have shown that SIAH1 regulates cell signaling pathways by promoting the ubiquitination and degradation of substrates. For example, SIAH1 can promote the ubiquitination of RECK, thereby affecting cell migration and invasion related to matrix metalloproteinase 9 (MMP9)[52]. In non-alcoholic fatty liver disease (NAFLD), SIAH1 regulates lipid metabolism by interacting with Sterol Carrier Protein 2 (Scp2) and promoting its ubiquitination and degradation[53]. These mechanisms indicate that SIAH1 not only plays a role in the occurrence and development of cancer but is also an important regulator of metabolic diseases. However, there is a lack of systematic literature reports on the relationship between the function of SIAH1 in the ubiquitination and deubiquitination processes and sepsis. This study found that low expression of SIAH1 may indicate immune dysregulation and metabolic defects in sepsis patients, and its expression level may be related to the severity of the disease. Low expression in sepsis causes dual damage through "attenuated autophagy + immune quiescence": on the one hand, it weakens cell stress protection, and on the other hand, it hinders immune activation, jointly promoting the progression of sepsis. Temporal-specific regulation targeting this gene may be a potential therapeutic strategy. SIAH1 is positively correlated with the infiltration of innate immune cells (monocytes, resting NK cells) but significantly negatively correlated with the markers of adaptive immune activation (T/B cell activation, dendritic cells, regulatory T cells), especially the strong negative correlation with NK cell activation ($r = -0.27$), suggesting that SIAH1 may promote the immunosuppressive state by inhibiting the acquired immune response. A spinal cord injury model revealed a new mechanism of SIAH1 in the inflammatory response: after lipopolysaccharide (LPS) stimulation, the formation of the GAPDH-SIAH1 complex in microglia increases, activating the NF-κB pathway and releasing pro-inflammatory factors such as IL-6 and TNF-α. Nrf2 can alleviate inflammation by inhibiting this complex - overexpression of Nrf2 reduces SIAH1 expression by 50% and decreases the microglia proliferation rate by 35%[54].In the GSEA analysis, high expression of SIAH1 is significantly enriched in cell autophagy (Autophagy/Mitophagy), ferroptosis, and FoxO signaling, indicating that it may affect the sepsis process by regulating cell metabolic stress and survival pathways. At the same time, it significantly inhibits pathways related to primary immunodeficiency, DNA replication, and autoimmunity, confirming its negative regulatory effect on immune activation, which is consistent with the results of immune infiltration. In summary, in sepsis, SIAH1 has dual or even opposite effects in different cell types and disease stages. The actual impact of low SIAH1 expression on the immune system is cell-type-specific and contradictory: in lymphoid cells, low SIAH1 expression leads to excessive apoptosis of T/B cells and abnormal activation of NK cells due to c-Myb accumulation; while in myeloid cells, its low expression alleviates inflammatory damage by blocking the GAPDH-SIAH1 complex [7, 55-57]. That is, in the early inflammatory stage of sepsis, high expression of SIAH1 in myeloid cells (such as macrophages /microglia) may activate the NF-κB pathway by forming the GAPDH-SIAH1 complex, promote the release of IL-6/TNF-α, and at the same time degrade key autophagy proteins to weaken the cell metabolic protection ability and cause abnormal activation of NK cells, jointly exacerbating inflammatory damage; inhibiting SIAH1 at this time can alleviate the pathological response. In the late immunosuppressive stage, low expression of SIAH1 in lymphoid cells (T/B cells) accelerates immune cell exhaustion and worsens immune paralysis due to the inability to effectively degrade pro-apoptotic proteins such as caspase-3. At this time, enhancing SIAH1 activity is needed to maintain lymphocyte homeostasis.

SUMO2 (small ubiquitin-like modifier 2), as an important member of the SUMO family, regulates the stability, localization, and function of target proteins through SUMOylation modification and plays a crucial role in inflammatory responses and immune regulation[58]. Rachael Barry et al. showed that the E3 SUMO ligase MUL1 mediates the SUMOylation of NLRP3 in the form of SUMO2/3 and inhibits the activation of the NLRP3 inflammasome[59]. However, Ying Qin et al. found that the E3 SUMO ligase TRIM28 specifically binds to NLRP3, promotes its SUMO1, SUMO2, and SUMO3 modifications, thereby inhibiting the ubiquitination and protein degradation of NLRP3 and promoting NLRP3 expression and inflammasome activation. TRIM28 and MUL1 may mediate NLRP3 SUMOylation at different lysine sites and regulate NLRP3 activity through different mechanisms [60]. In sepsis, Xinyong Liu et al. found that ginkgolic acid may promote inflammation and macrophage apoptosis in sepsis by inhibiting the SUMOylation process and increasing the activity of NF-κB p65[61]. From the immune infiltration analysis of SUMO2 in this study, it is significantly correlated with various immune cells: positively correlated with pro-inflammatory cells (monocytes, M0/M1 macrophages), but negatively correlated with some anti-inflammatory cells (resting mast cells, activated NK cells). GSEA results for SUMO2 show that the top-ranked pathways are neurodege- nerative diseases (Huntington's, Parkinson's), which appear unrelated to sepsis but are essentially linked to dysregulated mitochondrial quality control and are closely associated with immunometabolic reprogramming. Sepsis is a metabolically complex disease. During sepsis, cellular energy metabolism shifts from oxidative phosphorylation (OXPHOS) to glycolysis (the Warburg effect) —a transition that occurs in nearly all cell types. Glycolysis can enhance immune activation, but it is also a cause of

immunosuppression; excessive glycolysis instead leads to immune suppression. Mitochondrial dysfunction and metabolic disturbances are key drivers of sepsis mortality. Mitochondrial damage leads to metabolic reprogramming, which results in energy depletion and ROS burst, followed by exacerbated inflammation, which in turn causes further mitochondrial damage. This cycle ultimately leads to organ exhaustion, progressing to septic shock and multiple organ failure. Therefore, mitochondrial dysfunction is the root of metabolic reprogramming, and restoring OXPHOS depends on maintaining mitochondrial structure and function. In sepsis, SUMO2 may influence mitochondrial function, thereby regulating glycolysis and the inflammatory response, affecting cellular metabolic reprogramming and the progression of sepsis. Meanwhile, the activation of autoimmune pathways such as "graft-versus-host disease" and "autoimmune thyroiditis" indicates that there may be a mechanism similar to autoimmunity in sepsis. The low expression of SUMO2 in sepsis may participate in disease progression by regulating myeloid cell infiltration (pro-inflammatory), adaptive immune suppression (B/NK cells), and metabolic reprogramming (OXPHOS/ glycolysis). The enriched neurodegenerative and autoimmune pathways provide a new perspective for understanding the multi-organ damage in sepsis. The "pro-inflammatory" and "immune suppression" caused by SUMO2 may be the molecular basis of the dynamic process of "early hyperinflammation - late immune paralysis" in sepsis. Therapeutic strategies based on SUMO2 need to strictly consider spatiotemporal specificity. Targeting the SUMO2 regulatory network (such as enhancing its myeloid-specific expression or supplementing SUMOylation substrates) may simultaneously improve excessive inflammation and immune paralysis, providing new ideas for treatment.

UBE2C (Ubiquitin-conjugating enzyme E2 C) is a core component of the ubiquitin-proteasome system (UPS). As a member of the ubiquitin-conjugating enzyme E2 family, it is responsible for transferring ubiquitin molecules to specific substrates and mainly mediates lysine 11-linked polyubiquitination (K11-linked polyubiquitination). Its core functions include cell cycle regulation, maintenance of genomic stability, and cross-regulation of NF-κB signaling. UBE2C is a specific E2 enzyme of the anaphase-promoting complex/ cyclosome (APC/C), ensuring the normal exit from mitosis by degrading mitotic cyclins (e.g., Cyclin B1, Securin). In studies related to inflammatory diseases, UBE2C is considered a potential prognostic marker and therapeutic target [62, 63]. In this study, we found that UBE2C may drive immune imbalance through a dual mechanism of "promoting macrophage polarization and inhibiting T cell function" while activating metabolic stress and neurodegeneration-related pathways to exacerbate multi-organ damage. Further research should focus on verifying its specific role in macrophage metabolic reprogramming and T cell exhaustion and evaluating its potential as a staging diagnostic marker or immunotherapeutic target for sepsis. Immune infiltration analysis of UBE2C suggests a significant correlation with various immune cells, particularly a prominent positive correlation with macrophage subtypes. The correlation coefficients of M0, M1, and M2 macrophages range from 0.19 to 0.29, and the *p*-values are all highly significant. This may imply that UBE2C is involved in macrophage polarization or functional regulation. Some studies have also confirmed this. High expression of UBE2C is significantly positively correlated with M1 macrophage infiltration in sepsis patients ($r = 0.29$, $P < 1e-09$), suggesting that it may exacerbate the pathological process by promoting the activation of pro-inflammatory signaling pathways (e.g., IL - 6/JAK/STAT3)[64]. The most obvious negative correlations are with CD8+ T cells and naive CD4+ T cells, with correlation coefficients of -0.13 and -0.2, respectively. Positive correlations are concentrated in innate immune cells, while negative correlations tend to be with adaptive immune cells. UBE2C-mediated protein degradation may consume key proteins in immune cells (e.g., T cells), leading to immune exhaustion.Looking at the GSEA results, the top positively enriched pathways are neurodegenerative disease pathways, with Huntington's disease, Parkinson's disease, and prion disease ranking at the forefront, and all NES values are above 2.2. These pathways are involved in oxidative stress and mitochondrial function, potentially implicating UBE2C in cellular stress responses, possibly through the Warburg effect and regulation of mitochondrial dynamics, thereby influencing the progression of sepsis. The most strongly enriched signaling pathways are ribosome, cell cycle, and oxidative phosphorylation, suggesting that this gene may influence basic cell metabolism and proliferation. The most significantly negatively enriched pathways are related to transplant rejection and autoimmunity, especially the Th1/Th2 cell differentiation pathway with an NES value of -2.32, which corresponds to the negative correlation with T cells in immune infiltration.

To develop individualized treatment strategies targeting four characteristic UbRGs, we screened potential small-molecule drugs, TCMs, and their active ingredients. Based on the molecular docking results, Sirolimus showed high binding affinity to all four DE-UbRGs. Sirolimus is a well-known autophagy inducer and mTOR inhibitor[65, 66]. mTOR inhibition activates overall protein degradation through the ubiquitin-proteasome system and autophagy[67]. The mTOR signaling pathway plays a crucial role in the pathological process of sepsis[68-70]. Ubiquitination is involved in post-translational protein modification and plays an important role in the immune response, being closely related to the occurrence and progression of diseases[19, 71]. Some studies have shown that the role of basic fibroblast growth factor (bFGF) in myocardial ischemia/reperfusion (I/R) recovery is related to the inhibition of excessive autophagy and the increase in ubiquitinated protein clearance rate by activating the PI3K/Akt/mTOR signaling pathway[72]. Additionally, in a sepsis mouse model, Sirolimus alleviates lung injury by inhibiting the differentiation of Th17 cells through RORγt and STAT3 dysfunction[73]. Therefore, considering the existing research results, Sirolimus has known immunomodulatory properties and good safety. By inhibiting mTORC1, it may act through multiple coordinated pathways such as stabilizing mitochondrial function in a ubiquitination-dependent manner, regulating the inflammatory response, and enhancing antioxidant defense, making it a promising candidate drug for targeted ubiquitination therapy of sepsis. Subsequently, a low-dose regimen can be verified through sepsis models to avoid excessive immunosuppression. Lysine, as an essential amino acid involved in protein synthesis, can be used as a nutritional supplement for adjuvant therapy. Temozolomide plays a crucial role in the treatment of gliomas[74]. Downregulating UBE2C expression inhibits aerobic glycolysis by regulating p53 expression, thereby enhancing the sensitivity of glioma cells to Temozolomide[75]. However, there are case reports of a patient with glioblastoma receiving Temozolomide and radiotherapy being diagnosed with sepsis due to phlegmonous gastritis[76], and another patient with high-grade neuroendocrine tumors developing neutropenic sepsis after receiving PRRT combined with capecitabine or Temozolomide[77]. The specific mechanisms need further exploration. Pyrrolidonecarboxylic

acid, as a safe molecule, also warrants further exploration for its adjuvant treatment value. p-Chloroamphetamine and Cycloheximide, due to potential toxicity risks, are more suitable as tools for mechanism research rather than therapeutic drugs[78,79]. Alachlor[80] and Iproplatin[81] are not suitable for clinical research due to toxicity or efficacy issues, but their mechanism research still promotes the development of related fields. Therefore, some of the screened small-molecule compounds exhibit significant cytotoxicity or carcinogenic risk. They are recommended only for mechanistic studies and are not suitable as candidate drugs for sepsis treatment. Additionally, the immunosuppressant sirolimus and certain chemotherapeutic agents may increase the risk of secondary infections, and thus require structural optimization as well as systematic safety evaluation under rigorous standards. From the TCMs screening results, we obtained TCMs corresponding to four characteristic UbRGs, namely FBXO7 (Oenanthe javanica), SIAH (semen arecae and Areca Catechu L.), SUMO2 (Brassica rapa L.), UBE2C (Fructus Arctii lappae), along with the corresponding active ingredients of the above TCMs. However, TCMs contain numerous components, and some known components are toxic to the human body. Most of the time, purification of active ingredients is required. Therefore, this study only explores components with medicinal value. Through literature review and database retrieval, it was found that Oenanthe javanica has many active ingredients and has anti-inflammatory applications in traditional medicine[82], but there are few studies directly targeting sepsis. Previous studies have shown that FBXO7 inhibits the NF-κB pathway, regulates mitochondrial autophagy, and modulates the inflammatory response[47-50]. FBXO7 has the highest docking score with Kaempferol (-6.5 kcal/mol). Some studies have shown that Kaempferol reverses acute kidney injury in sepsis models by inhibiting macrophage infiltration in septic mice and suppressing the NF-κB/AKT signaling pathway[83,84]. Secondly, FBXO7 also has relatively stable binding ability with Isorhamnetin (-6.0 kcal/mol) and Quercetin (-6.1 kcal/mol). Yang JH et al. isolated Isorhamnetin from Oenanthe javanica. Studies on rats and lipopolysaccharide (LPS)-activated macrophages confirmed that Isorhamnetin prevents acute inflammation by blocking NF-κB activation[85]. Xue Y et al. found that Quercetin inhibits the activation of the NLRP3 inflammasome in epithelial cells triggered by Escherichia coli O157:H7, partly by preventing ROS production and activating autophagy[86]. Aming Sang et al. demonstrated that Quercetin inhibits oxidative stress - mediated endoplasmic reticulum stress by activating the SIRT1/AMPK pathway, alleviating sepsis - induced acute lung injury[87]. Therefore, the active ingredients of Oenanthe javanica (especially flavonoids) have multi - target anti - inflammatory and antioxidant properties, which meet the pathological needs of sepsis. Oenanthe javanica and its active ingredients may regulate the inflammatory response in sepsis and reduce organ damage (especially in the kidneys and lungs) by regulating the ubiquitination-related target FBXO7, inhibiting the NF-κB pathway, and maintaining mitochondrial homeostasis. However, there is currently no experimental evidence for the direct regulation of FBXO7 or ubiquitination by Oenanthe javanica, and further experiments are needed for verification.

We employed the same method to investigate the TCMs Arecae Semen and Arecae Pericarpium (Areca Catechu L.) in the SIAH mapping. Low-dose Arecae Semen, its extracts, and compounds exhibit pharmacological effects such as anthelmintic, antibacterial, anti-inflammatory, analgesic, antioxidant activities, and improvement of gastrointestinal function. The important mechanisms involved include reducing ROS production, inhibiting the activation of NADPH oxidase, increasing the activity of antioxidant enzymes, and influencing MAPK, AKT, TLR, NF-κB, Nrf -2, PI3K, and STAT3 signaling pathways[88-91]. Studies have shown that patients with septic shock and secondary organ dysfunction have reduced antioxidant status and increased lipid peroxidation[92]. X Terra et al. found that proanthocyanidins reduce LPS - induced ROS production and exert anti - inflammatory effects by inhibiting the activities of ERK1/2 and IKKb. Procyanidin B1 shows excellent binding ability with SIAH1 in molecular docking results, with a vina score of -9.3 kcal/mol. Jing Xing et al. demonstrated that Procyanidin B1 binds to the TLR4-MD-2 heterodimer, inhibits the downstream activation of MAPK and NF-κB signaling pathways, and exerts anti-inflammatory effects on LPS-treated THP1 cells[93]. In sepsis, Procyanidin B1 may bind to SIAH1, inhibit the ubiquitination and degradation of pro - inflammatory factors, block the excessive activation of NF-κB, and alleviate the inflammatory storm in sepsis. Its potential is clear, but the specific mechanism still needs to be clearly verified. It is worth noting that Procyanidin B1 has MW > 500, NorO > 10, NHorOH > 5, violating the Lipinski's rule of five for oral bioavailability. However, many drugs have exceeded the Lipinski's rules[94]. Procyanidin B1 shows a high affinity for SIAH1 (- 9.3 kcal/mol). As a natural polyphenolic compound, it can be metabolized and decomposed by gut microbiota into a series of low-molecular-weight polyphenol metabolites, which can then be absorbed and enter the systemic circulation[95,96]. Therefore, it still has high research value. The other two active ingredients, Catechin and ent-Epicatechin, have obvious biological activities such as antioxidant, anticancer, anti-inflammatory, antimutagenic, anti-aging effects, and improvement of liver function. Studies by Jung A. J., Joseph Shay et al. showed that they mainly exert anti-inflammatory and protective effects through ROS scavenging and Nrf2 antioxidant stress response[97-100]. Their specific roles in sepsis need further exploration. In general, the active ingredients of Arecae Semen may target the SIAH1-ubiquitination pathway, providing a new strategy for sepsis inflammation regulation. In particular, the antiinflammatory mechanism of Procyanidin B1 shows significant potential. However, since Arecae Semen has been classified as a Group 1 carcinogen by the WHO, and its fibrogenic effect is clear[101, 102]. Therefore, Arecae Semen raw materials cannot be directly used for treatment. Carcinogenic risks need to be avoided through ingredient purification/ structural modification, and its efficacy and mechanism in sepsis models need to be further verified.

As mentioned previously, SUMOylation is closely related to sepsis[58, 61]. SUMO2 and the potential TCM turnip (Brassica rapa L.), a traditional medicinal plant, have active components that regulate inflammation, oxidative stress, and immune responses through multiple targets[103-106]. However, there are no reports in the existing literature about its direct regulation of SUMOylation. The results of drug screening and molecular docking suggest a potential new mechanism. Among the five components in this study, Liquiritin deserves the most attention, with a high oral bioavailability (OB, 65.69%) and drug-likeness (DL, 0.74), and the optimal docking score (-6.5 kcal/mol). Studies by Shijiao Zheng[107], Shan-Qi Mou[108], Hongling Zhou[109], et al. have shown that Liquiritin can alleviate sepsis-related inflammation and organ damage in the heart, lungs, kidneys, etc. stimulated by lipopolysaccharide (LPS) by activating the Nrf2 signaling pathway, AMP-activated protein kinase-dependent signaling pathway, and inhibiting the

JNK/Nur77/c-Jun pathway. SUMO2 regulation may be a new target for the treatment of sepsis, but this is only a prediction and requires further experimental verification.In addition, Liquiritin and Quercetin are also active components of turnip. As mentioned before, Isorhamnetin can prevent acute inflammation by blocking NF-κB activation[85]; Quercetin can inhibit the activation of the NLRP3 inflammasome[86], activate the SIRT1/AMPK pathway to inhibit endoplasmic reticulum stress mediated by oxidative stress, and alleviate sepsis-induced acute lung injury[87]. Isorhamnetin and Quercetin may reduce the inflammatory response in sepsis by inhibiting NF-κB and reducing the SUMOylation of the NLRP3 inflammasome. β-sitosterol should not be ignored either. It can interfere with multiple cellular signaling pathways, including those related to the cell cycle, cell death, proliferation, survival, invasion, angiogenesis, metastasis, anti-inflammation, anti-cancer, liver protection, anti-oxidation, and cardiac protection. It shows no obvious toxicity in pharmacological screening, and its pharmacokinetic characteristics have been extensively studied [110]. β-sitosterol promotes the activation of antioxidant enzymes (such as superoxide dismutase, catalase, and glutathione) and inhibits lipid peroxidation by activating the Nrf2 and Nrf2/heme oxygenase-1 (HO-1) signaling pathways. It can also inhibit the expression of proteins involved in cellular signaling pathways (such as JAK3/STAT3 and NF-kB) and plays a key role in regulating the inflammatory/immune response[111,112]. For example, it can affect microglial polarization by inhibiting the TLR4/NF-κB signaling pathway, thereby reducing neuroinflammation and alleviating neuropathic pain[113].The anti-oxidative and anti-inflammatory effects of the active components in turnip (Brassica rapa L.) are highly relevant to the key pathophysiological processes in sepsis, such as oxidative stress and uncontrolled inflammation. The research suggests that these effects may be achieved through the regulation of SUMO2-mediated protein SUMOylation modification.

As a member of the ubiquitin-conjugating enzyme E2 family, UBE2C participates in the regulation of the cell cycle and inflammation. UBE2C is mapped to Fructus arctii, which is the fruit of Arctium lappa L. It contains many chemical constituents and has anti-cancer, anti-viral, anti-inflammatory, and anti-oxidant effects[114-116]. The study by Weifang Zhang et al. showed that Fructus arctii inhibits microglial activation and reduces the levels of inflammatory factors through the Let-7E-regulated Toll-like receptor (TLR) signaling pathway [117]. Arctigenin, an important component of Fructus arctii, has potent anti-inflammatory, anti-oxidant, and anti-viral effects [118, 119]. Studies by Supriya R Hyam et al. [120], Xianjuan Kou et al. [121], Xiang Xu et al. [122], and Xianbao Shi et al. [123] found that arctigenin inhibits key upstream pathways of NF-κB, such as PI3K/AKT, JAK-STAT, TLR4, and TNFR1, through multi-pathway crosstalk, and polarizes M1 macrophages into M2-like macrophages, ultimately alleviating various inflammations, such as in acute lung injury and cranial neuroinflammation, highlighting its broad-spectrum anti-inflammatory potential. Its mechanism of action is highly synergistic, providing a theoretical basis for the development of multi-target anti-inflammatory drugs. Arctigenin may block the ubiquitination modification of key inflammatory regulatory proteins such as IκBα and STAT3 by targeting the catalytic center of UBE2C; or it may reduce the expression of UBE2C by inhibiting the NF-κB/STAT3 pathway, disrupting the positive feedback loop of inflammation-ubiquitination. It has significant therapeutic potential in inflammation-related diseases, offering new ideas for the development of dual-target drugs for "ubiquitination-immune inflammation". It is worth noting that the OB value of arctigenin is only 8.05%, and pharmacokinetic studies have shown[124] that the significant first-pass metabolism of arctigenin hinders its in vivo and clinical efficacy after oral administration. However, due to its medicinal value, it was still included in this study, and the molecular docking results showed that it stably binds to UBE2C (-7.1 kcal/mol). It is recommended to administer it via a route other than oral. As another important active component of Fructus arctii, arctiin also has important immune-enhancing and anti-inflammatory effects. Arctiin improves glomerulonephritis by inhibiting NF-kappaB activation and nuclear translocation and reducing the levels of these pro-inflammatory cytokines[125]. The study by Bo Zhou et al. [126] showed that arctiin prevents LPS-induced acute lung injury by inhibiting the PI3K/AKT signaling pathway in mice. This suggests that it may target UBE2C for the treatment of sepsis complicated by acute kidney injury and acute lung injury, protecting renal tubular epithelial cells through a dual mechanism of anti-oxidation/anti-ubiquitination disorder. Additionally, C Ribeiro Nogueira et al. found that septic patients showed significant deficiencies in retinol and beta-carotene[127]. The study by Farouk K El-Baz et al.[128] showed that oral administration of beta-carotene can reduce cyclophosphamide (CP)-induced acute lung injury and inflammation. The molecular docking in this study showed that the vina score of beta-carotene with UBE2C is -7.5 kcal/mol, indicating stable binding. Perhaps beta-carotene can be used as an adjuvant treatment for sepsis-related conditions, reducing the inflammatory response and supplementing nutrition. Fructus arctii and its active components may regulate protein ubiquitination by targeting UBE2C, blocking the core inflammatory pathways of sepsis from the upstream and hitting the core mechanism of uncontrolled inflammation in sepsis, providing an innovative strategy for the development of a new generation of anti-sepsis drugs.

In this study, by integrating multi-omics data and employing machine learning algorithms, we optimized the screening and identification process for sepsis-related biomarkers. Through differential expression analysis, functional enrichment analysis, and integration of gene expression with clinical information, we performed extensive preliminary screening and further focused on four characteristic ubiquitination-related genes (UbRGs). The expression changes of these genes were validated using independent-sample PCR experiments, enhancing the credibility of the findings. Subsequently, we focused on immune infiltration analysis, GSEA, and drug screening for the four characteristic UbRGs, which revealed the critical roles of ubiquitination-related genes (UbRGs) in sepsis and helped identify novel potential intervention targets and therapeutic strategies. However, all findings from this study are exploratory and hypothesis-generating, and all gene–drug associations and mechanistic inferences require further validation through protein-level and functional experiments.

Nevertheless, this study has the following limitations: (1) Although public databases provided a relatively large sample size, detailed clinical information—such as the type of infectious pathogen and organ failure scores—was lacking, which may affect the clinical relevance and translational value of the findings. The 11 characteristic DE-UbRGs demonstrated some discriminative ability for distinguishing sepsis from control samples in internal validation within the current merged dataset; however, the AUC estimates were derived from internal data and have not been validated in independent external cohorts. Therefore, they should not be interpreted as confirmed clinical

diagnostic performance. Prospective, multi-center clinical cohorts are still needed to confirm the true generalizability of these 11 gene markers for sepsis discrimination. (2) The mechanistic validation is insufficient. The RT-qPCR experiments in the in vitro cell model only verified changes in target gene expression at the mRNA level and did not provide direct evidence regarding protein expression levels, E3 ligase activity, ubiquitination modification status, or causal relationships. Ubiquitination is a complex post-translational modification process involving a multi-enzyme cascade, and mRNA abundance does not simply correspond to protein function. Consequently, all mechanistic discussions in this study are hypotheses based on bioinformatic predictions and require further verification through experiments such as western blotting, co-immunoprecipitation (Co-IP), ubiquitin-ation assays, gene knockout/overexpression, and animal models. (3) Safety concerns regarding candidate drugs: Some of the screened small-molecule compounds (e.g., Areca catechu, Areca catechu and Cycloheximide) exhibit significant cytotoxicity or carcinogenic risk. They are recommended only for mechanistic studies and are not suitable as candidate drugs for sepsis treatment. Additionally, the immunosuppressant Sirolimus and certain chemotherapeutic agents may increase the risk of secondary infections, and their safety needs to be systematically evaluated under rigorous standards. (4) Limitations of the traditional Chinese medicine (TCM) screening model: The "single component−single target" analysis model employed in this study does not fully capture the holistic characteristics of TCM, such as multi-component synergy and multi-pathway regulation. Additionally, the screening criteria (e.g., oral bioavailability, drug-likeness) may exclude some potential active ingredients that require metabolic activation or can only be administered via parenteral routes. (5) Superficial validation of drug−target interactions: Current investigation of the drugs is limited to virtual screening and affinity calculations; the specific binding sites and regulatory mechanisms remain to be experimentally elucidated. Practical application of these drugs will require comprehensive and rigorous evaluations of pharmacodynamics, pharmacokinetics, toxicology, animal studies, and clinical trials, and their druggability may be further improved through formulations such as nanoformulations or sustained-release tablets.

Subsequent research can employ single-cell multi-omics technologies to resolve the spatiotemporal expression characteristics of ubiquitination-related genes across different stages of sepsis, further exploring the specific mechanisms of ubiquitination in each phase of sepsis, particularly in early intervention and treatment. In addition, studies should combine animal models and clinical samples to construct multi-omics integrated network models and systematically investigate the mechanisms linking ubiquitination and sepsis. By validating target functions, integrating ubiquitination modification omics to decipher global regulatory networks, and conducting pharmacokinetic-pharmacodynamic studies of active compon-ents along with in vitro and in vivo functional validation, the potential of the characteristic UbRGs as subtyping markers for sepsis and as a basis for personalized medicine can be further confirmed, thereby enhancing their clinical applicability.

Conclusion

sing machine learning, this study identified ubiquitination-related candidate genes linked to sepsis patholog-involving inflammation and immune microenvironment changes ─ and also preliminarily explored potential TCM monomers and small-molecule drugs. Of note, all findings are exploratory and hypothesis-generating. The proposed molecular mechanisms and drug−target interactions require subsequent validation at the protein level, via ubiquitination functional studies, and in animal models. Thus, this work offers initial candidate targets and a conceptual basis for further research on ubiquitination in sepsis, but does not represent validated clinical translation protocols.

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Data availability statement

1.Publicly available datasets were analyzed in this study. This data can be found here: Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database, including GSE3 2707, GSE54514, GSE69528, and GSE137340.

2.The components of TCMs were mainly obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) database (https://old.tcmsp-e.com/tcmsp.php), the HERB database (http://herb.ac.cn/).

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Author contribution

Lixiong Zeng (First Author) : Writing-Original Draft, Writing-Review & Editing, Conceptualization, Data Curation, Formal Analysis, Visualization, Methodology, Software; Yongbing Yu (First Author) ： Writing - Original Draft, Investigation, Validation, Formal analysis, Visualization, Software, Methodology; Junzhi Yang: Formal analysis, Validation, Methodology; Houyu Gan: Data Curation, Investigation, Methodology; Qiuli Chen: Data Curation, Investigation, Methodology; Xueyi Chen: Data Curation, Investigation, Visualization Jihua Feng: Formal analysis, Validation, Software, Funding acquisition; Kanglai Wei (Corresponding Author):Conceptualization, Resources,

Supervision, Validation, Writing-Original Draft, Writing-Review &Editing; Jianfeng Zhang (Corresponding Author)：Methodology, Writing - Review & Editing, Funding acquisition, Resources, Supervision, Project administration; Jie Yang (Corresponding Author)：Methodology, Writing - Review & Editing, Resources, Supervision, Project administration, Investigation, Conceptualization, Data Curation, Resources.

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Competing interests

The authors declare no competing interests.

Ethics declaration

Not applicable.

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Abbreviations:

DE-UbRGs: Differentially Expressed Ubiquitination-Related Genes; DEGs: Differentially Expressed Genes; DL: drug-likeness; FBXO7: F-Box Protein 7; GEO: Gene Expression Omnibus; GSEA: Gene Set Enrichment Analysis; LPS: lipopolysaccharide; OB: oral bioavailability; ROC: Receiver Operating Characteristic;RT-qPCR: reverse transcription-quantitative polymerase chain reaction; SIAH1: Siah E3 Ubiquitin Protein Ligase 1;SUMO2: Small Ubiquitin Like Modifier 2; TCMs: Traditional Chinese Medicines; UBE2C: Ubiquitin Conjugating Enzyme E2 C; UbRGs: Ubiquitination-Related Genes.