Identification of Potential Biomarkers for Nonsurvivor Sepsis Patients via Microarray Technology: A Study Based on GEO Datasets

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Research Article

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Abstract

**Background:** The mechanism of sepsis especially non-survivors has not yet been identified.

**Objective:** To identify the key genes concerned with non-survivor sepsis (NSS) and analyze its molecular mechanism.

**Methods:** The original data were obtained from the GEO database and then screened deferentially expressed genes (DEGs). GO and KEGG analysis were performed to analyze the functional annotation of DEGs. The protein-protein interaction (PPI) network and related analysis of hub genes were then carried out. Further, potential hub genes were confirmed in the lipopolysaccharide (LPS)-induced septic mice by western blotting and immunohistochemistry.

**Results:** we obtained 188 DEGs and 32 hub genes between NSS patients and healthy volunteers. Among of them, the top 10 hub genes including STAT1, ISG15, HERC1, EIF2AK2, RPL27, LY6E, IFI44L, XAF1, RSAD2 and HERC6 were studied, which predict sepsis based on receiver operator characteristic curve analysis. GO analysis found that these hub genes were enriched in positive regulation of biomedical process including translation, response to virus and suppression of mitochondrial depolarization, etc.; cell component including mitochondrial inner membrane; molecular function containing ligase activity, etc. The KEGG pathway analysis showed that these hub genes are obviously enriched in influenza A infection and leukocyte trans-endothelial migration. Furthermore, 20 mg/kg of LPS injection up-regulated the expression of ISG15, RPL27, LY6E and HERC6 in the lung tissues compared with control mice.

**Conclusion:** These identified 188 DEGs and 10 hub genes were associated with NSS, especially ISG15, RPL27, LY6E and HERC6 genes expressed in the lung as the most vulnerable organ.

Introduction

Sepsis is defined as infection-induced systematic inflammatory response syndrome with a presence of bacteria or highly suspicious infection foci(Schinkel et al., 2022). Pathological features of sepsis include septic shock and multiple organ injury or dysfunction, especially that in the lung(Saria, 2018, Singer et al., 2016). The available data showed that approximately 6 million population were died from sepsis-induced organs dysfunction per-year(Lewis et al., 2013). The morbidity and mortality rate of sepsis is highest in the world compared with other critical illness(Carchman et al., 2013).

Gene association studies have identified some factors that may result in genetic susceptibility to sepsis(Feng et al., 2019, Yang et al., 2018). The Surviving Sepsis Guidelines recommends to use procalcitonin as the potential bio-markers to evaluate the period of ongoing antimicrobial treatment and dysfunction of renal and coagulation(Rhodes et al., 2017, Singer et al., 2016). An ideal sepsis bio-marker should have high specificity and sensitivity in diagnosis and prognosis prediction, providing a fast and cost-effective bedside assay and confronted validation(Raeven et al., 2018). Gene expression analysis was widely used in clinical disease detection owing to its sensitivity, efficiency and cost-effectiveness.
Hence, genetic expression analysis of sepsis could provide a promising method for improving the success rate of emergency and severe treatment.

Various bio-informatics technology and analytical tools have been applied to explore disease processes, based on the identification of differently expressed genes (DEGs). For example, whole-blood gene expression analysis had identified 86 significantly down-regulated genes relating to systemic immune dysfunctions in septic patients (Parnell et al., 2013). To provide the novel bio-markers of nonsurvivor sepsis (NSS), bio-informatic methods are used to analyze the gene expression data from the Gene Expression Omnibus (GEO) database. Then, Gene Ontology (GO) analysis is performed to analyze the biological processes (BP), cell components (CC), molecular functions (MF) involved in identified DEGs, and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis is used to find out DEG-enriched cell pathways. Hub genes were obtained by filtering the significant modules from protein-protein interaction (PPI) network.

**Materials and methods**

**Statement of Ethics.** After the examination by the Ethics Committee of Guangxi Medical University Affiliated Cancer Hospital (Number: LW2020025), the relevant materials of human genetic resources that downloaded from the GEO database are conformed to the requirements of medical ethics according to the institutional and national guidelines for the care and use of participates.

**Microarray Dataset.** Using the available raw data in NCBI GEO database (http://www.ncbi.nlm.nih.gov/geo), we compared with gene expression profiles of the whole blood-derived RNA samples between NSS patients and healthy volunteers. The microarray expression dataset GSE54514 was obtained from Affymetrix GPL6947 platform (Affymetrix Illumina HumanHT-12 V3.0 expression beadchip), which was submitted by Parnell et al (Parnell et al., 2013) between September, 2013 and October, 2018. Different from Parnell et al, we emphasized on the DEGs for immune response, cell apoptosis, oxidative phosphorylation and neuroendocrine changes between the NSS patients and healthy volunteers.

**Identification of DEGs.** To read and normalized the original data, the packages Affy, Limma and Impute of R software (version: x64 3.2.1) were used. The missing values were treated via k-nearest neighbor method. According to the criterion of fold change (FC) > 2 and adjusted \( P \)-value < 0.05, DEGs between the NSS patients and healthy volunteers were screened and identified from the normalized data. Heat and volcano maps of DEGs were constructed using Pheatmap and Ggplot2 packages of R software.

**Functional annotation for DEGs.** The GO project developed a framework to show the genes function and biological characteristics of high-throughput genomes(Ashburner et al., 2000). KEGG pathway provided comprehensive analysis of cell pathways and connecting genomic function of the DEGs(Kanehisa and Goto, 2000). All DEGs were submitted to the online website DAVID (https://david.ncifcrf.gov/) to perform the GO enrichment and KEGG pathways with count > 5 and \( P \) value < 0.01.
**PPI network and modules analysis.** STRING database (https://cn.string-db.org/) was applied to assess the interactive relationships of DEGs, and the experimentally validated interactions were defined as statistically difference with a combined score more than 0.4. Using Cytoscape software (version 3.6.1) demonstrated the PPI networks between the DEGs. The Molecular Complex Detection (MCODE) app in Cytoscape software was performed to determine the most critical module on the PPI network according to the inclusion criteria as the MCODE scores >5, degree of cut-off = 2, node score cut-off = 0.2, k-score = 2 and maximum depth = 100.

**Identification and analysis of hub genes.** Using cyto-Hubba app in Cytoscape software to screen the hub genes with degree ≥10(Xu et al., 2019). GO enrichment and KEGG pathway of these hub genes were performed using the DAVID database again. Using receiver operator characteristic curve analysis, we determined the specialty and sensitivity of these hub genes for predicting NSS. Information and function of these hub genes was searched via BioGPS (http://biogps.org/#goto=welcome) and Uniprot database (https://www.uniprot.org)/.

**Animal models and sample collection.** Wild type C57BL/6J mice were obtained from the Animal Center of Guangxi Medical University (Nanning, China). The experimental operation flow and animal handling were both approved by the Institutional Animal Care and Use Committee of Guangxi Medical University. To verify the role of hub genes in the septic lung, 12 mice were intraperitoneally injected with lipopolysaccharide (LPS, 20 mg/kg)(Chang et al., 2018), and another 12 mice were received equivoluminal phosphate buffer saline (PBS) by intraperitoneal injection. After the treatment with LPS injection 24 h, the right primary bronchi in the trachea carina was ligatured, and then the left lung bronchoalveolar lavage was harvested by douching with ice-cold PBS. Further, an incision was made in the left ventricle and ice-cold PBS was used to wash left lungs until whitening was apparent. The right lung was obtained for paraffin embedding.

**Verification of animal model.** The wet/dry weight (W/D) ratio from right upper lobe of lung was calculated to estimate lung edema during LPS induced lung injury. The right lower lung lobe was fixed with 10% formaldehyde, embedded with paraffin and stained with hematoxylin and eosin. The degree of lung injury was scored according to alveolar congestion, hemorrhages, neutrophil infiltration and incrassation of the alveolar wall with a five-point score: 0, minimal injury, 1, mild injury, 2, moderate injury, 3, serious injury, and 4, maximal injury(Lin et al., 2018). Simultaneously, some lung samples were cut for transmission electron microscope (TEM) scanning to observe ultrastructural changes of lung epithelial cells.

**Immunohistochemistry techniques.** The sections were undergoing dewaxing, hydration, antigen retrieval and endogenous peroxidases blocking. Then, primary antibodies of hub genes coded proteins was used and their corresponding horseradish peroxidase-conjugated antibodies used as secondary antibody, with DAPI for nuclear staining. Simultaneously, the negative control slides were incubated with PBS instead of primary antibodies. Histological images were observed and saved using an Axio Scope A1 microscope (Zeiss, Oberkochen, Germany) and an AxioCam ICc3 camera (Zeiss).
The expression of these proteins were evaluated by a semi-quantitative scoring system using Image-Pro Plus 6.0 software (Media Cybernetics, Rockville, MD, USA). Briefly, staining intensity and percentage were semi-quantitative scored by the following criteria including no staining as negative = 0, yellow as mild = 1, light brown as moderate = 2, and dark brown as strong = 3. Then, five regions were randomly selected in one sample, and the average percentage of positive cells was calculated by counting the number of positive cells out of 200 cells. Samples were further grouped into the following categories: 0 (0%), 1 (1%–10%), 2 (11%–50%), 3 (51%–80%) and 4 (81%–100%). Finally, a final score of ≥3 was considered as high expression and a score <3 was regarded as low expression.

**Western blotting analysis.** Total proteins were extracted from whitened left lung tissues and then assessed by bicinchoninic acid assay to determine its concentration. The equivalent samples were separated using SDS-polyacrylamide gel. The proteins were then transferred onto a nitrocellulose membrane, which was blocked with 5% bovine serum albumin and 0.1% Tween-20 dissolved in Tris-buffered saline. The blocked membranes were incubated by primary antibodies of hub genes coded proteins and β-actin as internal reference at 4°C overnight. Using horseradish peroxidase-conjugated mouse anti-rabbit antibody as a secondary antibody incubated the cleaned membranes at the next day. The bands of each protein were scanned via a West Pico enhanced chemiluminescence kit (Thermo Fisher Scientific). These results were assessed by a semi-quantitative scoring system using Image Lab 4.1 software (Bio-Rad, Hercules, CA, USA).

**Statistical analysis.** The data were analyzed by SPSS 22.0 software. All quantitative data were showed as mean ± SD. Independent sample t test was carried out to analyze the comparison between control and septic group. The statistical difference was defined as P value less than 0.05.

**Results**

**Identified DEGs between NSS and healthy blood samples.** The presentation of the identified DEGs was performed by all nodes in volcano plot between NSS and healthy blood samples (Fig. 1A). The nodes were marked as blue or red, based on the cut-off criterion (fold change≥2 and adjusted P value < 0.05). The up-regulated and down-regulated DEGs, between NSS blood and healthy blood, were shown as red dots or blue dots, respectively. Fig. 1B and 1C showed the circular cluster map of top 10 up-regulated and down-regulated DEGs. Finally, 105 up-regulated and 83 down-regulated DEGs were screened and identified from the GSE54514 dataset. The heat map of the top 10 up-regulated and down-regulated DEGs was displayed in Fig. 1D.

**GO Functional enrichment and KEGG pathway analysis.** The results of GO categories demonstrated that the identified DEGs were significantly enriched in such BP as type I interferon (IFN) signaling pathway, translation, movement of cell or subcellular component, ephrin receptor signaling pathway, defense response to virus, apoptosis, positive regulation of nuclear factor (NF)-κB transcription factor activity (Fig. 2A-B), Enrichment of DEGs in CC included the extracellular exosome, cytosol, membrane, early endosome, cytoplasm, extracellular matrix, focal adhesion and mitochondrion (Fig. 2C), and in MF included protein
binding, protein complex binding and structural constituent of ribosome (Fig. 2D). The KEGG pathway analysis results showed that the DEGs were mainly enriched in Fc Gamma R-mediated phagocytosis (Fig. 2E). Fig. 2F and 2G showed the network of GO analysis classified with terms and P value respectively.

**PPI and module network construction.** Framing of the PPI network and determination of significant modules were conducted with 336 edges and 114 nodes in Fig. 3A. According to previous defined criteria, we identified two clusters of the most important module on the PPI network map (Fig. 3B and C). The first cluster included 96 edges and 15 nodes (MCODE scores = 13.714), and another cluster possessed 52 edges and 12 nodes (MCODE scores = 9.455).

**Hub genes screen and analysis.** With degrees ≥10, there were of 32 hub genes identified, consisting of 162 edges and 32 nodes in the constructed PPI network as shown in Fig. 4A. The expression and PPI network of top 10 hub genes were performed in Fig. 4B-C. These hub genes were analyzed using GO enrichment and KEGG pathway. The results of these analyses showed that hub genes were significantly enriched in such BP as translation, response to virus, suppression of mitochondrial depolarization and extrinsic apoptotic signaling pathway in absence of ligand, protein ubiquitination related to ubiquitin-dependent protein catabolism, release of cytochrome c from mitochondria, innate immune response, and endodermal cell differentiation (Fig.4D-E). In the CC results, hub genes were mainly enriched in the mitochondrial inner membrane (P = 0.038). As for variations in the MF, hub genes were mainly enriched in ligase activity, structural constituent of ribosome and cytochrome-c oxidase activity (Fig.4D-E). KEGG pathways demonstrated that hub genes were significantly enriched in such cell pathway as influenza A infection and leukocyte transendothelial migration (Fig.4F-G).

Here, the top ten hub genes were STAT1, ISG15, HERC1, EIF2AK2, RPL27, LY6E, IFI44L, XAF1, RSAD2 and HERC6. The relative expression of these hub genes was performed in Fig. 5A and 5B. Table 1 showed the detailed information and function. To determine accurate thresholds of hub genes to predict NSS, receiver operator characteristic curves were performed. The expression of these hub genes involved in a diagnosis of NSS (0.7<AUC<1 and P ≤ 0.05) as shown in Table 2, and the receiver operator characteristic curves of these hub genes were presented in Fig. 5C-L.

**Administration of LPS 20 mg/kg triggers acute lung edema and injury.** Mice treated with LPS 20 mg/kg caused an increase in the ratio of W/D and pathological score compared with control group (Fig. 6A and B). Although only one mouse died in control group at the seventh day, a significant difference in postoperative survival between the two groups for 1-week survival was observed (P = 0.014, Fig. 6C). Obviously, 20 mg/kg of LPS injection favored infiltration of neutrophils and accumulation of protein-rich fluid in the alveolar space, and disorder of lung structure compared with control mice (Fig. 6D). Furthermore, ultrastructure of lung epithelial cells following 20 mg/kg of LPS injection showed karyopyknosis, disorder structure of cell membrane and organelles edema (Fig. 6E).

**Expression of ISG15, RPL27, LY6E and HERC6 in the septic and control mice.** According to the results of Table 2, the relatively high-expression hub genes in the lung with NSS were ISG15, RPL27, LY6E and HERC6. The assessment of Western blotting showed that 2.0 mg/kg of LPS injection upregulated the
expression of ISG15, RPL27, LY6E and HERC6 (Fig. 7A-D). Simultaneously, the results of IHC showed the same trends for the expression of these genes (Fig. 7E-G).

**Discussion**

Inflammation and multiple organ injury are the most common and prominent feature of sepsis, in particular NSS (Taboada et al., 2010). Since lacking of treatment strategy to significantly attenuate inflammation and organ injury, current treatment for sepsis mainly focuses on antimicrobial treatment and multi-organ support. Due to high mortality and high incidence of complications, the exploration of the molecular mechanism that involves in sepsis especially NSS progress and risk prediction of severe organ damage favors considerable importance to the diagnosis, treatment and prevention of NSS.

In the present study, several bio-informatics methods were used to analyze original data and identify hub genes in NSS. The microarray data GSE54514 was used to screen DEGs and 188 DEGs were finally discovered that shared top 10 hub genes: STAT1, ISG15, HERC1, EIF2AK2, RPL27, LY6E, IFI44L, XAF1, RSAD2 and HERC6. Among them, ISG15, RPL27, LY6E and HERC6 were relatively high-expressed hub genes in the lung, showing the better specificity between samples of NSS or healthy volunteers.

It showed that the resistance to endotoxin-induced shock was improved in STAT1-deficient mice (Herzig et al., 2012). Park et al. (Park et al., 2018) found that histone deacetylase 4 controls the acetylation of high mobility group box 1 in LPS-stimulated macrophages via the Janus kinase/STAT1 pathway. Biflorin favors the negative regulation of pro-inflammatory factor expression through p38 mitogen-activated protein kinase and STAT1 deactivation in macrophages as a result to protect mice from endotoxin shock (Lee et al., 2016). In the LPS-treated monocytes, miR-30a suppressed the release of interleukin-10 by targeting STAT1-MD-2, that is a LPS-binding protein formed a heterodimer with Toll-like receptor (Wang et al., 2014). Therefore, these results suggested that STAT1 plays a deleterious role in sepsis especially endotoxin induced sepsis and accelerate the progress of inflammation and multiple organ injury.

HERC1 regulates the ERK pathway by acting C-RAF for inhibiting cell proliferation, contain a putative BH3 domain to bind to BAK for UV-induced apoptosis and interact with tuberous sclerosis complex 2 protein for regulating cell growth (Holloway et al., 2015, Chong-Kopera et al., 2006, Schneider et al., 2018). EIF2AK2-mediated stress response regulated by TIA1 RNA-binding protein family is involved in the fidelity of mRNA translation and maturation, and RNA-stress-sensing pathways in human cell (Meyer et al., 2018). Another study showed that EIF2AK2 is an activator of the NLRP3 inflammasome to influence the occurrence and development of inflammatory bowel disease (Varghese et al., 2015). Gomez-Carballa et al. (Gomez-Carballa et al., 2019) found that IFI44L favored superior results than those got from the 2-transcript test to discriminate between viral from bacterial infection in febrile children. Interestingly, the upregulation of XAF1 protein comprise an positive effect of β-cell apoptosis and interferon-β production because of metabolic endotoxemia-induced macrophages activation and infiltration in islets (Tsuruta et al., 2018). Furthermore, RSAD2 is positively associated with mature dendritic cells function and essential for its maturation through the IRF7-mediated signaling pathway for T cell-mediated immunity (Jang et al., 2018).
These hub genes showed a crucial role for cell growth, proliferation and apoptosis by regulation of inflammation and immunity, which maybe the reason of differential expression between the NSS patients and healthy volunteer.

Using BioGPS (http://biogps.org/#goto=welcome) and Uniprot database (https://www.uniprot.org/) to obtain the information and function of the hub genes, we found that ISG15, RPL27, LY6E and HERC6 were displayed positive effect in lung injury. Furthermore, in mice undergoing LPS-induced lung injury, the expression of these four hub genes were both up-regulated compared with control mice. ISG15 plays a crucial role in innate immune response as intracellular protein modifier and extracellular signaling molecule to stimulate the secretion of interferon-γ (Perng and Lenschow, 2018, Swaim et al., 2017).

Human intracellular ISG15 is also served as a substrate for ISGylation-mediated antiviral immune response to prevent interferon-α/β over-amplification and auto-inflammation (Zhang et al., 2015). These studies were both concentrated on the role of ISG15 in the cancer cell growth and viral resistance, but whether ISG15 functions as a crucial role in sepsis remains unclear.

RPL27 is a mitochondrial ribosomal protein that functions in proper rRNA processing and maturation of 28S and 5.8S rRNAs (Wang et al., 2015). However, there were no studies found to demonstrate the role of RPL27 in cell growth, inflammation or immunity. Xu et al (Xu et al., 2014) showed that the regulatory LY6E pathway serve as one of negative regulation of counterpoise monocyte activation in LPS-stimulated monocytes, which might be induced by the impaired gastrointestinal tract during ongoing HIV-1 infection. LY6E pertains to the class of interferon-inducible host factors that increase viral infectivity without inhibition of interferon antiviral activity (Mar et al., 2018, Bacquin et al., 2017, Yu et al., 2019) which may be contributed to the poor prognosis of sepsis. Previous studies (Arimoto et al., 2015, Oudshoorn et al., 2012) showed that murine HERC6 plays a crucial function in protein ISGylation to improve the interferon-β promoter and confer antiviral activity.

Although the present work involves systematic and rigorous bio-informatics analysis, there still are some limitations. First, the sample size was relatively small and a larger data sample is required for further validation. Then, the molecular and experimental results of this study are based on the common target lung and results of other target organs such as heart and liver were necessary.

**Conclusion**

Using several bio-informatics methods obtained 188 DEGs and 10 hub genes related to NSS (STAT1, ISG15, HERC1, EIF2AK2, RPL27, LY6E, IFI44L, XAF1, RSAD2 and HERC6) from the GEO database, especially ISG15, RPL27, LY6E and HERC6 highly expressed in the sepsis-induced lung injury in mice. Although there are several contradictions and ambivalence of the roles these hub genes played in NSS, the present study demonstrates that exploration and elucidation of these areas would provide new potential targets and prospects for the diagnosis, treatment and prediction of NSS.

**Abbreviations**
GEO, Gene Expression Omnibus, GWES, genome-wide expression studies, DEGs, differentially expressed genes, GO, gene ontology, KEGG, Kyoto Encyclopedia of Genes and Genomes, PPI, protein–protein interaction, FC, fold change, MCODE, Molecular Complex Detection, BP, biomedical process, CC, cell component, MF, molecular function, PBS, phosphate buffer saline, W/D, wet/dry weight, HE, hematoxylin and eosin, TEM, transmission electron microscope, BCA, bicinchoninic acid, NF-κB, nuclear factor-κB, TLR, Toll-like receptor, AUC, area under curve, ODT, optimal diagnostic threshold, STAT1, signal transducer and activator of transcription 1, ISG15, ISG15 ubiquitin like modifier, HERC1, H HECT domain and RCC1-like domain-containing protein 1, EIF2AK2, eukaryotic translation initiation factor 2 alpha kinase 2, RPL27, ribosomal protein L27, LY6E, lymphocyte antigen 6 family member E, IFI44L, interferon induced protein 44 like, XAF1, XIAP associated factor 1, RSAD2, radical S-adenosyl methionine domain containing 2, HERC6, HECT and RLD domain containing E3 ubiquitin protein ligase family member 6.

**Declarations**

**Acknowledgments**

We sincerely acknowledge Parnell GP and his colleagues for their original work on expression profiling by array. We thank all co-authors for support from data collecting to revising manuscript.

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**Availability of data and materials**

The used and analyzed dataset during the current study was available from the corresponding author based on reasonable request.

**Author's contribution**

Ren Jing conceived and designed the study. Yan Ma and Shichao Shan was a major contributor in writing the manuscript and submitting the manuscript. Cheng Luo carried out the animal experiments, Zhaokun Hu collected the dataset and analyzed the data, Jianlan Mo made substantial contributions to research conception, and designed the draft of the research process. All authors read and approved the final manuscript.

**Patient consent for publication**

Not applicable.

**Competing interests**
The authors declared no competing interests.

References


Tables

Table 1 Summaries for the top 10 hub genes.
<table>
<thead>
<tr>
<th>No.</th>
<th>Gene symbol</th>
<th>Full name</th>
<th>UniProtKB ID</th>
<th>High-expression position</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>STAT1</td>
<td>signal transducer and activator of transcription 1</td>
<td>P42224 (STAT1_HUMAN)</td>
<td>Cardiac myocytes, CD4+ T cells, CD8+ T cells, etc.</td>
<td>It is a signal transducer and transcription activator that regulates cellular responses to interferon, cytokine KITLG/SCF and other cytokines and growth factors.</td>
</tr>
<tr>
<td>2</td>
<td>ISG15</td>
<td>ISG15 ubiquitin like modifier</td>
<td>P05161 (ISG15_HUMAN)</td>
<td>Lymphoma burkitts (Daudi), lung, 721 B lymphoblasts, etc.</td>
<td>It mediates the innate immunity to viral infection either via ISGylation or action as a free or unconjugated protein.</td>
</tr>
<tr>
<td>3</td>
<td>HERC1</td>
<td>HECT domain and RCC1-like domain-containing protein 1</td>
<td>Q15751 (HERC1_HUMAN)</td>
<td>Profrontal cortex, CD8+ T cells, amygdala, CD4+ T cells, etc.</td>
<td>It affects membrane trafficking via some guanine nucleotide exchange factor activity and its ability to bind clathrin.</td>
</tr>
<tr>
<td>4</td>
<td>EIF2AK2</td>
<td>eukaryotic translation initiation factor 2 alpha kinase 2</td>
<td>P19525 (E2AK2_HUMAN)</td>
<td>Trigeminal Ganglion, fetal thyroid, tonsil, etc.</td>
<td>It is an interferon-induced dsRNA-dependent serine/threonine-protein kinase for mediating innate immunity to viral infection, signal transduction, apoptosis, cell proliferation and differentiation.</td>
</tr>
<tr>
<td>5</td>
<td>RPL27</td>
<td>ribosomal protein L27</td>
<td>P61353 (RL27_HUMAN)</td>
<td>Lymphoma burkitts (Daudi), bronchial epithelial cells, lung, etc.</td>
<td>It is a component of the large ribosomal subunit for proper rRNA processing and maturation.</td>
</tr>
<tr>
<td>6</td>
<td>LY6E</td>
<td>lymphocyte antigen 6 family member E</td>
<td>Q16553 (LY6E_HUMAN)</td>
<td>Lung, 721 B lymphoblasts, liver, heart, etc.</td>
<td>It is involved in T-cell development and modulation of nicotinic acetylcholine receptors activity.</td>
</tr>
<tr>
<td>7</td>
<td>IFI44L</td>
<td>IFN induced protein 44 like</td>
<td>Q53G44 (IFI44L_HUMAN)</td>
<td>721 B lymphoblasts, tonsil, CD33+ myeloid, etc.</td>
<td>It presents a low antiviral activity against hepatitis C virus.</td>
</tr>
<tr>
<td>8</td>
<td>XAF1</td>
<td>XIAP associated factor 1</td>
<td>Q6GPH4 (XAF1_HUMAN)</td>
<td>CD33+ myeloid, CD56+ NK</td>
<td>It is a negative regulator of members of the inhibitor of apoptosis</td>
</tr>
</tbody>
</table>
Table 2 Receiver operator characteristic curve analysis of the top 10 hub genes.

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>NSS</th>
<th>AUC</th>
<th>P-value</th>
<th>ODT</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>STAT1</td>
<td>0.938</td>
<td>0.002</td>
<td>9.759</td>
<td>1.000</td>
<td>0.778</td>
<td></td>
</tr>
<tr>
<td>ISG15</td>
<td>0.963</td>
<td>0.001</td>
<td>8.041</td>
<td>1.000</td>
<td>0.889</td>
<td></td>
</tr>
<tr>
<td>HERC1</td>
<td>0.926</td>
<td>0.002</td>
<td>9.033</td>
<td>0.889</td>
<td>0.889</td>
<td></td>
</tr>
<tr>
<td>EIF2AK2</td>
<td>0.815</td>
<td>0.024</td>
<td>9.897</td>
<td>0.889</td>
<td>0.778</td>
<td></td>
</tr>
<tr>
<td>RPL27</td>
<td>0.969</td>
<td>0.001</td>
<td>12.507</td>
<td>1.000</td>
<td>0.889</td>
<td></td>
</tr>
<tr>
<td>LY6E</td>
<td>0.840</td>
<td>0.015</td>
<td>8.684</td>
<td>1.000</td>
<td>0.667</td>
<td></td>
</tr>
<tr>
<td>IFI44L</td>
<td>0.889</td>
<td>0.005</td>
<td>7.175</td>
<td>1.000</td>
<td>0.667</td>
<td></td>
</tr>
<tr>
<td>XAF1</td>
<td>0.926</td>
<td>0.002</td>
<td>8.014</td>
<td>1.000</td>
<td>0.889</td>
<td></td>
</tr>
<tr>
<td>RSAD2</td>
<td>0.887</td>
<td>0.007</td>
<td>7.444</td>
<td>0.889</td>
<td>0.889</td>
<td></td>
</tr>
<tr>
<td>HERC6</td>
<td>0.975</td>
<td>0.001</td>
<td>7.585</td>
<td>1.000</td>
<td>0.889</td>
<td></td>
</tr>
</tbody>
</table>

NSS, nonsurvive sepsis; AUC, area under curve; ODT, optimal diagnostic threshold.

Graphic Abstract
The original microarray dataset was obtained from the GEO database and then screened deferentially expressed genes (DEGs). GO and KEGG analysis were performed to analyze the functional annotation of DEGs. The protein-protein interaction (PPI) network was performed to identify hub genes. Among these hub genes, we focused on the ISG15, HERC1, RPL27 and LY6E with higher specificity. ISG15 mediates the innate immunity to viral infection either via ISGylation or action as a free or unconjugated protein. HERC6 is a E3 ubiquitin-protein ligase which accepts ubiquitin from E2 ubiquitin-conjugating enzyme in the form of a thioester and then directly transfers the ubiquitin to targeted substrates. RPL27 is a component of the large ribosomal subunit for proper rRNA processing and maturation. LY6E is involved in T-cell development and modulation of nicotinic acetylcholine receptors activity. In lipopolysaccharide (LPS)-induced septic mice, the proteins translated by these four genes were highly expressed in the lung tissues.

**Figures**
Figure 1

Identified DEGs between blood samples from healthy control individuals and patients with NSS. (A) A volcano plot showing the all identified DEGs between NSS and control blood samples via analysis of the GSE54514 dataset with R software. The X-axis presents the log-scaled fold-change and the Y-axis indicates the negative logarithm of adjusted P-value. Each symbol shows a gene. The red symbols indicate upregulated genes, while blue symbols present downregulated genes. (B) Circular cluster map of up-regulated DEGs. (C) Circular cluster map of down-regulated DEGs. (D) A heatmap showing the top 20 DEGs between NSS and control blood samples via analysis of the GSE54514 dataset with R software.

**Figure 2**

**Enrichment function analysis of all identified DEGs.** Detailed information relating to enrichment function in the (A) GO chord of top 10 DEGs, (B) biological processes, (B) cellular components, (C) molecular function and (E) KEGG pathway for all identified DEGs. (F) Network of GO analysis classified with terms.
(G) Network of GO analysis classified with P value. NSS, nonsurvival sepsis, DEGs, differently expressed genes.

![Diagram of protein-protein interaction network and two significant module networks]

**Figure 3**

**Protein-protein interaction network and two significant module networks.** (A) The PPI network presenting the intricate relationships between all identified DEGs. (B) The first significant module network determined based on the analysis of protein-protein interaction network. (C) The second significant module network identified based on the analysis of protein-protein interaction network.
Figure 4

Protein-protein interaction network and enrichment function analysis of the screened hub genes. (A) The protein-protein interaction network of the total of 32 genes as hub genes with degrees ≥10. (B) A heatmap showing the correlations between these hub genes. The color represents the intensity of the correlation. It indicates a positive correlation between the values of 10.2511~20.5022 while a negative correlation between the values of 0~10.2511. The larger the absolute value represents the stronger the correlation. (C) The protein-protein interaction network of these hub genes. (D) Biological processes analysis for hub genes. (E) GO chord of top 10 hub genes. (F) KEGG pathway analysis for hub genes. (G) Network of GO analysis classified with terms.
**Figure 5**

Relative analysis of the top 10 hub genes including STAT1, ISG15, HERC1, EIF2AK2, RPL27, LY6E, IFI44L, XAF1, RSAD2 and HERC6. (A) and (B) Expression of these hub genes in the blood sample from healthy control individuals and patients with NSS. (C-L) Receiver operator characteristic curves indicating that these top 10 hub genes could sensitively and specifically predict NSS. NSS, nonsurvival sepsis, STAT1, signal transducer and activator of transcription 1, ISG15, ISG15 ubiquitin like modifier, HERC1, H HECT domain and RCC1-like domain-containing protein 1, EIF2AK2, eukaryotic translation initiation factor 2 alpha kinase 2, RPL27, ribosomal protein L27, LY6E, lymphocyte antigen 6 family member E, IFI44L, interferon induced protein 44 like, XAF1, XIAP associated factor 1, RSAD2, radical S-adenosyl methionine domain containing 2, HERC6, HECT and RLD domain containing E3 ubiquitin protein ligase family member 6. *P < 0.05 vs. CON group, **P < 0.01 vs. CON group, ***P < 0.001 vs. CON group.
Figure 6

Septic lung injury in mice treated with PBS (CON) or 20 mg/kg LPS. (A) Lung edema was evaluated by calculating the wet/dry weight ratio. (B) Pathological scores were used based on the results of hematoxylin and eosin staining. (C) Survive cure between the CON and Sepsis group. Both of these experiments were repeated three times. (D) Histology of lung tissues were stained with hematoxylin and eosin. (E) Transmission electron micrographs of type II alveolar cells. Both of these experiments were repeated three times. *$P < 0.05$ vs. CON group.
Figure 7

Protein expression of ISG15, RPL27, LY6E and HERC6 by IHC in mice treated with PBS (CON) or 20 mg/kg LPS (Sepsis). (A) Levels of ISG15. (B) Levels of RPL27. (C) Levels of LY6E. (D) Levels of HERC6. (E) Average percentage of positive cells in the Sham, ISG15 and RPL27 expression. (F) Average percentage of positive cells in the Sham, LY6E and HERC6 expression. (G) Protein expression of these hub genes by
IHC. Both of these experiments were repeated three times. *$P < 0.05$ vs. CON group, **$P < 0.01$ vs. CON group, ***$P < 0.001$ vs. CON group.