Bioinformatic Identification of Hub Genes and Biological Pathways for Sepsis-Associated Acute Lung Injury

Chao Zhang

Chongqing Medical University Pediatric College: Chongqing Medical University Affiliated Children's Hospital

Feng Xu (✉ xufengmeddoctor@163.com)

Chongqing Medical University <https://orcid.org/0000-0002-1122-0581>

Fang Fang

Chongqing Medical University

Research

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Abstract

Background: Sepsis-associated acute lung injury (ALI) is a potentially lethal complication associated with a poor prognosis and high mortality worldwide, especially in the outbreak of COVID-19. However, the fundamental mechanisms of this complication were still not fully elucidated. Thus, we conducted this study to identify hub genes and biological pathways of sepsis-associated ALI, mainly focus on two pathways of LPS and HMGB1.

Methods: Gene expression profile GSE3037 were downloaded from Gene Expression Omnibus (GEO) database, including 8 patients with sepsis-induced acute lung injury, with 8 unstimulated blood neutrophils, 8 LPS- induced neutrophils and 8 HMGB1-induced neutrophils. Differentially expressed genes (DEGs) identifications, Gene Ontology (GO) function analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis, Gene Set Enrichment Analysis (GSEA) and protein-protein interaction (PPI) network constructions were performed to obtain hub genes and relevant biological pathways.

Results: We identified 534 and 317 DEGs for LPS- and HMGB1-induced ALI, respectively. The biological pathways involved in LPS- and HMGB1-induced ALI were also identified accordingly. By PPI network analysis, we found that ten hub genes for LPS-induced ALI (CXCL8, TNF, IL6, IL1B, ICAM1, CXCL1, CXCL2, IL1A, IL1RN and CXCL3) and another ten hub genes for HMGB1-induced ALI (CCL20, CXCL2, CXCL1, CCL4, CXCL3, CXCL9, CCL21, CXCR6, KNG1 and SST). Furthermore, by combining analysis, the results revealed that genes of TNF, CCL20, IL1B, NFKBIA, CCL4, PTGS2, TNFAIP3, CXCL2, CXCL1 and CXCL3 were potential biomarkers for sepsis-associated ALI.

Conclusions: Our study revealed that ten hub genes associated with sepsis-induced ALI were TNF, CCL20, IL1B, NFKBIA, CCL4, PTGS2, TNFAIP3, CXCL2, CXCL1 and CXCL3, which may serve as genetic biomarkers and be further verified in prospective experimental trials.

Background

Sepsis is a potentially lethal complication associated with a poor prognosis and high mortality [1]. It is estimated that nineteen millions of patients affected by sepsis worldwide annually, and the actual mortality of sepsis have unacceptably reached as high as 32.6%![2] Meanwhile, sepsis is often complicated with acute lung injury (ALI), which is manifested as acute respiratory distress syndrome (ARDS), even as subsequent multiple organ dysfunction syndrome (MODS), especially in the situation of COVID-19 infection [3]. ARDS, as well as MODS, is considered as a milestone that predict poorer prognosis and higher mortality. Unfortunately, treatments of sepsis-induced acute lung injury are still limited [4]. Moreover, mechanisms of this disease have not been fully elucidated. Thus, it is critically important to investigate the mechanism of sepsis induced ALI to find out novel therapeutic targets.

The role of lipopolysaccharide (LPS) and high mobility group box-1 (HMGB1) in sepsis-associated ALI received more and more concerns in recent years. Several studies were conducted to explore relevant

pathways for LPS and HMGB1, alone or together, that cause or aggravate sepsis-associated ALI [5–8]. However, there are still a lack of bioinformatics evidence exploring the potential hub genes and biological pathways for LPS- and HMGB1- induced sepsis-associated ALI. To fill in the gap of this field, we conducted the bioinformatics analysis by extracting GSE3037 datasets from GEO database, attempting to identify the hub genes and pathways involving in sepsis-associated ALI, mainly focus on these two pathways of LPS and HMGB1.

Materials And Methods

Sepsis Induced ALI Datasets

Expression profile data of GSE3037 was extracted from Gene Expression Omnibus (GEO) database via microarray GPL96 platform. GSE3037 datasets include 8 patients with sepsis-induced ALI. The baseline data of these patients were listed in Table 1. Blood neutrophils of these eight patients were cultured with LPS, cultured with HMGB1 or left unstimulated. Therefore, 8 unstimulated blood neutrophils samples (GSM66867-GSM66874), 8 HMGB1-induced samples (GSM66875-GSM66882) and 8 LPS-induced samples (GSM66883-GSM66890) were collected for further analysis.

Table 1
Baseline data of extracted sepsis-induced acute lung injury patients

Sample no.	sex	age	Site of infection	Sepsis shock at ICU?
1	Male	66	Urosepsis	No
2	Male	34	Pneumonia	Yes
3	Male	43	Pneumonia	No
4	Male	44	Cellulitis	No
5	Male	41	Pneumonia	No
6	Female	42	Pneumonia	Yes
7	Male	43	Pneumonia	Yes
8	Female	50	Pneumonia	No

Identifications of DEGs

Gene expression matrix files were extracted and further analyzed, referring to “hgu133a.db” annotations, which translated raw microarray data to gene expression data. Differential expression analysis was performed by “limma” [9] with criteria for adjust *P*-value < 0.1 and |log fold change (FC)|>1.2.

Enrichment analysis of DEGs

1. GO function and KEGG pathway analysis of upregulated and downregulated DEGs were performed by *metascape* (<https://metascape.org/>) [10] (minimum overlap:3, *P*-value cut-off:0.01 and minimum

enrichment:1.5). The further chordal graph and heatmap analysis was conducted by *GOpilot* software [11].

GSEA analysis

Gene Set Enrichment Analysis (GSEA) was performed to study the enrichment of a gene set in a large number of genes, which often consists of multiple gene sets [12]. We chose “h.all.v7.2.symbols.gmt” for reference and set times of permutation as 1000 and type of permutation as “gene set”. The enrichment was considered as significant when $FDR < 0.05$. Meanwhile, Normalized Enrichment Score (NES) was applied to assess the extent of enrichment.

PPI network construction and modular analysis

Search Tool for the Retrieval of Interacting Genes (STRING) online database (<https://string-db.org/>) [13] was performed to evaluate of protein–protein interaction (PPI). *Cytoscape* [14] and Molecular Complex Detection (MCODE; cut-off: scores > 3 and nodes > 4) was used to finalize module screening. Top ten hub genes were identified by further analysis via *CytoHubba*.

Results

Identification of DEGs for LPS- and HMGB1-induced pathways

Gene expression value in LPS and HMGB1-induced neutrophils samples was similar with the value in unstimulated neutrophils samples, respectively (Fig. 1). For LPS-induced ALI, volcano plots and heatmap revealed that a total of 534 DEGs were identified, with 296 up-regulated DEGs and 238 down-regulated DEGs (Fig. 2). For HMGB1-induced ALI, the results revealed that totally 317 DEGs were identified, with 158 up-regulated DEGs and 159 down-regulated DEGs (Fig. 3).

GO function analysis

GO function analysis revealed that relevant biological process for LPS-induced ALI included cellular response to lipid, neutrophil chemotaxis, regulation of DNA-binding transcription factor activity (Fig. 4A). For molecular function, receptor ligand activity, protein C-terminus binding and hormone activity (Fig. 4B) were obtained. Corresponding cellular components included endocytic vesicle membrane, transcription factor complex, peroxisome, etc. (Fig. 4C). Besides, for HMGB1-induced ALI, relevant biological process included response to chemokine, cellular response to lipopolysaccharide and fever generation (Fig. 5A). For molecular function, receptor ligand activity, protein kinase regulatory activity and cytokine binding (Fig. 5B) were identified. Relevant cellular components included endoplasmic reticulum lumen, extracellular matrix, Schaffer collateral CA1 synapse and microtubule organizing center part, etc. (Fig. 5C).

KEGG pathways analysis

For LPS-induced ALI, KEGG pathway analysis revealed that the significant enrichment pathways included IL-17 signaling pathway, Cytokine-cytokine receptor interaction, NF- κ B signaling pathway, etc. (Fig. 6). Meanwhile, for HMGB1-induced ALI, KEGG pathway analysis revealed that the significant enrichment pathways involved cytokine-cytokine receptor interaction, TNF signaling pathway and cancer pathways, etc. (Fig. 7).

GSEA analysis

For LPS-induced ALI, GSEA analysis showed the significant enrichment biological processes/states involved TNFA-signaling via NF- κ B, inflammatory response, apoptosis, ultraviolet response up, hypoxia and cholesterol homeostasis (Fig. 8). In addition, for HMGB1-induced ALI, GSEA analysis showed the significant enrichment biological processes/states involved TNFA-signaling via NF- κ B, inflammatory response, apoptosis, ultraviolet response, hypoxia and KRAS signaling up (Fig. 9).

PPI network construction and hub genes identification

For LPS-induced ALI, we identified 10 modules in PPI network (Fig. 10A-10B). Using CytoHubba, we identified 10 potential hub genes related to LPS-induced ALI, including CXCL8, TNF, IL6, IL1B, ICAM1, CXCL1, CXCL2, IL1A, IL1RN and CXCL3 (Fig. 10C). Meanwhile, for HMGB1-induced ALI, we also identified ten modules in PPI network (Fig. 11A-11B). We identified ten hub genes related to HMGB1-induced ALI, including CCL20, CXCL2, CXCL1, CCL4, CXCL3, CXCL9, CCL21, CXCR6, KNG1 and SST (Fig. 11C).

Combining analysis

We subsequently performed a combining analysis to extract the overlapping data between LPS-induced and HMGB1-induced ALI. Then a total of 88 DEGs were overlapping (Fig. 12A). By PPI network construction, notably, we found a significant module in PPI network (Fig. 12B-C). In this module, genes of TNF, CCL20, IL1B, NFKBIA, CCL4, PTGS2, TNFAIP3, CXCL2, CXCL1, CXCL3 were identified (Fig. 12D). In addition, we also performed GO function analysis, KEGG pathways analysis and GSEA analysis for combining DEGs, the results were not shown.

Discussion

The present study is the first study to conduct a comprehensive bioinformatics analysis to explore hub genes and biological pathways related to LPS and HMGB1 triggered sepsis-associated ALI. The present study identified 534 and 317 DEGs for LPS- and HMGB1-induced ALI, respectively. The biological pathways involved in LPS- and HMGB1-induced ALI were also demonstrated accordingly. By conducting PPI network analysis, we found that ten hub genes for LPS-induced ALI (CXCL8, TNF, IL6, IL1B, ICAM1, CXCL1, CXCL2, IL1A, IL1RN and CXCL3) and another ten hub genes for HMGB1-induced ALI (CCL20, CXCL2, CXCL1, CCL4, CXCL3, CXCL9, CCL21, CXCR6, KNG1 and SST). Notably, by combining analysis, the results revealed a module of genes TNF, CCL20, IL1B, NFKBIA, CCL4, PTGS2, TNFAIP3, CXCL2, CXCL1 and CXCL3 were potential biomarkers for sepsis-associated ALI.

To further explore the mechanisms of LPS-triggered sepsis-associated ALI, we searched the literatures for the functions of LPS relevant 10 genes (CXCL8, TNF, IL6, IL1B, ICAM1, CXCL1, CXCL2, IL1A, IL1RN and CXCL3). The functions of these ten genes were summarized in Table 2. The cellular components encoded by these 10 genes were all previously proved to be participated in the progression of inflammations in sepsis patients or animal models [15–24]. For chemokine ligand family, CXCL1, CXCL2, CXCL3 and CXCL8, the common functions are all chemoattractant for neutrophils [15, 20–21, 24]. And for interleukin family, IL1A, IL1B, ILRN and IL6, their common functions are rapid increasing in levels and evoking “cytokine storm” [17–18, 22–23]. For ICAM1, stimulated by LPS, resulted in increases in neutrophil-neutrophil adherence and aggregation [19]. And for TNF, the most common mediator of sepsis, elicit the release of inflammatory mediators [16]. LPS may stimulate these cellular components to aggravate the progression of sepsis-induced lung injury.

Table 2

Hub genes and their functions in inflammation of sepsis based on previous literatures (for LPS-induced ALI)

Hub genes	Cellular components	Reference	Functions mentioned in the literatures
CXCL8	Chemokine (C-X-C motif) ligand 8	<i>T M Cunha et al</i> ^[15]	Recruiting leukocytes from the blood into tissues during inflammation
TNF	Tumor necrosis factor	<i>Lucy K Reiss et al</i> ^[16]	Eliciting the release of inflammatory mediators, and mediator of sepsis itself
IL6	Interleukin 6	<i>Tanaka T et al</i> ^[17]	Excessive synthesis of IL-6 while fighting environmental stress leads to an acute severe systemic inflammatory response known as 'cytokine storm'
IL1B	Interleukin-1-B	<i>Varljen T et al</i> ^[18]	Proinflammatory mediator whose level is rapidly increased during infection
ICAM1	Intercellular-adhesion-molecule-1	<i>Ode Y et al</i> ^[19]	An increased expression of ICAM-1 on neutrophils after stimulation with LPS has been shown, which resulted in significant increases in neutrophil-neutrophil adherence and aggregation
CXCL1	Chemokine (C-X-C motif) ligand 1	<i>Paudel S et al</i> ^[20]	CXCL1 induces neutrophil influx during bacterial infections, in addition, it is a central player in host defense, granulopoiesis, and mobilization of neutrophils during sepsis.
CXCL2	Chemokine (C-X-C motif) ligand 2	<i>De Filippo et al</i> ^[21]	CXCL2 is secreted, especially by mast cells, monocytes, and macrophages at the site of inflammation, and is an important chemotactic for neutrophils during infections
IL1A	Interleukin-1-A	<i>Zhu W et al</i> ^[22]	IL-1 signaling via the type 1 IL-1 receptor leads to multiple inflammatory effects including vasodilation, increased vascular permeability
IL1RN	Interleukin-1-RN	<i>Meyer et al</i> ^[23]	C allele of a synonymous coding variant (rs315952-C) in IL1RN is associated with reduced risk for ARDS and increased plasma IL-1ra
CXCL3	Chemokine (C-X-C motif) ligand 3	<i>Rebollo J</i> ^[24]	CXCL3 has been shown to act as a chemoattractant for neutrophils to and for cerebellar progenitor cells

Besides, we also searched previous literatures and screened for the studies with the ten hub genes for HMGB1 triggered ALI. The cellular components encoded by these 10 genes were all previously proved to be participated in the progression of inflammations in sepsis patients or animal models. [20–21, 24, 25–31] For chemokine ligand family, CXCL1, CXCL2, CXCL3 and CXCL9, the common functions are all chemoattractant for neutrophils. [25, 28–29] For CC chemokine family, CCL4 plays key roles in

chemotaxis as well as enhancement of the inflammatory response, while CCL21 regulates leukocyte trafficking and homing to the lymph nodes. [26, 28] In addition, CCL20 is an early trigger of neutrophil cell recruitment and activation of T cells in the small intestine. [27] For CXCR6, it could mediate iNKT-cell accumulation, clustering and arrest within the liver in several inflammatory conditions. [29] For KNG1, it could accelerate the progress of cellular inflammation. [30] Besides, SST has a protective effect on intestinal barrier dysfunction through the suppression of NF- κ B. [31] HMGB1 may stimulate all these cellular biomarkers to aggravate the progression of sepsis-induced lung injury. These functions of ten hub genes for HMGB1-induced ALI were listed in Table 3.

Table 3

Hub genes and their functions in inflammation of sepsis based on previous literatures (for HMGB1-induced ALI)

Hub genes	Cellular components	Reference	Functions mentioned in the literatures
<i>CXCL9</i>	Chemokine (C-X-C motif) ligand 9	<i>Cavalcanti et al</i> [15]	CXCL9 induces the CXC chemokines which act preferentially on T and B lymphocytes and natural killer cells (NK)
<i>CCL4</i>	Chemokine (C-C motif) ligand 4	<i>Nowak et al</i> [16]	CCL4 is a member of the CC chemokine family, and is produced by numerous cell types including monocytes, neutrophils, T and B lymphocytes, NK cells, and dendritic cells. It plays a key role in chemotaxis as well as enhancement of the inflammatory response
<i>CCL20</i>	Chemokine (C-C motif) ligand 20	<i>Kitagawa Y et al</i> [17]	CCL20 has been identified as an early trigger of neutrophil cell recruitment and activation of T cells in the small intestine.
<i>CCL21</i>	Chemokine (C-C motif) ligand 21	<i>Castro R et al</i> [18]	The regulation of leukocyte trafficking and homing to the lymph nodes (LNs)
<i>CXCR6</i>	Chemokine (C-X-C motif) receptor 6	Young et al [19]	CXCR6 has been shown to mediate iNKT-cell accumulation, clustering and arrest within the liver in several inflammatory conditions
<i>CXCL1</i>	Chemokine (C-X-C motif) ligand 1	<i>Paudel S et al</i> [20]	CXCL1 induces neutrophil influx during bacterial infections, in addition, it is a central player in host defense, granulopoiesis, and mobilization of neutrophils during sepsis.
<i>CXCL2</i>	Chemokine (C-X-C motif) ligand 2	<i>De Filippo et al</i> [21]	CXCL2 is secreted, especially by mast cells, monocytes, and macrophages at the site of inflammation, and is an important chemotactic for neutrophils during infections
<i>KNG1</i>	Kininogen-1	<i>Hu Q et al</i> [22]	As a pro-inflammatory cytokine, KNG1 has been demonstrated to accelerate the progress of inflammation. Inhibition of KNG1 could relieve cellular inflammation
<i>SST</i>	Somatostatin	<i>Xu X</i> [23]	SST has a protective effect on intestinal barrier dysfunction through the suppression of NF- κ B
<i>CXCL3</i>	Chemokine (C-X-C motif) ligand 3	Rebollo J [24]	CXCL3 has been shown to act as a chemoattractant for neutrophils to and for cerebellar progenitor cells

Finally, we searched previous publications and screened for studies with the ten potential key genes for sepsis-associated ALI. As we described previously, TNF, CCL20, IL1B, CCL4, CXCL2, CXCL1, CXCL3 were listed in Table 2 and Table 3. However, NFKB1A, PTGS2 and TNFAIP3 were newly recognized in the combining analysis, as shown in Table 4.

Table 4

Hub genes and their functions in inflammation of sepsis based on previous literatures (for sepsis-associated ALI)

Hub genes	Reference	Functions mentioned in the literatures
<i>NFKB1A</i>	<i>Yenmis et al</i> [32]	NFKB1A have a genetic effect on the development of a variety of inflammatory diseases associated with altered immune response
<i>PTGS2</i>	<i>Li N et al</i> [16]	LPS increased the levels of ferroptotic markers involving prostaglandin endoperoxide synthase 2 (PTGS2)
<i>CCL20</i>	<i>Jae-Min Yuk et al</i> [17]	Toll-like receptor (TLR)-induced inflammation could be promoted by Tnfaip3 transcription and fine-tuning of metabolic reprogramming in macrophages

In spite that we identified several novel genes that may involve in the progression of sepsis-associated ALI, there are still several limitations in this study. Firstly, lack of experimental verification is the main drawback of this study. Further explorable studies in vivo or in animals should be conducted to investigate the weighted value of these hub genes. Secondly, due to limited sample size of neutrophils of sepsis patients, we failed to conduct a survival analysis based on these data. Nevertheless, our study still provided substantial implications for diagnosing and treating sepsis-induced ALI.

Conclusions

Bioinformatics results showed that ten hub genes of LPS-induced sepsis-associated ALI were CXCL8, TNF, IL6, IL1B, ICAM1, CXCL1, CXCL2, IL1A, IL1RN and CXCL3, while another ten hub genes of HMGB1-induced sepsis-associated are CCL20, CXCL2, CXCL1, CCL4, CXCL3, CXCL9, CCL21, CXCR6, KNG1 and SST. By combining analysis, we found that the key genes for sepsis-induced ALI are TNF, CCL20, IL1B, NFKBIA, CCL4, PTGS2, TNFAIP3, CXCL2, CXCL1 and CXCL3, which may serve as genetic biomarkers for diagnosing and treating sepsis-associated ALI. In addition, these findings should be further verified in subsequent experimental studies.

Abbreviations

ALI: acute lung injury; ARDS: acute respiratory distress syndrome; DEGs: differentially expressed genes; FC: fold change; GEO: gene expression omnibus; GO: gene ontology; GSEA: gene set enrichment analysis; HMGB1: high mobility group box-1; KEGG: kyoto encyclopedia of genes and genomes; LPS: lipopolysaccharide; MCODE: molecular complex detection; MODS: multiple organ dysfunction syndrome; NES: normalized enrichment score; PPI: protein-protein interaction; STRING: search tool for the retrieval of interacting genes.

Declarations

Ethical Approval and Consent to participate

Not applicable.

Consent for publication

All authors are consented and approve the publication of this article.

Availability of supporting data

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

CZ performed data analysis work and wrote the manuscript. FX designed the study and assisted in writing this manuscript. And FF edited this manuscript. All three authors fully read and approved the final version of manuscript.

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Authors' information

Chao Zhang^{1,2,3}, MD, PhD, Feng Xu^{1,2,3} MD, PhD, Fang Fang^{1,2,3} MD, PhD

¹ Department of Pediatric Intensive Care Unit, Children's Hospital of Chongqing Medical University, Chongqing, China

² Ministry of Education Key Laboratory of Child Development and Disorders, National Clinical Research Center for Child Health and Disorders (Chongqing), Chongqing, China

³ China International Science and Technology Cooperation base of Child Development and Critical Disorders, Chongqing Key Laboratory of Pediatrics, Chongqing, China

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Figures

Figure 1

Gene expression value in HMGB1-induced neutrophils samples (red color), LPS-induced neutrophils (blue color) and in unstimulated neutrophils (green color). a Before adjusting, b After adjusting.

Figure 2

Volcano plots (a) and heatmap (b) of identified DEGs of LPS-induced ALI. In volcano plots, the orange color plots represent up-regulated DEGs and blue color plots represent down-regulated DEGs.

Figure 3

Volcano plots (a) and heatmap (b) of identified DEGs of HMGB1-induced ALI. In volcano plots, the orange color plots represent up-regulated DEGs and blue color plots represent down-regulated DEGs.

Figure 4

GO function analysis revealed the relevant biological process (BP) (a), molecular function (MF) (b) and cellular components (CC) (c) for LPS-induced ALI.

Figure 5

GO function analysis revealed the relevant biological process (BP) (a), molecular function (MF) (b) and cellular components (CC) (c) for HMGB1-induced ALI.

Figure 6

Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis for LPS-induced ALI.

Figure 7

Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis for HMGB1-induced ALI.

Figure 8

GSEA analysis revealed of enrichment biological processes/states of LPS-induced ALI including TNFA-signaling via NF-kB (a), inflammatory response (b), apoptosis (c), ultraviolet response up (d), hypoxia(e) and cholesterol homeostasis (f).

Figure 9

GSEA analysis revealed of enrichment biological processes/states of HMGB1-induced ALI including TNFA-signaling via NF-kB (a), inflammatory response (b), apoptosis (c), ultraviolet response up (d), hypoxia(e) and KRAS-signaling (f).

Figure 10

PPI network construction: a. PPI network of LPS induced ALI, b. PPI network of LPS-induced ALI after clustering analysis, c. PPI network for top ten hub genes.

Figure 11

PPI network construction: a. PPI network of HMGB1-induced ALI, b. PPI network of HMGB1-induced ALI after clustering analysis, c. PPI network for top ten hub genes.

Figure 12

a Overlapping data of DEGs for both HMGB1-induced ALI and LPS-induced ALI, b PPI network for sepsis-associated ALI; c Module highlighted in the PPI network; d. Hub genes for sepsis-associated ALI.