

Identification of differentially expressed genes and signaling pathways in neutrophils during sepsis-induced immunosuppression via bioinformatics analysis

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To the Editor: Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection. Sepsis deeply perturbs immune homeostasis, and impairs innate and adaptive immunity by directly altering the lifespan, production, and function of the effector cells responsible for homeostasis.^[1] A previous study by Demaret *et al*^[2] reported that the phase of sepsis-induced immunosuppression is characterized by a markedly altered neutrophil chemotaxis (functional and chemokine receptor expressions), oxidative burst, lactoferrin content, and an increased number of circulating immature granulocytes, and these aspects were associated with an increased risk of death after septic shock. However, little is known about the key genes and pathways of neutrophils during the sepsis-induced immunosuppression phase. Therefore, this calls for the use of bioinformatics analysis to identify differentially expressed genes (DEGs) and signaling pathways during sepsis-induced immunosuppression, with the overarching goal of elucidating the role of neutrophils in sepsis-induced immunosuppression.

In this study, the GSE64457 gene expression dataset, comprising the gene expression profile of 15 sepsis-induced immunosuppression patients and eight healthy volunteers, was downloaded from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>). Analysis of differential gene expression was performed using limma package in the Bioconductor package (available online: <http://www.bioconductor.org/>). The related operating instruction codes were put into R software (version 4.0.3, The R Foundation for Statistical Computing, <http://www.r-project.org/>), followed by analysis of DEGs in sepsis-induced immunosuppression and normal samples using the limma software package. Notably, the DEGs were filtered according to log₂ fold

change (FC) > 1 and corrected *P* value < 0.05 after adjustment using false discovery. Next, the Database for Annotation, Visualization and Integrated Discovery online tool was used to perform Gene Ontology (GO) annotations and GO enrichment of the DEGs with regards to the biological process (BP), molecular function (MF), and cellular component (CC). Moreover, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed using the KEGG Orthology Based Annotation System (KOBAS) 3.0 online analysis database (<http://kobas.cbi.pku.edu.cn/>). Finally, the protein-protein interaction network was constructed using STRING database (<https://string-db.org/>), and the CYTOSCAPE software (version 3.7.2, 1999 Free Software Foundation, Inc., Boston, MA, USA) was used to identify interactions and pathway relationships between the proteins encoded by DEGs in neutrophils during sepsis-induced immunosuppression.

Batch normalization of the GSE64457 dataset resulted in identification of 407 DEGs (corrected *P* value < 0.05, log₂ FC > 1), of which 227 were upregulated and 180 were downregulated [Figure 1A]. Figure 1B shows the significant results of the GO enrichment analysis of DEGs in sepsis-induced immunosuppression. With regards to BPs, the DEGs were mainly enriched in inflammatory response (GO: 0006954), canonical glycolysis (GO: 0061621), and positive regulation of nitric oxide biosynthetic process (GO: 0045429). In the MF group, the genes were significantly enriched in phospholipase inhibitor activity (GO: 0004859), phospholipase A2 inhibitor activity (GO: 0019834), and major histocompatibility complex class II protein complex binding (GO: 0023026). In the CCs group, the genes were mainly enriched in extracellular

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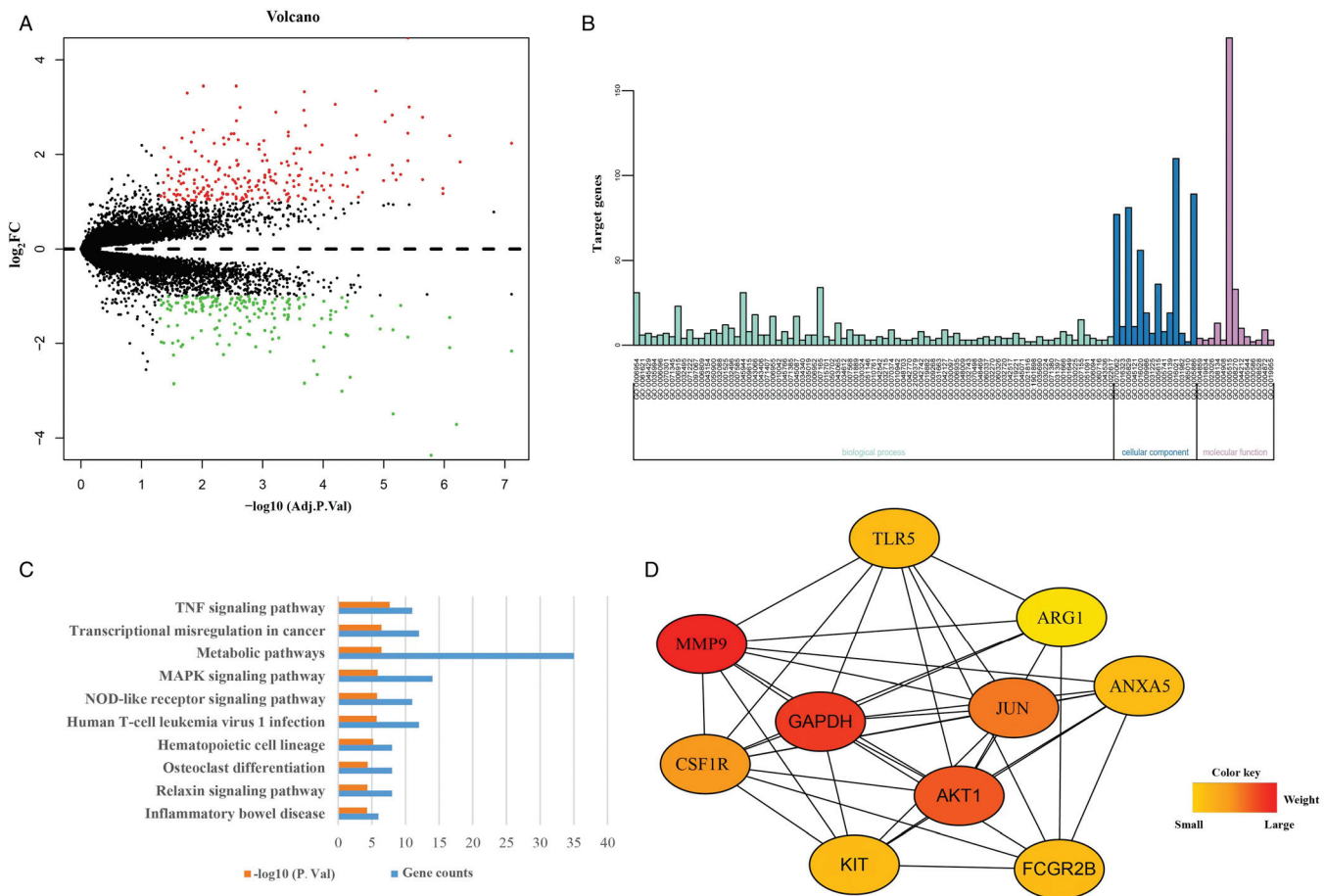


Figure 1: Bioinformatics analysis of neutrophils in sepsis-induced immunosuppression. (A) Differential expression of the gene samples. The red points represent upregulated genes screened on the basis of $\log_2 \text{FC} > 1.0$ and a corrected P value of < 0.05 . The green points represent downregulated genes screened on the basis of $\log_2 \text{FC} < -1.0$ and a corrected P value of < 0.05 . The black points represent genes with no significant difference. (B) GO analysis divided DEGs into three functional groups: BP, CC, and MF. (C) The top ten most significantly enriched pathways by KEGG enrichment analysis using all candidate DEGs. (D) The PPI network of top ten genes. According to the rank of connection degree (number) for each gene, which is represented by the different degrees of color (from red to yellow), the darker the color of the gene, the greater the role of the gene in the PPI network. Red, saffron yellow, and yellow represent hub genes. AKT1: Protein kinase B α ; ANXA5: Annexin A5; ARG1: Arginase 1; BP: Biological process; CC: Cellular component; CSF1R: Colony stimulating factor 1 receptor; DEG: Differentially expressed genes; FC: Fold change; FCGR2B: Fc fragment of immunoglobulin G receptor IIb; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; GO: Gene ontology; JUN: JUN antigen; KEGG: Kyoto Encyclopedia of Genes and Genomes; KIT: c-Kit receptor tyrosine kinase; MAPK: Mitogen-activated protein kinase; MF: Molecular function; MMP9: Matrix metalloproteinase 9; NOD: Nucleotide oligomerization domain-containing protein; PPI: Protein-protein interaction; TLR5: Toll like receptor 5; TNF: Tumor necrosis factor.

exosome (GO: 0070062), basolateral plasma membrane (GO: 0016323), and cytosol (GO: 0005829). These results suggest that most DEGs in neutrophils in sepsis-induced immunosuppression are significantly enriched in inflammatory response, respiratory burst, metabolic processes, and extracellular exosome. Furthermore, the most significantly enriched pathways of the DEGs were subjected to KEGG analysis. Results showed that the DEGs were enriched in 97 signaling pathways, of which tumor necrosis factor signaling pathway, transcriptional misregulation in cancer, metabolic pathways, and mitogen-activated protein kinase signaling pathway were the significantly enriched pathways [Figure 1C]. Moreover, all candidate DEGs were imported into the STRING database and calculated online to determine the interaction network among proteins encoded by candidate DEGs. After removing the discrete and partially connected nodes, the PPI network data of DEGs were imported into the CytoHubba of CYTOSCAPE, followed by construction of a complex network of the DEGs. It is worth noting

that CytoHubba provides 12 analytical methods for identifying hub objects and sub-networks from a complex interactome. In this study, we extracted the top 20 results analyzed using the 12 methods. Next, the frequency of the genes that appeared together in > 5 methods was counted, and it resulted in documentation of 21 genes. Finally, the mass of each of these 21 genes was calculated, followed by visualization of a PPI network of proteins encoded by the top ten genes in CYTOSCAPE. Analyses of PPI networks and hub genes revealed that the most important hub DEGs were *MMP9*, *GAPDH*, *AKT1*, *JUN*, *CSF1R*, *FCGR2B*, *TLR5*, *KIT*, *ANXA5*, and *ARG1* [Figure 1D].

Neutrophils, the first line of defense in protecting the body from infection, have been shown to modulate sepsis-induced immunosuppression. Therefore, this study performed an integrated analysis on the gene expression profiles of neutrophils during sepsis-induced immunosuppression. The main aim of the study was to identify the DEGs and related key pathways for the neutrophils in this

disease. Results obtained from the limma package identified 407 DEGs, including 227 upregulated genes and 180 downregulated genes. Furthermore, GO analysis and KEGG pathways analysis were conducted to determine the functions of the genes. GO and KEGG analysis results were consistent with the knowledge that sepsis-induced immunosuppression exists concomitantly with persistent inflammation, and enables the development of persistent, recurrent, secondary, and nosocomial infections, thereby leading to poorer outcomes and increased long-term mortality.^[3] In addition, results showed that the production and release of essential effector molecules associated with the inflammatory response were significantly impaired. Canonical glycolysis was significantly enriched because immune cells use considerable amounts of energy for both their house-keeping functions and their specialized activities. On the other hand, resting immune cells have low energy needs and derive most of their adenosine triphosphate (ATP) from oxidative phosphorylation (OXPHOS). Notably, activated leukocytes require a substantial increase in energy production and thus rely on the induction of glycolysis to carry out their functions. Therefore, the shift from OXPHOS to glycolysis enables the cells to rapidly produce ATP.

In addition, the PPI network of proteins encoded by DEGs identified the top ten genes associated with neutrophils in sepsis-induced immunosuppression, including *MMP9*, *GAPDH*, *AKT1*, *JUN*, *CSF1R*, *FCGR2B*, *TLR5*, *KIT*, *ANXA5*, and *ARG1*. Among them, *MMP9*, *GAPDH*, *JUN*, *TLR5*, *ANXA5*, and *ARG1* were upregulated, while the others were downregulated. GO analysis showed that all of these genes were involved in the most important BPs, MFs, and CCs, and were mainly implicated in multiple pathways identified by the KEGG analysis conducted in this study. Earlier studies demonstrated that these genes can act on immune cells and regulate their immune responses, inflammatory responses, and chemotaxis.^[4] Furthermore, some of these genes, such as *AKT*, *JUN*, and *CSF1R*, are involved in the proliferation, differentiation, development, phagocytosis, and activation of neutrophils. The results of PPI network were consistent with the knowledge that even in patients with sepsis-induced immunosuppression, the function of neutrophils in the immune responses, inflammatory responses, and chemotaxis capacity is reserved, thereby leading to persistent inflammation. Besides, the upregulated *JUN* can promote proliferation of neutrophils. However, *CSF1R* is linked to the development of neutrophils *in vivo*. A previous study by Dai *et al*^[5] reported that downregulation of *CSF1R* may lead to reduced cavity development of the bone marrow, loss of some progenitor populations, monocytopenia, and reduced neutrophils population in the bone

marrow. Thus, patients with sepsis-induced immunosuppression are always associated with an increased number of circulating immature granulocytes.

In conclusion, this study has identified the key genes and pathways involved in the sepsis-induced immunosuppression specific neutrophil phenotype. Bioinformatics analysis results are consistent with findings reported in previous cell and animal models and human studies. We speculate that *GAPDH*, *AKT1*, *JUN*, *MMP9*, *CSF1R*, *FCGR2B*, *TLR5*, *KIT*, *ANXA5*, and *ARG1* may be the key genes that modulate the progression of sepsis-induced immunosuppression specific circulating neutrophil activation. The findings of this study may provide novel insights into the development of promising targets for the diagnosis and treatment of sepsis-induced immunosuppression in the future.

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Conflicts of interest

None.

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