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Exploring the Interactions Between Mitochondria-Related Genes and the Immune Microenvironment in Sepsis: A Bioinformatics Study

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ABSTRACT

Background: Sepsis is a critical condition with a high mortality rate, and the underlying mechanisms are not fully understood. Mitochondria and immune inflammation play key roles in sepsis, but their interactions are not well characterized. In this study, we used bioinformatics to explore the interactions between mitochondria-related genes and the immune microenvironment in sepsis.

Methods: We obtained mRNA expression profile datasets from NCBI GEO and analyzed them to identify differentially expressed genes (DEGs). We then identified mitochondria-associated DEGs (MitoDEGs) by crossing the DEGs with mitochondrial line-associated genes. Gene ontology (GO) enrichment analysis and Kyoto Gene and Genome Encyclopedia (KEGG) pathway analysis were performed to investigate the role of MitoDEGs in sepsis. We constructed a protein–protein interaction (PPI) network and identified central MitoDEGs associated with sepsis. Additionally, we used NetworkAnalyst to predict transcription factors and construct a ceRNA regulatory network to predict miRNAs and lncRNAs interacting with MitoDEGs. We also analyzed the pattern of immune infiltration in sepsis and investigated the relationship between MitoDEGs and immune cell infiltration.

Results: We identified 49 MitoDEGs, with PPI network analysis revealing 10 hub genes. We constructed a ceRNA network predicting miRNAs and lncRNAs interacting with key genes. Immunoassays showed changes in immune cell infiltration in the sepsis microenvironment, and Spearman analysis revealed significant correlations between hub MitoDEGs and specific immune cell types.

Conclusion: Our study provides insights into the interaction between mitochondrial metabolism and the immune microenvironment in sepsis. These findings may help identify new targets for medical intervention in sepsis.

INTRODUCTION

Sepsis is a severe syndrome of the systemic inflammatory response caused by infection that can lead to multiorgan failure and shock and is associated with increased mortality. In 2017, nearly 49 million cases of sepsis were reported worldwide, with a mortality rate of 22.5%, accounting for almost 20% of all deaths worldwide Seymour et al. (2019). Despite extensive research on sepsis, its mortality rate remains high Pfalzgraff et al. (2019). As the molecular mechanisms underlying its development remain unclear, sepsis remains a significant clinical burden Vincent et al. (2022). Therefore, exploring the underlying pathogenesis of sepsis and identifying effective therapeutic agents and strategies can help improve the prognosis of patients with septic AKI and thus reduce clinical mortality

Mitochondria play a crucial role in metabolic transition Ramond et al. (2019). They are essential for maintaining cellular homeostasis and are metabolically active organelles with finely regulated kinetics responsible for maintaining mitochondrial integrity and function Glancy et al. (2020), Friedman et al. (2014). Mitochondria are integral to the regulation of cellular metabolism and ATP production through the tricarboxylic acid cycle and respiratory chain. In addition, mitochondrial dynamics and function influence many intracellular signals involved in cellular processes such as cell growth, proliferation, differentiation, motility, intercellular communication, tissue regeneration, and apoptosis. As a vital organelle that provides energy for cellular activities in eukaryotic cells, mitochondrial dysfunction can severely affect cell synthesis and metabolism, disrupt

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cellular functions, and lead to insufficient energy supply to tissues and organs, resulting in abnormal organ function and tissue damage Verdonk et al. (2017), Pan et al. (2018). Recently, it has been recognized that sepsis is closely related to mitochondrial dysfunction. The mechanisms of mitochondrial damage in sepsis include oxidative stress injury Malik et al. (2013), Bhatti et al. (2017), mitochondrial calcium overload Marchi et al. (2018), Boyman et al. (2020), and mtDNA damage Quan et al. (2020). These mitochondrial damage mechanisms can cause cell death through the apoptotic pathway and exacerbate the damage caused by sepsis to the organism.

There is mounting evidence indicating that immune dysfunction plays a significant role in the development of sepsis. Sepsis-related immunosuppression involves various cell types and is characterized by the release of anti-inflammatory cytokines, immune cell death, T-cell depletion, and overproduction of immunomodulatory cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) Torres et al. (2022). In sepsis-induced immunosuppression, an increase in Treg numbers in peripheral blood is linked to long-term mortality in sepsis patients Venet et al. (2004). Activated T cells and mast cells have been shown to produce and secrete interleukin-4 (IL-4), an anti-inflammatory factor that induces CD4+ T cells to differentiate into T helper 2 (Th2) cells, promotes autocrine signaling to produce other anti-inflammatory cytokines through positive feedback, and inhibits the release of pro-inflammatory cytokines Hotchkiss et al. (2013). An observational retrospective study demonstrated that monitoring lymphocyte count, monocyte count, and neutrophil-lymphocyte ratio (NLR) could predict the severity and mortality of abdominal infection sepsis patients Liu et al. (2021). Monitoring the immune status of sepsis is crucial for assessing the prognosis of sepsis patients and protecting organ function in a timely manner. Both mitochondrial metabolism and immune responses are crucial in the development of sepsis. However, there is a lack of research on the dynamic interplay between the mitochondrial and immune microenvironment in sepsis, which requires further exploration.

In this study, we analyzed the differentially expressed genes (DEGs) in three sepsis patient datasets (GSE26378, GSE57065, and GSE95233) obtained from the NCBI Gene Expression Omnibus database (GEO) using the limma package. We then combined the mitochondria-related DEGs (MitoDEGs) with the mitochondria-related genes in the GeneCards database to identify mitochondria-related differentially expressed genes (MitoDEGs). We analyzed how mitochondria-related genes contribute to the development of sepsis and their correlation with immune infiltration. We also explored the relationship between key mitochondria-related genes and immune infiltration, providing new ideas for potential therapeutic targets and clinical management of mitochondrial function changes in sepsis.

MATERIALS AND METHODS

Microarray Data Retrieval

The sepsis dataset was obtained from the public repository NCBI GEO (http://www.ncbi.nlm.nih.gov/geo) Barrett et al. (2013) using the search term "sepsis." We further filtered the datasets by sequencing type (transcriptional), animal species (human), and sample source (blood). GSE26378 ([HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array) was generated from the GPL570 platform and includes 21 normal samples and 81 sepsis patient samples Wynn et al. (2011). GSE57065 ([HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array) was also generated from the GPL570 platform and comprises samples from 25 normal groups and 82 sepsis patient samples Tabone et al. (2019). Finally, GSE95233 ([HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array) was generated by the GPL570 platform and includes 22 normal group samples and 51 sepsis patient samples Venet et al. (2017).

Acquisition of microarray data and identification of differentially expressed genes (DEGs)

We employed the "limma" package in R software to identify differentially expressed genes (DEGs) in normal and septic samples from the GSE26378, GSE57065, and GSE95233 datasets. Genes with adjusted P values<0.05 and |log2 (Fold-change) |≥1 were considered DEGs. Using GIFtS as the screening condition, we selected the top 205 genes from the GeneCards database as mitochondria-associated genes. The MitoDEGs were obtained by intersecting the DEGs with mitochondria-related genes in the three datasets. The volcano map was generated using the R packages "ggplot2" Gustavsson et al. (2022) and "Complex Heatmap" Gu et al. (2016). The expression profile of MitoDEGs in normal and septic samples was visualized using the R package "ggplot2" Gustavsson et al. (2022) to create a radar map.

Functional enrichment analysis

To evaluate the function of differentially expressed mitochondria-associated genes, we performed gene ontology (GO) Gene et al. (2015) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Wrzodek et al. (2011) pathway enrichment analysis on the MitoDEGs using the R package "clusterProfiler" Yu et al. (2012).

We considered items with P<0.05 in the Benjamini–Hochberg test to be statistically significant. The results were visualized using Chordal and Circle plots with the R packages "ggplot2"" Gustavsson et al. (2022) and "GOplot" Walter et al. (2015).

Analysis of protein–protein interactions (PPI) and identification of hub genes

We conducted PPI analysis of overlapping MitoDEGs using the STRING database (https://string-db.org/) Szklarczyk et al. (2021) with a threshold set to a composite score of ≥0.4, and downloaded the files in tsv format. The resulting interactions were visualized as a network using Cytoscape 3.10.0 Doncheva et al. (2019). We filtered the hub MitoDEGs using the plugins MCODE and CytoHubba in Cytoscape 3.10.0.

Prediction of the central MitoDEGs-transcription factor (TF) network

We used the NetworkAnalyst online tool Xia et al. (2015) to construct gene regulatory networks for hub gene-transcription factor (gene-TF) interaction networks. To construct the gene-TF network, we used TF and gene target data obtained from the JASPAR database Castro-Mondragon et al. (2022) and imported them into Cytoscape 3.10.0 for visualization.

Construction of the ceRNA regulatory network

The hub MitoDEGs were imported into eight databases, including the mirTarBase database (https://maayanlab.cloud/Harmonizome/resource/MiRTarBase), using R software in combination with experimental methods to validate the relationships and predict the relationship with hub MitoDEG-related miRNAs. We obtained lncRNA–miRNA interaction data from the starBase database (https://starbase.sysu.edu.cn/) and screened target lncRNAs based on clipExpNum >10. Finally, we visualized the results in Cytoscape software.

Immune infiltration analysis

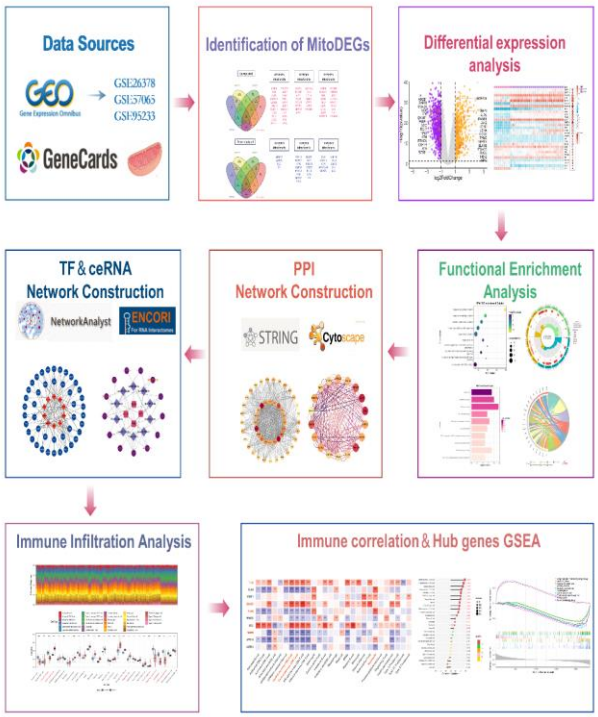
To address the heterogeneity induced by the different platforms of the gene matrices in the original datasets GSE23678 and GSE57065, we combined them after normalization and elimination of batch effects using the R package "sva" Leek et al. (2012). We used the normalized gene expression matrices for further immuno infiltration analysis. We excluded the GSE95233 dataset from the analysis due to its significantly smaller sample size compared to the other two datasets, which could lead to biased results. We analyzed 28 immune cell infiltrates in normal and septic samples using the R package "ssGSEA" ($P<0.05$) Subramanian et al. (2005). We detected differentiated immune cells in normal and septic samples using box line plots. We also performed correlation analysis of the 28 immune cells using R software. Furthermore, we conducted correlation analysis between the 28 immune cells and hub MitoDEGs using the R package "Corrplot" (Spearman correlation analysis function). We presented the correlations between hub MitoDEGs and the 28 immune cells as lollipop plots. Finally, we performed functional enrichment analysis using the GO pathway dataset from GSEA.

RESULTS

Identification and analysis of mitochondria-related differentially expressed genes in sepsis

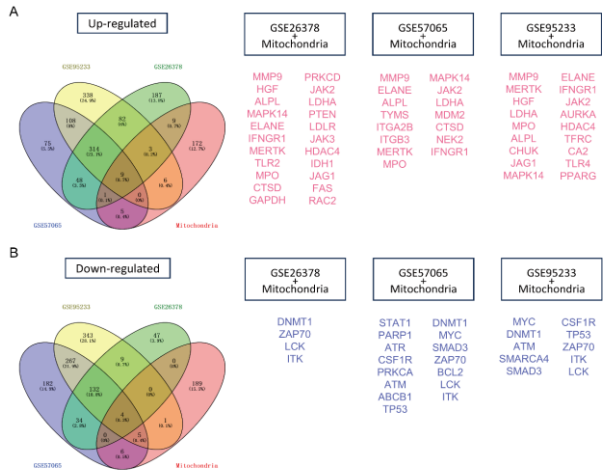
The overall experimental procedure is depicted in Figure 1. We downloaded three sepsis-related datasets, GSE26378, GSE57065, and GSE95233, from the GEO database and analyzed DEGs from sepsis samples and normal control samples using the "limma" package after quality control, normalization, and background correction. We used an adjusted P value <0.05 and |log2-fold change| ≥1 as thresholds to obtain differentially expressed genes. The difference analysis revealed 879 DEGs in the GSE26378 dataset, of which 653 genes were up-regulated and 226 genes were downregulated in the DCM samples compared with the normal samples; 1190 DEGs in the GSE57065 dataset, including 560 upregulated genes and 630 down-regulated genes; and 1621 DEGs in the GSE95233 dataset, including 860 up-regulated genes and 761 downregulated genes.

Figure 1: Flowchart of the multistep screening strategy on bioinformatics data



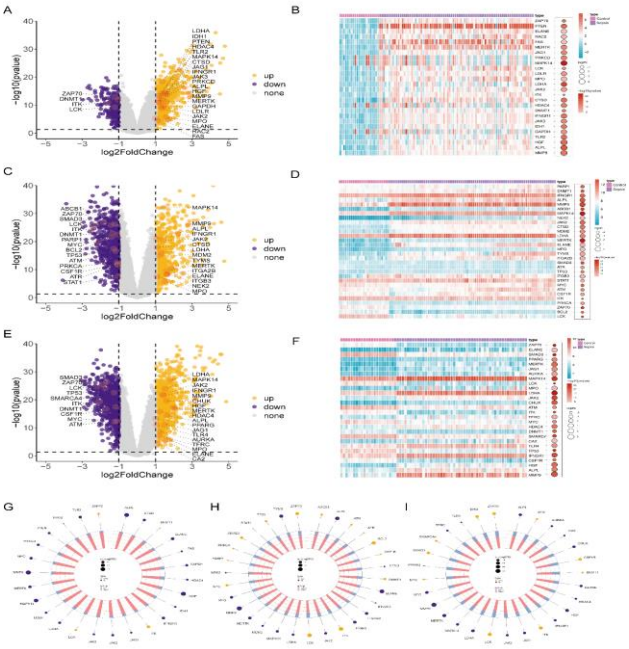
We retrieved mitochondria-related genes from the GeneCards database and selected genes overlapping with DEGs as MitoDEGs from the three datasets. There were a total of 26 MitoDEGs in the GSE26378 dataset (22 upregulated and 4 downregulated), 30 MitoDEGs in the GSE57065 dataset dataset (14 upregulated and 16 downregulated), and 26 MitoDEGs (18 upregulated and 10 downregulated) in the GSE95233 dataset (Figure 2A, B).

Figure 2: MitoDEGs in sepiss. (A, B) Venn diagrams show the number of upregulated and downregulated DEGs that overlap between GSE26378, GSE57065, GSE95233, and mitochondria-related genes.



We visualized the MitoDEGs as volcano plots and heatmaps (Figure 3A-F). Additionally, we visualized the proportion of each MitoDEG in the sample as a circular histogram (Figure 3G-I). Merging the MitoDEGs for each dataset yielded 67 overlapping MitoDeGs, of which 35 genes were upregulated and 32 genes were downregulated in the sepsis samples compared to the normal samples.

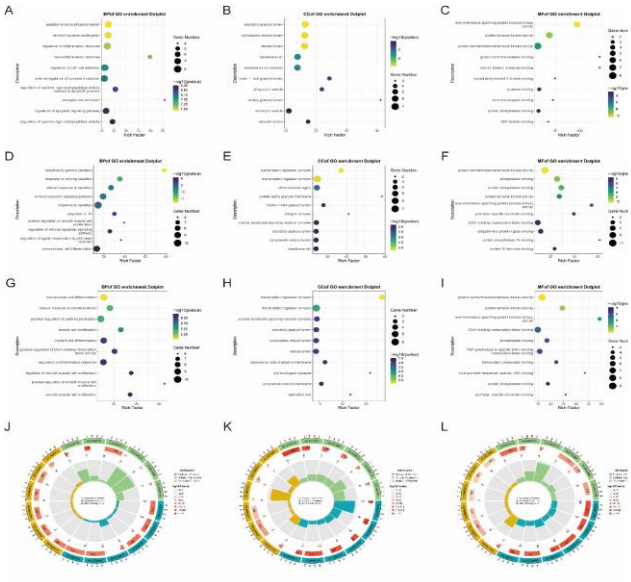
Figure 3: The results of MitoDEG analysis in sepsis. (A, B)are volcano plots and clustered heatmaps of MitoDEGs in GSE26378; (C, D)are volcano plots and clustered heatmaps of MitoDEGs in GSE57065; (E, F)are volcano plots and clustered heatmaps of MitoDEGs in GSE95233; (G-I)are GSE26378 , GSE57065 , GSE95233 grouped expression radar plots.



Functional and pathway enrichment analysis of MitoDEGs

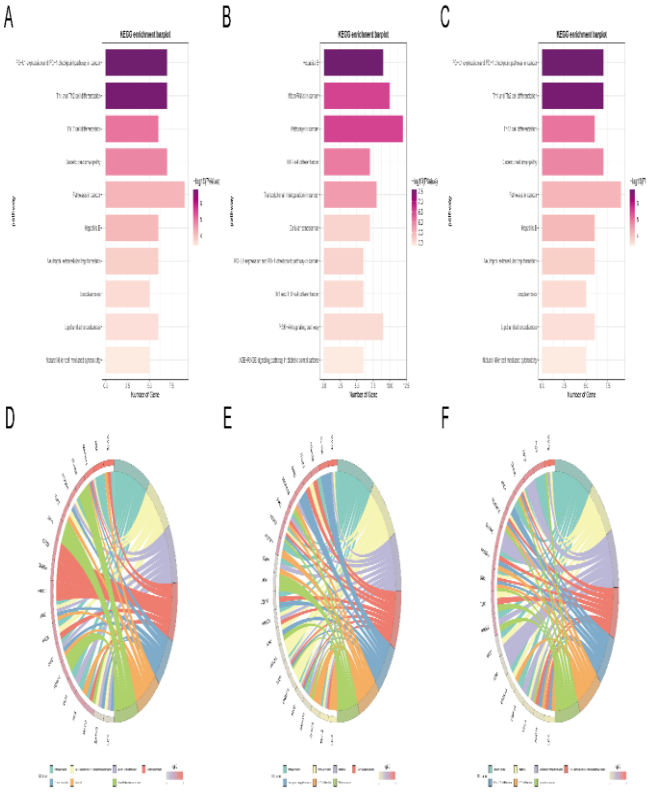
We performed further functional enrichment of MitoDEGs using GO and KEGG pathway analyses. GO analysis revealed that in biological processes (BP), regulation of inflammatory response, positive regulation of cytokine production, and in cellular component (CC), secretory granule lumen, cytoplasmic vesicle lumen were significantly enriched in several datasets. In molecular function (MF), protein tyrosine kinase activity, protein serine/threonine/tyrosine kinase activity, and protein phosphatase binding were significantly enriched in several datasets (Figure 4A-I).

Figure 4: GO enrichment analyses of MitoDEGs from GSE26378, GSE57065 and GSE95233. (A-C) The enriched GO terms of MitoDEGs in GSE26378; (D-F) The enriched GO terms of MitoDEGs in GSE57065; (G-I) The enriched GO terms of MitoDEGs in GSE95233; (J-L) for GSE26378, GSE57065, GSE95233 GO enrichment circle map; BP biological process, CC cellular component, MF molecular function.



The most enriched KEGG pathways of MitoDEGs were mainly involved in PD-L1 expression and the PD-1 checkpoint pathway in cancer, Th1 and Th2 cell differentiation, Th17 cell differentiation, and hepatitis B pathways (Figure 5A-F).

Figure 5: KEGG enrichment analyses of MitoDEGs from GSE26378, GSE57065 and GSE95233. (A, D) KEGG pathway enrichment results in GSE26378; (B, E) KEGG pathway enrichment results in GSE57065; (C,H) KEGG pathway enrichment results in GSE95233.



PPI network analysis and hub MitoDEG identification

We merged the MitoDEGs of each dataset to obtain 49 overlapping MitoDEGs, in which 33 genes were upregulated and 16 genes were downregulated in the sepsis samples compared with the normal samples.

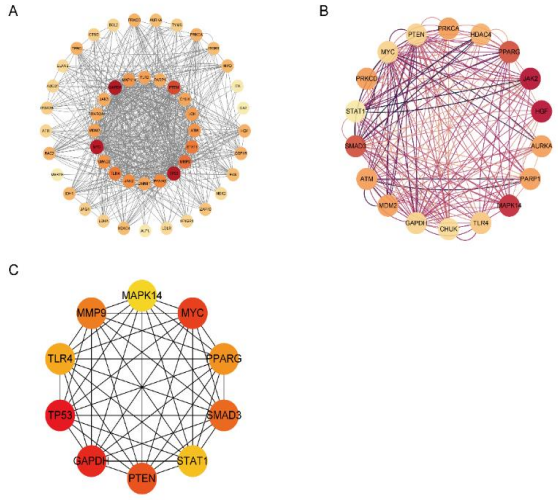
We imported the MitoDEGs into the String database and constructed a network graph with 49 nodes, 320 edges, and an average node degree of 13.1 based on the default implementation criteria of the String database.

We downloaded the network data from the String database and imported it into Cytoscape software for visualization (Figure 6A).

We used the plug-in MCODE of Cytoscape to identify the significant module and obtain a network graph containing 18 MitoDEGs (Figure 6B).

Using the MCC algorithm in the plug-in CytoHubba, we identified 10 candidate hub genes from the PPI network, including TP53, GAPDH, MYC, PTEN, SMAD3, MMP9, PPARG, TLR4, STAT1, and MAPK14 (Figure 6C).

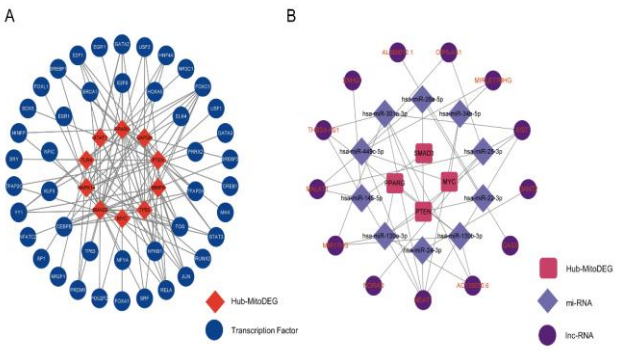
Figure 6: PPI network analysis and hub MitoDEGs identification. (A)PPI network of MitoDEG; (B)A key cluster with 18 genes was further chosen as hub genes by MCODE; (C)Top 10 hub genes explored by CytoHubba.



Hub MitoDEGs-TF network and ceRNA regulatory network

We used the NetworkAnalyst website to construct a gene regulatory network including a network of 44 transcription factors interacting with hub MitoDEGs (Figure 7A). In R, we used eight databases, including ENCORI, miRDB, miRWalk, RNA22, RNAInter, TargetMiner, TargetScan, and miRTarBase to predict the miRNAs of Hub MitoDEGs. We found TP53, SMAD3, PTEN, and MMP9 in 8 databases, and a total of 10 miRNAs were predicted. Subsequently, we obtained 13 target lncRNAs by the starBase database. We constructed a ceRNA regulatory network consisting of 3 mRNAs, 10 miRNAs, and 13 lncRNAs and visualized it in Cytoscape software (Figure 7B).

Figure 7: Hub MitoDEGs-TF network and ceRNA regulatory network. (A)Hub MitoDEGs-TF regulatory network: the red squares represent hub MitoDEGs, and the blue circles represent TFs; (B)ceRNA regulatory network: the red square represents hub MitoDEGs, the purple square represents miRNA, and the purple circle represents lncRNA.

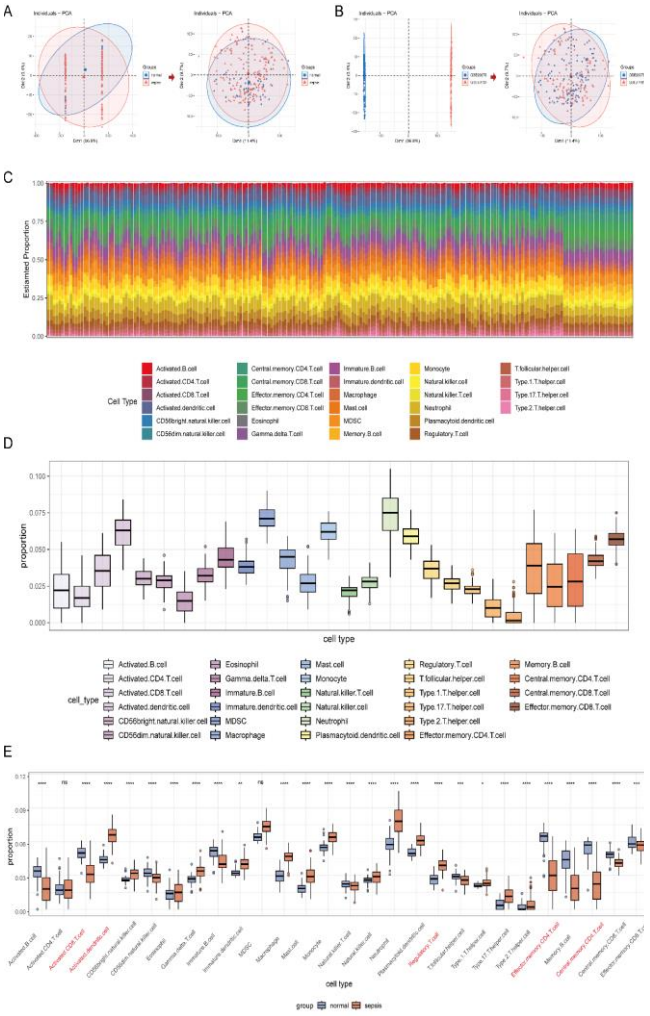


Immune cell infiltration in sepsis

We combined the sepsis datasets GSE26378 and GSE57065 after batch effect removal.

After batch correction, we obtained and normalized the integrated sepsis dataset, including 164 sepsis samples and 46 normal samples. The differences between the two datasets were significantly reduced after removal of batch effects (Figure 8A, B). To better understand the differences in immune function in sepsis, we applied the ssGSEA algorithm to analyze the immune infiltration of GSE26378 and GSE57065 samples. The results revealed significant differences between sepsis samples and normal samples in terms of infiltration of 26 immune cell types ($p<0.05$). Among them, activated dendritic cells and neutrophils were more abundantly infiltrated in sepsis samples, and effector memory CD4 T cells and activated CD8 T cells were more abundantly infiltrated in normal samples (Figure 8C-E). Analysis of multiple correlations between infiltrating immune cells in sepsis revealed the strongest synergistic effect of central memory CD4 T cells and effector memory CD4 T cells, followed by central memory CD4 T cells and activated CD8 T cell (Figure 9A).

Figure 8: Infiltration of immune cell types compared between sepsis samples and normal samples. (A, B) PCA of two original sepsis datasets before batch-effect correction and PCA of the integrated sepsis dataset after batch-effect correction; (C)Stacked bar chart of immune cells; (D, F) Box plot of immune cell proportions.



Hub MitoDEG correlation with immune cells and GSEA enrichment analysis

We conducted correlation analysis between hub MitoDEGs and ssGSEA immune infiltration, which showed that most genes were significantly correlated with immune cells (Figure 9B). Among them, TP53 was positively correlated with effector memory CD4 T cells, MDSC4 and negatively correlated with type 17 T helper cells (Figure 10A); SMAD3 was positively correlated with effector memory CD4 T cells and negatively correlated with mast cell and activated dendritic cells (Figure 10B); PTEN was positively correlated with neutrophils and negatively correlated with activated CD4 T cells and effector memory CD4 T cells (Figure 10C); MMP9 was positively correlated with mast cells, activated dendritic cells, activated CD8 T cells, and effector memory CD4 T cells (Figure 10D). GASE enrichment analysis of the four hub MitoDEGs showed that the respirasome and respiratory chain complex were significantly enriched in TP53 and MMP9, and azurophil granules, specific granule, tertiary granules, and primary lysosomes were significantly enriched in SMAD3 and PTEN (Figure 10E-H).

Figure 9: Immune correlation analysis. (A)The correlation matrix of immune cell proportions; (B)The correlation between hub MitoDEGs and immune cells.

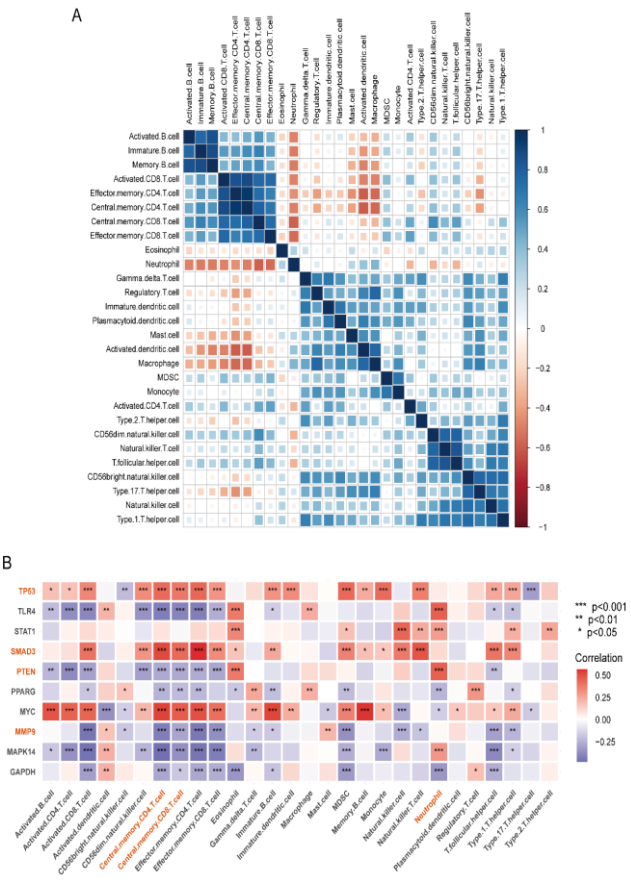
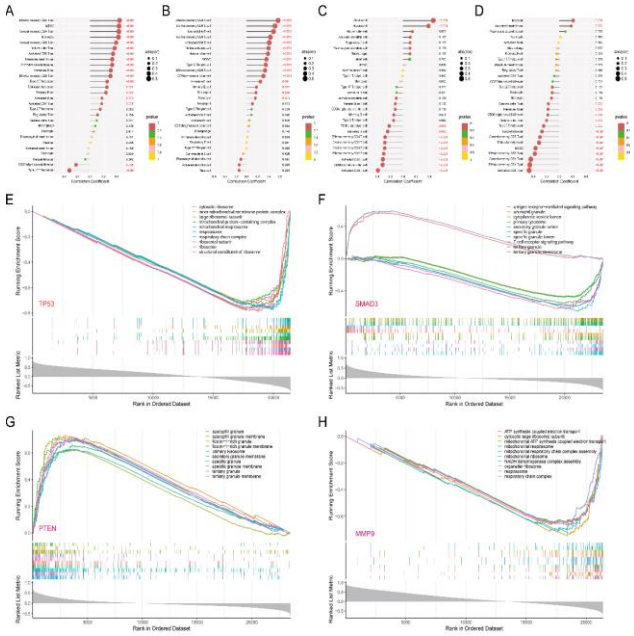


Figure 10: GSEA analysis of hub MitoDEGs. (A-D) The lollipop chart of P53, SMAD3, PTEN, and MMP9 demonstrates the correlation between genes and immune cells; (E-H) Gene Set Enrichment Analysis (GSEA) of GO pathway enrichment for the TP53, SMAD3, PTEN, and MMP9 high-expression group versus the low-expression group.



DISCUSSION

Sepsis is a condition characterized by organ dysfunction resulting from a dysregulated body response to infection, with high morbidity and mortality. Despite its clinical significance, the pathogenesis of sepsis remains unclear, and specific therapeutic drugs are currently unavailable. Mitochondria, the cellular energy supply unit, play a crucial role in various diseases. Studies have demonstrated that during sepsis, mitochondria undergo structural and functional changes in different organs. Mitochondrial energy deficiency, oxidative stress, fusion-fission imbalance, reduced autophagy, and biological functions are all implicated in the progression of sepsis McCall et al. (2022). In this study, we utilized multiple bioinformatics approaches to identify differentially expressed genes (DEGs) from three sepsis-related microarray datasets obtained from GEO. We then intersected these DEGs with mitochondria-related genes to obtain mitochondria-related differentially expressed genes. Multiple studies have reported alterations in mitochondrial function and mitochondria-driven cellular pathways in sepsis, and our study suggests that sepsis-induced mitochondrial alterations may contribute to the pathophysiology of sepsis-induced organ failure Stanzani et al. (2019), Thiessen et al. (2017). CD4+ T cells play a crucial role in sepsis-induced immunosuppression. Th1, Th2, Treg, and Th17 cell subsets are involved in the regulation of Inflammation Xu et al. (2020). Sepsis-induced immunosuppression is associated with a significant depletion of CD4+, CD8+, and B cells Hotchkiss et al. (2013). Alterations in immune cell metabolism have emerged as a critical driver of sepsis-induced immunosuppression Cheng et al. (2016). Extensive defects in immune cell energy metabolism underlie immunosuppression in sepsis, which is associated with mitochondrial dysfunction Cheng et al. (2016). Therefore, the aim of this study was to analyze the regulatory role of mitochondrial and immune dysregulation in the development and progression of sepsis and to explore relevant targets. The findings of this study may contribute to our better understanding of mitochondrial metabolism, immunity, and their interactions in sepsis.

In our study, we utilized the authoritative proteomic database GeneCards to obtain mitochondria-related genes and identified 10 hub MitoDEGs with strong relevance to sepsis. We then used eight databases to predict miRNAs associated with these hub MitoDEGs. Data analysis revealed four genes, TP53, SMAD3, PTEN, and MMP9, all of which had corresponding miRNAs in the databases searched.

TP53 is a transcription factor that regulates DNA stability and cell growth normality. Its localization in mitochondria is crucial for mitochondrial dysfunction through posttranslational modification of key regulatory proteins essential for mitochondrial dynamics, mitochondrial reactive oxygen species (ROS) scavenging, and mtDNA maintenance Chen et al (2016), Saleem et al (2013). TP53 gene mutations or downregulation of expression not only inhibit mitochondrial respiratory activity but also affect the permeability and regulatory sensitivity of the outer mitochondrial membrane Nakano et al. (2001). Studies have demonstrated that TP53 activation and associated signaling are involved in the development of sepsis-induced multiple organ dysfunction syndrome (MODS) Guo et al. (2013). TP53 appears to mediate sepsis-induced end-organ failure Hotchkiss et al. (2000), and interrupting the association of TP53 with mitochondria reduces mortality in sepsis models Mukherjee et al. (2023). SMAD3 is a receptor regulatory protein that is activated by serine kinase phosphorylation and subsequently signals to downstream targets Yang et al. (2019). Previous studies have reported increased mortality in SMAD3-deficient mice in the context of lipopolysaccharide (LPS)-stimulated sepsis, suggesting that SMAD3 reduces the sensitivity of vital organs to LPS Lv et al. (2014). SMAD3 plays a crucial role in regulating inflammatory immune homeostasis by modulating inflammatory immune cells such as macrophages and T lymphocytes. Activation of Smad3 protein inhibits macrophage activity, thus attenuating the inflammatory response Werner et al. (2000).

Additionally, SMAD3 mediates immune regulatory functions, including effector T lymphocytes, helper T cells (Th cells), and Tregs Billing et al. (2017). PTEN is a tumor suppressor gene with bispecific phosphatase activity Ortega-Molina et al. (2013). PTEN is capable of inhibiting disease progression by antagonizing tyrosine kinases and other phosphatases responsible for angiogenesis, cell survival, and other biological processes Liu et al. (2018). PTEN is also associated with the PI3K/protein kinase B (AKT) signaling pathway Zhang et al. (2018). Numerous experiments have demonstrated that increased ROS induced by oxidative stress can activate PTEN as a signaling molecule, thereby inhibiting the PI3K/AKT pathway and regulating cell survival signals such as apoptosis Zhang et al. (2021). Studies have shown that myocardial autophagy can be inhibited to attenuate sepsis-induced cardiac dysfunction by silencing PTEN expression Sang et al. (2020). Matrix metalloproteinases (MMPs) are tissue remodeling enzymes involved in the processing of various biomolecules Mori et al. (2019). Among them, the translocation of MMP9 to mitochondria can be mediated by the molecular chaperones heat shock protein (Hsp)-60 and 70 and mitochondrial membrane translocase Kowluru et al. (2021). MMP9 is localized in the cytoplasmic lysates of leukocytes, astrocytes, and macrophages. Intracellular MMP9 may play a role in Toll-like receptor 4-mediated regulation of innate immunity and inflammation Zhang et al. (2015). MMP9 has been found to be involved in sepsis, and downregulation of MMP9 due to preconditioning could exert a protective effect against damage caused by excessive inflammation in a mouse model of sepsis Huang et al. (2019). Mitochondria play a crucial role in immunity, and mitochondrial biogenesis, fusion, and division have a role in immune cell activation Mills et al. (2017). In our study, we analyzed immune cell infiltration using the ssGSEA algorithm and found higher enrichment of effector memory CD4 T cells, central memory CD4 T cells, and activated CD8 T cells in normal samples than in sepsis samples. Dysregulation of CD8+ and CD4+ T cells is a key component of immune dysfunction in sepsis Yang et al. (2014). The immune imbalance associated with sepsis can be indicated by abnormal numbers of T-cell subsets. It was found that the CD4+/CD8+ T-cell ratio was significantly increased in sepsis patients compared to healthy controls Tschaikowsky et al. (2002). A reduced CD4+/CD8+ T-cell ratio, which correlates with the severity of sepsis, can lead to rapid loss of CD8+ T cells in a mouse model of sepsis Condotta et al. (2013). The progressive reduction in CD4+ T cells in sepsis may impair the patient's ability to eradicate infection and increase their vulnerability to other invading pathogens Hotchkiss et al. (2001). Our study also revealed a significant enrichment of activated dendritic cells (DCs) and neutrophils in sepsis samples. DCs are important antigen-presenting cells and play a crucial role in the immune response by linking innate and adaptive immunity Poehlmann et al. (2009). Altered DC numbers and dysfunction are important causes of immunosuppression and secondary infection in sepsis Li et al. (2019). In animal models, the upregulation of DC cell surface markers in the spleen after the onset of sepsis is an important mechanism for the development of "immune paralysis" in septic mice Guo et al. (2021). Neutrophils also play a crucial role in the development of sepsis and have receptors that recognize pathogen-associated or damage-associated molecular patterns, initiating signaling cascades and producing inflammatory mediators during sepsis, leading to amplification of the inflammatory response Bianchi et al. (2007). During sepsis, most immune cells tend to undergo apoptosis, creating an immunosuppressive environment, while neutrophils show an increased lifespan, and delayed apoptosis can lead to prolonged inflammation Sonego et al. (2016). Additionally, the narrow migration of neutrophils is impaired, leading to their restriction to the vasculature and causing overwhelming vascular inflammation through the release of cytokines, ROS, and neutrophil extracellular traps (NETs) Metzler et al. (2011). Sepsis also enhances the expression of programmed cell death ligand 1 (PD-L1) on neutrophils, triggering lymphocyte apoptosis through direct contact and ultimately promoting sepsis-induced immunosuppression Wang et al. (2015).

Extensive defects in the energy metabolism of immune cells underlie immunosuppression in sepsis, which is associated with mitochondrial dysfunction Cheng et al. (2016). Correlation analysis in our study showed that TP53 and SMAD3 were positively correlated with effector memory CD4 T cells, while PTEN and MMP9 were negatively correlated with effector memory CD4 T cells. SMAD3 was negatively correlated with activated dendritic cells, and PTEN was positively correlated with neutrophils. These findings are consistent with the fact that effector memory CD4 T cells were significantly enriched in normal samples, while activated dendritic cells and neutrophils were significantly enriched in sepsis samples, indicating a strong association between mitochondria-related genes and immune cells in sepsis.

In our study, bioinformatics analysis of sepsis datasets revealed the interaction between mitochondria and the immune microenvironment. Screening and validation of hub MitoDEGs for TP53, SMAD3, PTEN, and MMP9 provided potential molecular targets for in-depth exploration of immunometabolic treatment of sepsis. Despite the rigorous bioinformatic analysis performed in our study, further experiments are needed to validate the effects of mitochondria-related genes on the immune microenvironment of sepsis. Therefore, the specific mechanisms of immunometabolic regulation in sepsis still need to be

explored further.

CONCLUSIONS

In conclusion, our comprehensive bioinformatics analysis revealed differences in mitochondria-related genes and immune cell infiltration between normal and sepsis samples. We identified crosstalk between mitochondria-associated genes and immune cell infiltration in the sepsis dataset. Four hub MitoDEG genes were screened and validated, where PTEN and MMP9 were highly expressed in sepsis, while TP53 and SMAD3 were expressed at low levels. Importantly, TP53 and SMAD3 were positively correlated with effector memory CD4 T cells, while PTEN and MMP9 were negatively correlated with effector memory CD4 T cells. These findings suggest that TP53, SMAD3, PTEN, and MMP9 are coregulatory molecules of immune metabolism in sepsis.

DECLARATIONS

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The GEO database (https://www.ncbi.nlm.nih.gov/geo/) and GeneCards database (https://www.genecards.org/) were used in this study.The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

WM and HL designed the study, processed the bioinformatics data, and wrote the manuscript. All authors reviewed and approved the final version.

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