MEDICAL SCIENCE MONITOR

DATABASE ANALYSIS

e-ISSN 1643-3750
© Med Sci Monit, 2020; 26: e923881
DOI: 10.12659/MSM.923881

Received: 2020.02.28
Accepted: 2020.04.06
Available online: 2020.04.21
Published: 2020.06.23

Bioinformatics-Based Study to Investigate Potential Differentially Expressed Genes and miRNAs in Pediatric Sepsis

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Source of support: This work was supported by grant no. 81960363 founded by the National Science Foundation of China, and by the Doctoral Scientific Research Foundation of Dali University (no. KYBS201721), and by the Foundation of Yunnan Provincial Department of Education (no. 2019Y0284)

Background: Sepsis is an extremely common health issue with a considerable mortality rate in children. Our understanding about the pathogenic mechanisms of sepsis is limited. The aim of this study was to identify the differential expression genes (DEGs) in pediatric sepsis through comprehensive analysis, and to provide specific insights for the clinical sepsis therapies in children.

Material/Methods: Three pediatric gene expression profiles (GSE25504, GSE26378, GSE26440) were downloaded from the Gene Expression Omnibus (GEO) database. The difference expression genes (DEGs) between pediatric sepsis and normal control group were screened with the GEO2R online tool. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of the DEGs were performed. Cytoscape with CytoHubba were used to identify the hub genes. Finally, NetworkAnalyst was used to construct the targeted microRNAs (miRNAs) of the hub genes.

Results: Totally, 160 overlapping upward genes and 61 downward genes were identified. In addition, 5 KEGG pathways, including hematopoietic cell lineage, *Staphylococcus aureus* infection, starch and sucrose metabolism, osteoclast differentiation, and tumor necrosis factor (TNF) signaling pathway, were significantly enriched using a database for labeling, visualization, and synthetic discovery. In combination with the results of the protein–protein interaction (PPI) network and CytoHubba, 9 hub genes including ITGAM, TLR8, IL1β, MMP9, MPO, FPR2, ELANE, SPI1, and C3AR1 were selected. Combined with DEG-miRNAs visualization, 5 miRNAs, including has-miR-204-5p, has-miR-211-5p, has-miR-590-5p, and has-miR-21-5p, were predicted as possibly the key miRNAs.

Conclusions: Our findings will contribute to identification of potential biomarkers and novel strategies for pediatric sepsis treatment.

MeSH Keywords: Computational Biology • Hu Paraneoplastic Encephalomyelitis Antigens • MicroRNAs • Sepsis

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/923881

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Background

Sepsis in children is regarded as the leading global cause of high mortality [1]. Every year, an estimated 1.2 million children are diagnosed with sepsis, with the mortality rate ranging from 4% to 50% [2–4]. Recently, expert consensus guidelines on the treatment of sepsis in children were proposed to assist clinicians in assessing pediatric sepsis and septic shock [5]. However, in view of the non-specific signs and symptoms of sepsis, the diagnosis and treatments of sepsis remain immensely complex.

Blood-based non-invasive biomarkers can be the key to personalized medicine in sepsis whereby patients receive specific treatments due to their identifiable molecular signatures [6]. Diverse biomarkers have been assessed in the diagnosis of pediatric sepsis [7–9]. However, none of these biomarkers has excellent specificity and/or sensitivity in the clinical practice. Procalcitonin (PCT) and C-reactive protein (CRP) have been treated as biomarkers for diagnosing sepsis in children because of their relatively better specificity [10]. Nevertheless, no gold standard biomarker currently exists for utility in routine medical settings. Therefore, identification of new biomarkers is urgently needed.

High-throughput technologies have been applied for the potential biomarker discoveries [11]. Clinical bioinformatics as an emerging strategy is considered one of the promising and pivotal approaches to overcome clinical challenges in early diagnosis, competent therapies, and predictive prognostication of cancer patients [12]. Such strategies have been used extensively for investigations in liver cancer, gastric cancer, and other types of cancer [13–15]. Also, an increasing number of novel biomarkers in some non-tumor diseases have been identified via bioinformatics analysis [16–18].

With this in mind, we made a preliminary attempt to elucidate biomarkers and the molecular mechanisms underlying sepsis. In this investigation, we downloaded 3 original datasets containing messenger RNA (mRNA) expression profiles of pediatric sepsis from the Gene Expression Omnibus (GEO) datasets for analysis. Then we identified differential expression genes (DEGs) between pediatric sepsis samples and normal samples. Subsequently, we built a protein–protein interaction (PPI) network and performed functions of genes and pathway enrichment analysis of the DEGs. Finally, potential microRNAs (miRNAs) associated with DEGs were predicted by constructing a miRNA-gene regulatory network through Cytoscape software.

Material and Methods

Microarray data processing

Three microarray datasets (GSE25504, GSE26378, GSE26440) of pediatric sepsis and control samples were collected from the GEO database (*https://www.ncbi.nlm.nih.gov/geo/*) after a careful review. All data were freely downloaded via the internet. Details regarding these 3 gene expression profiles used for this analysis are presented in Table 1. Among them, GSE25504 was based on Illumina HumanHT-12 V3.0 expression beadchip. GSE26378 and GSE26440 were based on Agilent GPL570 platform ([HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array).

Differentially expressed genes (DEGs) selection

GEO2R (*http://www.ncbi.nlm.nih.gov/geo/geo2r*) was applied to perform DEGs analysis between serum samples from pediatric sepsis and control groups, and corrected *P*-value calculations to obtain |logFC|. Genes with correcting *P*-value <0.05 and |logFC| ≥1.0 were deemed as DEGs. An online visualization software Funrich (*http://funrich.org/*) was used to generate the Venn diagram of DEGs.

Functional and KEGG enrichment analysis of DEGs

Functional enrichment of DEGs was analyzed in 3 categories of the Gene Ontology (GO) domains: biological process (BP), cellular component (CC), and molecular function (MF). The KEGG database contains pathway datasets involving biological functions, diseases, chemicals, and drugs. In this investigation, significantly upregulated and downregulated DEGs combined sepsis microarray data were analyzed using an online software (Enrichr, *http://amp.pharm.mssm.edu/Enrichr/*). A *P* value <0.05 was taken to be a significantly enriched term.

Table 1. Details for GEO pediatric sepsis data.

Reference	GEO	Platform	Control	Sepsis
Dickinson P (2014)	GSE25504	GPL6947	35	27
Wong HR (2011)	GSE26378	GPL570	21	82
Wong HR (2011)	GSE26440	GPL570	32	78

GEO – Gene Expression Omnibus.

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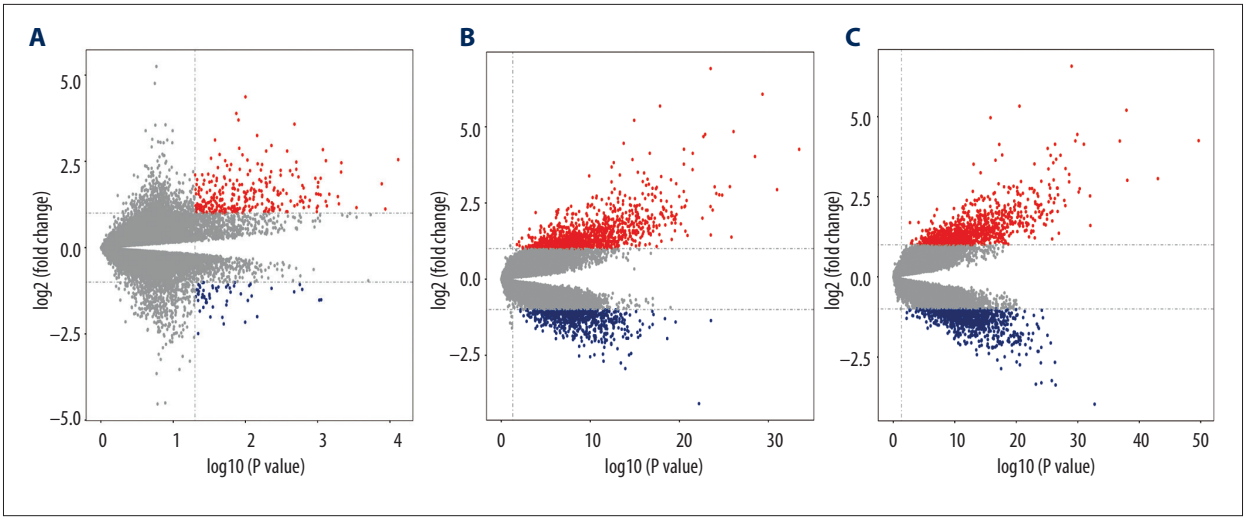

Figure 1. Volcano plot representing differential expression genes (DEGs) between control groups and sepsis groups. (**A–C**) Shows DEGs in GSE25504, GSE26378, and GSE26440 dataset, respectively.

Protein–protein interaction (PPI) establishment and identification of hub genes

An online tool (Search Tools for the Retrieval of Interacting Genes, STRING, *https://string-db.org/*) was used to analyze protein interactions. A confidence score >0.40 was used to screen the PPI pairs. On this basis, Cytoscape software was applied to construct the PPI network topology. The CytoHubba is a plug-in tool for Cytoscape to calculate the Degree of network topology.

MiRNAs associated with hub genes

The top 9 hub genes were mapped to corresponding miRNAs using NetworkAnalyst 3.0 (*https://www.networkanalyst.ca/*), a visual online platform which helps in finding miRNA-gene interactions in Gene Regulatory Networks. The miRNAs having degree cutoff value=1.0 were found for each of hub genes. Lastly, these hub genes and miRNA were plotted via Cytoscape 3.7.2.

Results

Sepsis DEGs identification

Three datasets gene expression profiles (GSE25504, GSE26378, and GSE26440) were retrieved from those present in the GEO database including both healthy and sepsis samples. Among them, GSE25504 included 35 healthy samples and 28 sepsis samples, GSE26378 included 21 healthy samples and 82 sepsis samples, and GSE26440 included 32 healthy samples and 78 sepsis samples. According to the criteria of $P<0.05$ and $|\log FC| \geq 1.0$, 600 DEGs in total were obtained from GSE25504, containing 403 upregulated genes and 197 downregulated genes. The 1700 DEGs were obtained when the gene chip GSE26378 was filtered. Among them, 1065 genes were upregulated, and 635 genes were downregulated. In GSE26440 dataset, 1883 DEGs were obtained, 906 of which were upregulated and 977 of which were downregulated. All DEGs were compared between normal controls and sepsis samples. The gene expression profile of each 3 of DEGs containing 2 sets of sample data is shown in Figure 1.

These genes were further filtered and the Venn diagrams representing these gene were plotted. As presented in Figure 2, 221 DEGs were found to be remarkably differentially expressed among the 3 groups, with 160 upregulated genes and 61 downregulated genes (Table 2).

Functional classification and pathway enrichment of DEGs

The major GO functional terms of the DEGs, including biological process (BP), molecular function (MF), cellular component (CC) ontologies, are illustrated as Figure 3. The top 5 significant GO-BP terms for DEGs were principally associated with neutrophil degranulation, neutrophil activation involved in immune response, neutrophil mediated immunity, membrane raft assembly, and complement receptor mediated signaling pathway (Figure 3A, Supplementary Table 1). The result showed that DEGs were primarily enriched in GO-MF, including the activities of complement receptor, phosphofructokinase activity, interleukin-1 receptor binding, C-C chemokine binding, and UDP-glucosyltransferase activity (Figure 3B, Supplementary Table 2). The analysis of GO-CC indicated that DEGs significantly enriched in specific granule, tertiary granule, T cell receptor complex, tertiary granule membrane, and specific granule lumen (Figure 3C, Supplementary Table 3). These results suggest that leukocyte degranulation, leukocyte mediated immunity, and leukocyte activation involved in immune

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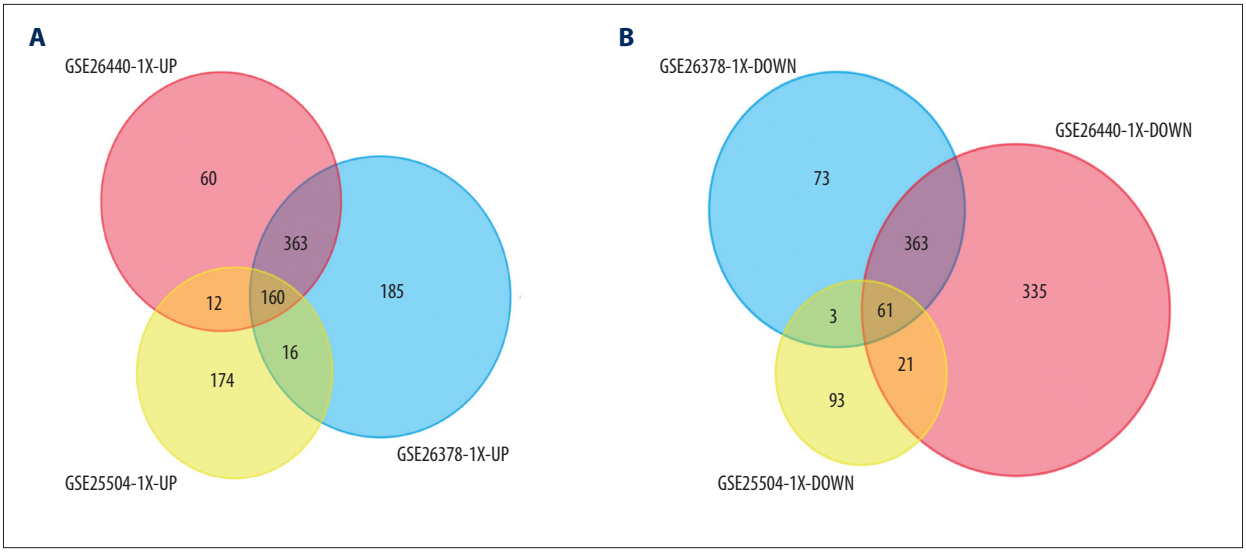

Figure 2. Venn diagrams showing the overlaps of numbers of differential expression genes (DEGs) between 3 selected Gene Expression Omnibus (GEO) datasets. (**A, B**) illustrate overlap of upregulated and downregulated genes in GSE25504, GSE26378, and GSE26440 dataset, respectively.

Table 2. Screening DEGs in pediatric sepsis patients by integrated microarray.

DEGs	Gene terms
Upregulated	MMP8 CD177 OLFM4 ARG1 MCEMP1 ANXA3 HP RETN LTF ANKRD22 MMP9 LCN2 VNN1 GPR84 CEACAM1 S100A12 CA4 CYSTM1 CLEC4D SOCS3 TCN1 RGL4 GYG1 PFKFB3 FCGR1B S100P IRAK3 MGAM LILRA5 IL18R1 LRG1 ADGRG3 DYSF ALPL PFKFB2 HK3 CKAP4 PGLYRP1 CD163 ZDHHC19 PADI4 TLR5 PGD NLRC4 SERPINB1 SLC2A3 GADD45A KIF1B ACSL1 ADM BCL6 SORT1 PROK2 STOM SAMSN1 PSTPIP2 PYGL B4GALT5 FCER1G ACER3 C3AR1 CST7 MKNK1 RNASE2 TNFAIP6 IL1RN SIPA1L2 THBS1 FKBP5 IL18RAP SLC22A4 OSCAR PLBD1 CDA FLOT1 SIGLEC5 FAR2 UGCG PGS1 SERPINA1 FAM129A ELANE DEFA4 TSPO ITGAM LILRA3 NCF4 CD55 BASP1 ORM1 ETS2 SIRPA FLOT2 ATP9A MARCKS TLR8 RAB31 FFAR2 CEBPD CCR1 PLIN3 AQP9 CEACAM6 C5AR1 FPR2 CTSD MPO SLC25A37 SEMA4A AGTRAP STXBP2 MSRB1 TRIB1 ALOX5AP HCK TIMP1 GK FGR SPI1 RHAG ADGRE1 MMP25 CD14 CSF3R LILRB3 CREB5 GCA ALPK1 GRN KCNJ2 FPR1 NBEAL2 NAMPT PRKCD CLEC1B STX3 MTF1 NFE2 CEBPB P2RX1 UBE2H ZYX IDI1 FES DENND3 RNF24 G6PD VPS9D1 RALB VNN2 ASAP1 MS4A3 CTSA GRINA GNA15 STX11 BAZ1A SHKBP1 RHOG IL1B
Downregulated	CD6 GIMAP1 FCRLA KDM2B PIK3IP1 CD8A SKAP1 PCED1B BCAS4 RPL22 PKIA LEF1 DYRK2 SPOCK2 NMT2 ETS1 LDHB CLEC2D PRKCQ-AS1 CD96 ATM FAM102A ITM2A MAL CD3G CD27 CCR7 C12orf57 AUTS2 HLA-DMB PAQR8 RFTN1 EVL PLEKHA1 CLC IL7R PRKCH PASK CD3D FCMR FAM117B CCL5 TRAF5 THEMIS RNF125 ABLIM1 CD2 BCL11B GPR183 RASGRP1 SAMD3 GZMK CD247 NELL2 GZMA TRAT1 GPR18 SH2D1A LRRN3 KLRB1 KLRF1

DEGs – differentially expressed genes.

response are the most significantly enriched functional terms for GO-BP, GO-MF, and GO-CC. The top 5 significant KEGG pathways of DEGs were enriched in human hematopoietic cell lineage, *Staphylococcus aureus* infection, starch and sucrose metabolism, osteoclast differentiation, and tumor necrosis factor (TNF) signaling pathway (Figure 3D, Supplementary Table 4).

PPI network construction and hub genes identification

On the basis of STRING database, the PPI analysis of DEGs were performed and visualized by Cytoscape as presented in Figure 4. The top 9 genes, including ITGAM, TLR8, IL1B, MMP9, MPO, FPR2, ELANE, SPI1, and C3AR1, were taken to as potential hub genes based on the node degree score generated via Cytoscape. These 9 hub genes are presented in Figure 5. The result shows that the integrin alpha m (ITGAM, degree 66) is the most significant gene, followed by Toll-like receptors (TLR8,

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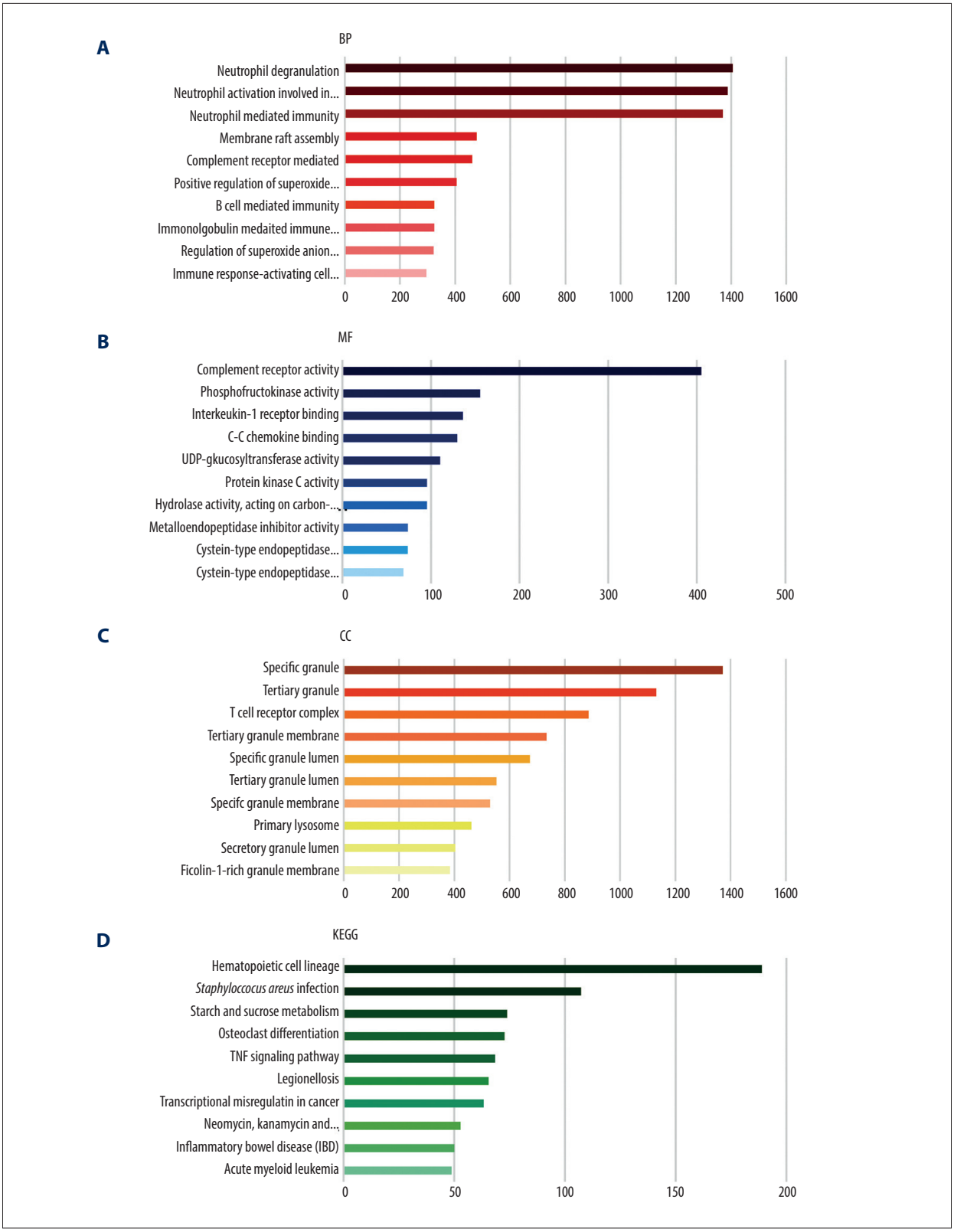

Figure 3. Gene Ontology (GO) functional and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of differential expression genes (DEGs). GO functional analysis showing enrichment of DEGs in (**A**) biological process, (**B**) molecular function, and (**C**) cellular component. (**D**) KEGG pathway enrichment analysis of DEGs.

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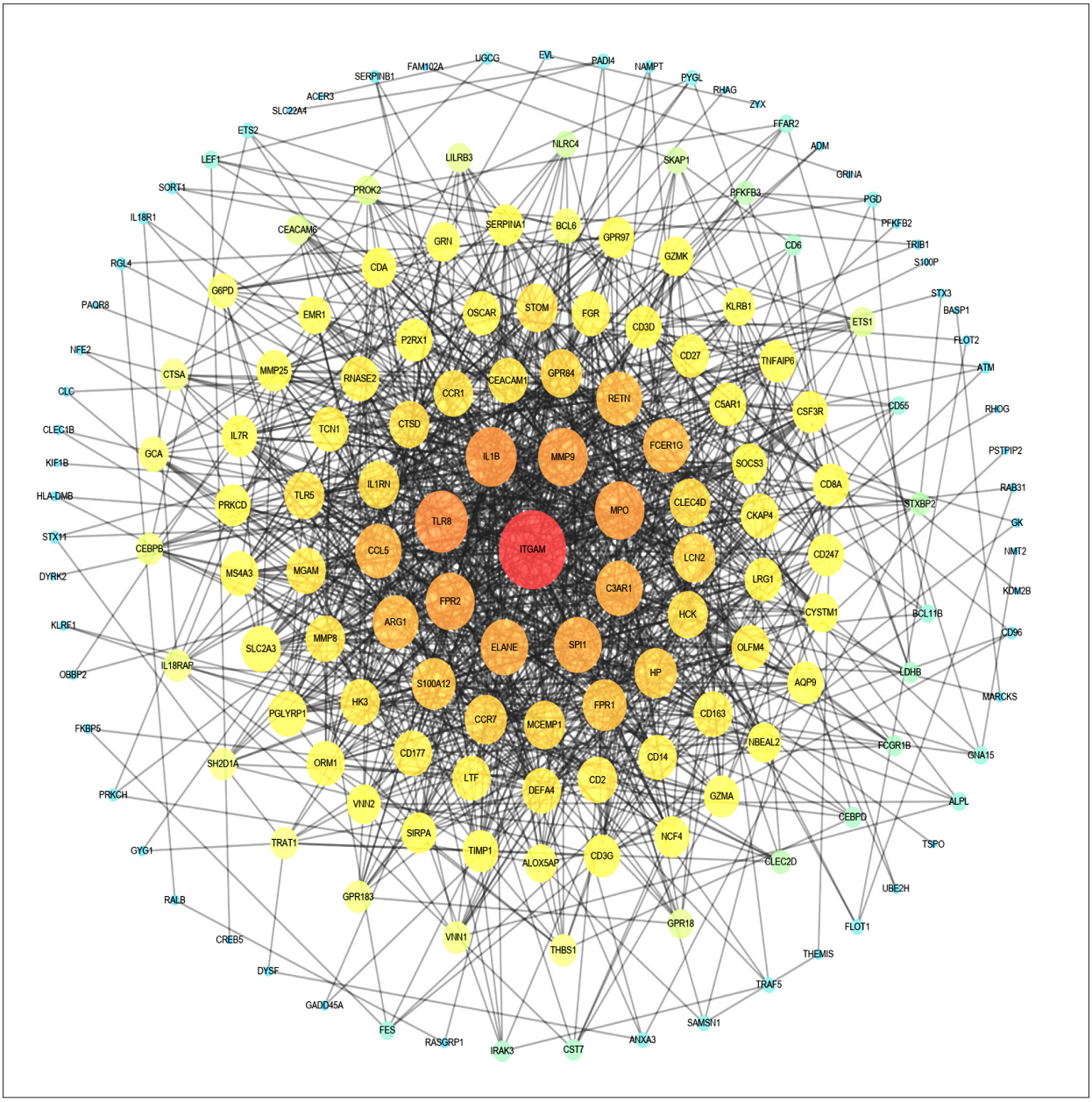

Figure 4. Protein–protein interaction network of 160 upregulated and 61 downregulated genes were analyzed using Cytoscape software. The network includes 219 nodes and 1062 edges. The edges between 2 nodes represent the gene-gene interactions. The size and color of the nodes corresponding to each gene were determined according to the degree of interaction. Color gradients represent the variation of the degrees of each gene from high (red) to low (blue). The closer to the red node, the higher connectivity between 2 nodes.

degree 43), interleukin 1 beta (IL1β, degree 40), matrix metalloproteinases-9 (MMP9, degree 38), bone marrow peroxidase (MPO, degree 38), formyl peptide receptor 2 (FPR2, degree 37), neutrophil elastic enzyme (ELANE, degree 35), transcription factors pu.1 (SPI1, degree 35) 1, and complement component 3a receptor 1 (C3AR1, degree 34).

Integrated miRNA/gene regulatory networks

MiRNAs are known to regulate targeted genes transcription inhibition or abrogate protein translation. The significantly differential miRNAs and genes regulatory networks was constructed using Cytoscape software. The target miRNAs were predicted based on NetworkAnalyst databases. The top 9 DEGs and their corresponding regulatory miRNAs molecules

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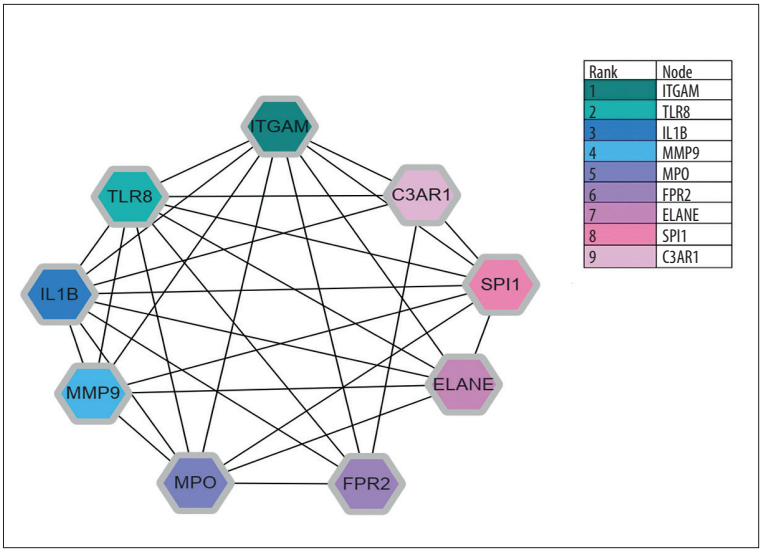

Figure 5. Protein–protein interaction network for the top 9 hub genes. Node color indicates the number of degrees. The top 9 ranked hub genes are depicted using a pseudocolor scale. Green color stands for highest degree, and pink color represents lowest degree.

are shown in Figure 6. For instance, among 9 DEGs, ITGAM, IL1B, and MMP9 could be predicted as common targets of 2 miRNAs (has-miR-204-5p and has-miR-211-5p). Two miRNAs (has-miR-590-5p and has-miR-21-5p) could be bind to 3 target genes (ITGAM, IL1B, and C3AR1). However, these findings need further validation.

Discussion

The pathogenesis of sepsis is multifactorial, with environmental and genetic factors interacting to produce some pathological features. In current investigation, 221 DEGs in total were identified, consisting of 160 upregulated genes and 61 downregulated genes. The results of GO functional classification indicated that the DEGs were mainly enriched in leukocyte degranulation, leukocyte mediated immunity, and leukocyte activation involved in immune response. In the PPI network of DEGs, 9 (ITGAM, TLR8, IL1β, MMP9, MPO, FPR2, ELANE, SPI1, and C3AR1) out of 221 genes had high degrees. These 9 genes were all upregulated in pediatric patients with sepsis. Most of these 9 hub genes have been shown to have key effects on leukocyte infiltration, and activation.

ITGAM (namely CD11b) regulates activation, adhesion, and migration of leucocytes from blood to the sites of injury [19,20]. Previous data by others suggested that increased expression of ITGAM on polymorphonuclear neutrophils (PMN) was correlated with the reduced survival of sepsis [21]. Blocking of ITGAM has been shown to significantly inhibit LPS-induced endotoxin shock and microbial sepsis [22].

TLR8, a member of Toll-like receptors (TLRs) family, is well known for its capacity to sense single stranded RNA (ssRNA) from viruses such as hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and influenza virus. More recently, some investigations have confirmed that TLR8-mediated RNA recognition plays a vital role in sensing of *S. aureus*, *Escherichia coli*, and *Borrelia burgdorferi* [23–25]. Bacterial infection-driven cell activation was abrogated by TLR8 inhibition and implied an anti-inflammatory potential of TLR8 blockage in sepsis [24]. However, whether TLR8 can be served as a biomarker for sepsis needs to be investigated further.

It has been established that IL1β has a powerful proinflammatory effect on host defense against some microbial pathogens [26]. It has been known that the IL-1 pathway regulates inflammation, angiogenesis, hematopoiesis, and cognition [27]. A clinal trial done in 2016 showed that the use of IL1 receptor inhibition was related to significant improvement of the 28-day survival of patients with sepsis [28]. Thus, IL1β may also contribute to the sepsis development.

MMP9 plays a vital role in encouraging inflammation and inhibiting platelet aggregation [29]. Previous studies have shown that the MMP9 levels in septic patients were significantly higher than that in healthy controls [30–32]. In further study, MMP9 expression levels was evaluated as prognostic biomarkers of sepsis, further indicating the potential for MMP9 in the pathophysiology of sepsis. MMP9 gene level disorders may participate in the pathophysiological process of sepsis and may act as a new molecular target for pediatric patients with sepsis diagnosis and prognosis.

Myeloperoxidase (MPO) is a sign of neutrophils infiltration and a sign of oxidative stress [33]. During sepsis, the production of MPO outpaces the body's antioxidant defenses. This imbalance can lead to direct mitochondrial damages, which has been reported to result in sepsis-mediated organ failure [34,35].

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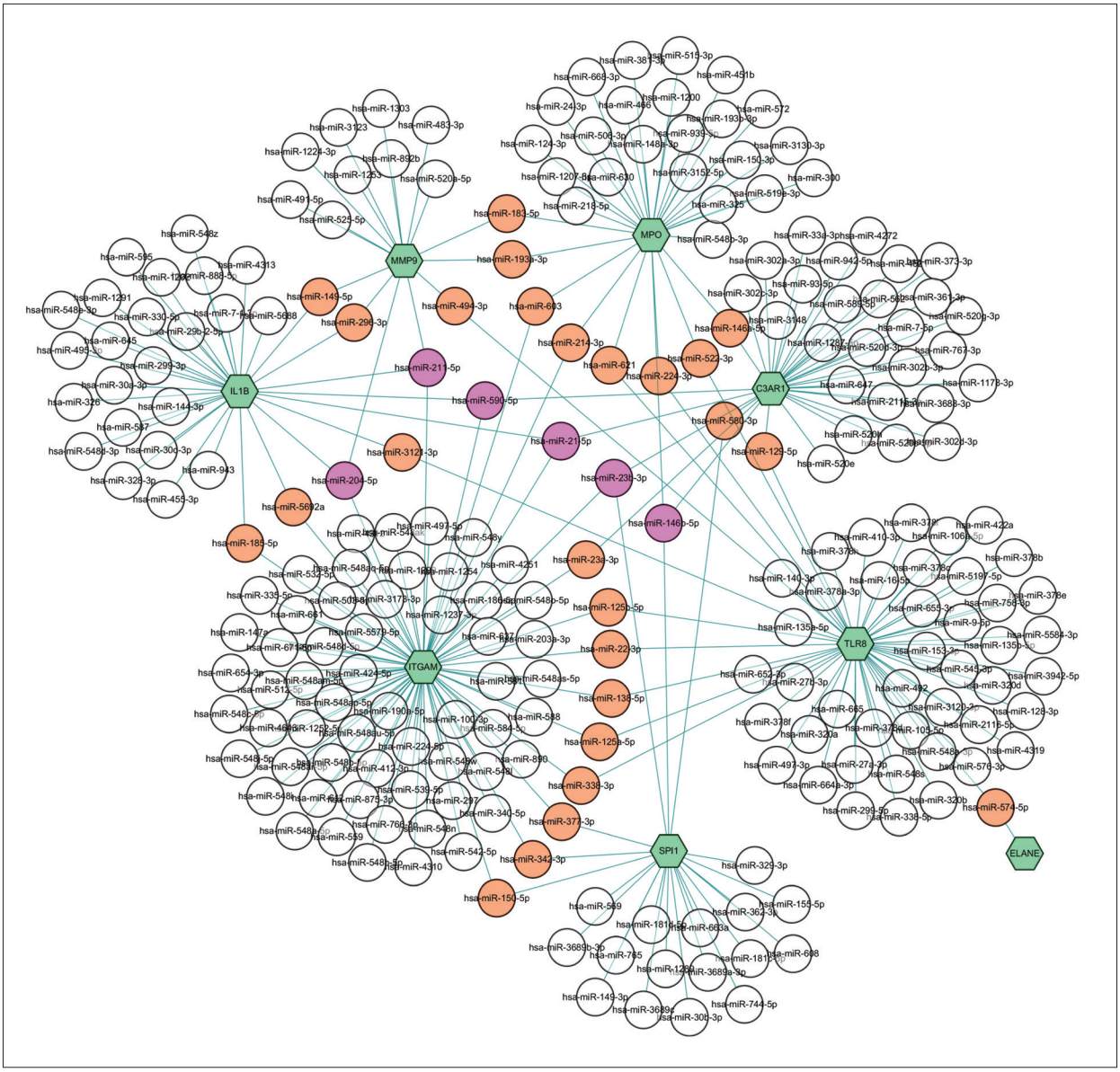

Figure 6. Integrated miRNA-DEGs networks for the top 9 hub genes. Green hexagons represent 9 hub genes. White circles represent miRNA which has low connectivity with hub genes. Red circles represent miRNA which has moderate connectivity with hub genes, and purple circles represent miRNA which has high connectivity with hub genes. miRNA-DEG – microRNA-differential expression genes.

Furthermore, other observations suggest that decreasing oxidative stress can serve as a safeguard strategy for mitochondria during sepsis [36,37]. MPO may therefore be a potentially new treatment target for pediatric sepsis.

Lipoxin A4 (LXA4), a kind of eicosanoid, is generated from arachidonic acid by sequential action of lipoxygenases [38]. Accumulating evidence have shown that activation of LXA4 receptor (ALX)/formyl peptide receptor 2 (FPR2) is essential in experimental sepsis models [39,40]. Additionally, ALX/FPR2 deficiency in mice abrogates bacterial clearance and provokes the host response in polymicrobial sepsis [41], which implies that ALX/FPR2 regulates anti-inflammatory reactions and may be a potential treatment option for pediatric sepsis.

Elastase neutrophil expressed (ELANE) regulates the LPS-induced response during injury and infection [42]. A recent study showed that ELANE participated in the maturation of neutrophil granulocytes [43].These findings suggest that the ELANE gene may play a key role in the sepsis development.

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Salmonella pathogenicity island 1 (SPI1/PU.1) is a pivotal regulator of myeloid lineage specification during hematopoiesis in mammals. Zhang and colleagues confirmed that SPI1 activation was the main cause of bone marrow suppression during sepsis [44]. Moreover, Karpurapu et al. have shown that the attenuated lung inflammation and myeloperoxidase activity were associated with favorable survival in LPS-challenged PU.1-deficient mice [45]. These results imply that suppressive SPI1 may provide a new complementary treatment option against sepsis.

C3a/C3aR axis is considered as an important pathway mediating inflammatory responses [46]. C3aR-deficient mice exhibit an increased lethality to endotoxin shock, which implied that C3aR act as a protective anti-inflammatory role in endotoxin-shock [47]. Napier and colleagues have reported that C3aR expression is dramatically enhanced in patients with severe sepsis [48]. Therefore, C3aR may contribute to endotoxemia severity and disease outcome, and may be used as an alternative therapeutic target for sepsis.

MiRNAs are involved in the regulating gene expression by degrading their target genes and weaken their translations. After reviewing previous studies, we found that numerous research studies in the field of sepsis have investigated the interconnection between miRNAs and hub genes. For instance, Li and associates indicated that downregulation of miR-204/miR-211 could result in candidemia-induced kidney injuries [49]. MiR-590 contributed to sepsis-induced acute kidney injury (AKI) expression to abrogate translation of the target genes [50]. MiR-21 has been shown to increase more than 30-fold in sepsis [51]. Besides, Zhang et al. recently reported that inhibition of miR-23b could be beneficial for polymicrobial sepsis-induced cardiac dysfunction [52]. Whereas, miR-146b protected against sepsis-induced mice myocardial injury [53]. Our present results indicated that several miRNAs, including miR-204, miR-211, miR-590, miR-21, miR-23b, and miR-146b, may play a pivotal role in pediatric sepsis. However, the functions of miR-204, miR-211, miR-590, miR-21, miR-23b, and miR-146b in pediatric sepsis need to be further defined. Additionally, research that focuses on the genes and miRNAs in pediatric sepsis are still limited.

It is evident that gene-miRNA regulatory networks greatly contribute to the pathophysiological process of pediatric sepsis, a finding that can help us better understand the mechanisms pediatric sepsis and provide effective and novel therapeutic strategies for pediatric sepsis. Nonetheless, our study was not without limitations, such as 1) only the top 9 hub genes was involved in our current study; 2) lack of research on detailed molecular mechanisms that the hub genes and miRNAs regulate in pediatric sepsis; 3) lack of research of some function studies about hub genes and miRNAs in the constructed networks. Even so, the results of this study remain meaningful, as these hub genes and miRNAs may provide more potential molecular biomarkers, and may improve early diagnosis, therapy, and prognosis of pediatric sepsis patients in the future.

Conclusions

Our findings suggested that compared with the healthy control group, the expressions of ITGAM, TLR8, IL1β, MMP9, MPO, FPR2, ELANE, SPI1, and C3AR1 in patients with sepsis were significantly upregulated, which may have an important influence on the pathophysiological mechanism of sepsis. Some potential target miRNAs (has-miR-204-5p, has-miR-211-5p, has-miR-590-5p, and has-miR-21-5p) were also predicted. Identification of these genes and miRNAs may contribute to the development of early diagnostic strategies, prognostic markers, and therapeutic targets for sepsis. However, experimental research is still necessary to validate the functions of these molecules in sepsis.

Conflicts of interest

None.

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Supplementary Data

Supplementary Table 1. GO biological process terms for DEGs between the control and pediatric sepsis groups.

Index	Name	P-value	Adjusted P-value	Odds ratio	Combined score
1	Neutrophil degranulation (GO: 0043312)	3.277e-51	1.672e-47	12.09	1405.58
2	Neutrophil activation involved in immune response (GO: 0002283)	5.617e-51	1.433e-47	11.99	1387.48
3	Neutrophil mediated immunity (GO: 0002446)	9.578e-51	1.629e-47	11.89	1369.74
4	Membrane raft assembly (GO: 0001765)	0.00002597	0.004734	45.25	477.76
5	Complement receptor mediated signaling pathway (GO: 0002430)	0.000002892	0.001342	36.20	461.67
6	Positive regulation of superoxide anion generation (GO: 0032930)	0.000004505	0.001642	32.91	405.11
7	Immunoglobulin mediated immune response (GO: 0016064)	0.00007155	0.01074	33.94	323.93
8	B cell mediated immunity (GO: 0019724)	0.00007155	0.01106	33.94	323.93
9	Regulation of superoxide anion generation (GO: 0032928)	0.000009593	0.002577	27.85	321.74
10	Immune response-activating cell surface receptor signaling pathway (GO: 0002429)	0.000002132	0.001209	22.62	295.44

Supplementary Table 2. GO molecular function terms for DEGs between the control and pediatric sepsis groups.

Index	Name	P-value	Adjusted P-value	Odds ratio	Combined score
1	Complement receptor activity (GO: 0004875)	0.000004505	0.005186	32.91	405.11
2	Phosphofructokinase activity (GO: 0008443)	0.002461	0.3147	25.86	155.33
3	Interleukin-1 receptor binding (GO: 0005149)	0.0005490	0.1580	18.10	135.88
4	C-C chemokine binding (GO: 0019957)	0.003257	0.3124	22.62	129.57
5	UDP-glucosyltransferase activity (GO: 0035251)	0.004158	0.3190	20.11	110.26
6	Hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in linear amidines (GO: 0016813)	0.005159	0.3493	18.10	95.33
7	Protein kinase C activity (GO: 0004697)	0.005159	0.3712	18.10	95.33
8	Metalloendopeptidase inhibitor activity (GO: 0008191)	0.007458	0.4518	15.08	73.88
9	Cysteine-type endopeptidase inhibitor activity (GO: 0004869)	0.0002446	0.09383	8.87	73.78
10	Cysteine-type endopeptidase inhibitor activity involved in apoptotic process (GO: 0043027)	0.002270	0.3265	11.31	68.87

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Supplementary Table 3. GO cellular component terms for DEGs between the control and pediatric sepsis groups.

Index	Name	P-value	Adjusted P-value	Odds ratio	Combined score
1	Specific granule (GO: 0042581)	1.250e-32	5.575e-30	18.67	1371.13
2	Tertiary granule (GO: 0070820)	1.939e-29	4.324e-27	17.11	1130.94
3	T cell receptor complex (GO: 0042101)	1.923e-10	7.795e-9	39.59	885.78
4	Tertiary granule membrane (GO: 0070821)	8.636e-17	9.629e-15	19.84	733.66
5	Specific granule lumen (GO: 0035580)	4.868e-15	3.618e-13	20.43	673.46
6	Tertiary granule lumen (GO: 1904724)	7.091e-13	3.514e-11	19.74	552.36
7	Specific granule membrane (GO: 0035579)	3.583e-15	3.196e-13	15.91	529.26
8	Primary lysosome (GO: 0005766)	0.000002892	0.00009213	36.20	461.67
9	Secretory granule lumen (GO: 0034774)	1.576e-20	2.343e-18	8.85	403.53
10	Ficolin-1-rich granule membrane (GO: 0101003)	6.019e-11	2.684e-9	16.32	384.05

Supplementary Table 4. KEGG pathway enrichment terms for DEGs between the control and pediatric sepsis groups.

Index	Name	P-value	Adjusted P-value	Odds ratio	Combined score
1	Hematopoietic cell lineage	1.011e-8	0.000003115	10.26	188.93
2	*Staphylococcus aureus* infection	0.00001001	0.001028	9.32	107.25
3	Starch and sucrose metabolism	0.0006476	0.02849	10.06	73.83
4	Osteoclast differentiation	0.00001197	0.0009217	6.41	72.68
5	TNF signaling pathway	0.00003052	0.001880	6.58	68.43
6	Legionellosis	0.0003494	0.01794	8.23	65.48
7	Transcriptional misregulation in cancer	0.000007366	0.001134	5.35	63.25
8	Neomycin, kanamycin and gentamicin biosynthesis	0.05405	0.3542	18.10	52.81
9	Inflammatory bowel disease (IBD)	0.0007588	0.02597	6.96	50.01
10	Acute myeloid leukemia	0.0008138	0.02279	6.86	48.77

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