Prediction of immune factors and signaling pathways in lung injury induced by LPS based on network analysis

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ABSTRACT

Objective: To construct a regulatory network involved in acute lung injury, so as to provide a new theoretical basis and research ideas for studying the relationship between inflammatory factors and immune proteins to collectively regulate the occurrence of acute lung injury.

Method: By using Meta-analysis, GO, KEGG and other methods notarized and constructed the regulatory network pathways of cytokine cascade and lung injury induced by LPS.

Results: The result of Meta-analysis showed that the correlation between CD14, TNF-α, IL-6 gene and acute lung injury was statistically significant. GO analysis and KEGG analysis showed that acute lung injury contained CD14, TNF-α, IL-6 and other involved factors in the induced process of LPS, these inflammatory factors and immune proteins jointly regulate the process of disease development.

Conclusion: CD14 receptor is an important receptor involved in mediating LPS-activated cells, and is a high-affinity LPS receptor. LPS stimulates inflammatory effector cells to bind to LPS receptor-CD14 to activate intracellular signal cascade. Direct or indirect involvement of pathogenic factors enable cytokine caused by induction form a particularly complex network of cytokine regulatory pathways, of which the inflammatory factors TNF-α and IL-6 are simultaneously involved in LPS-mediated and CD14-mediated cytokine cascades.

1. Introduction

Acute lung injury (ALI) is an inflammatory reaction produced by various pathogenic factors (such as infection, trauma, etc.) in lung tissue through direct or indirect action. Severe ALI is prone to develop into acute respiratory distress syndrome (ARDS) (Wang, 2014). Diffuse alveolar damage, pulmonary vascular endothelial cells (PVEC) and extensive alveolar epithelial damage are the main pathological features of ALI (Ma et al., 2013). With the people's deeper understanding of the regulation of acute lung injury on immune factors, more experimental studies have shown that CD3+, CD4+, CD8+, NK cells, B cells and other immune and inflammation-related factors play a very important role in the pathogenesis of the disease (Shi and Ren, 2013). The scholars at home and abroad tends to study the regulatory network of immune cytokines, and how immune cytokines play a regulatory role in lung injury, as the result, immune dysfunction has become a hot spot for medical researchers.

This study intended to explore the relationship between various immune cytokines such as CD3+, CD4+, CD8+, NK cells and B cells and lung injury through meta-analysis (meta-analysis), combined with bioinformatics methods such as GO and KEGG database, it systematically comprehensively analyzed the related regulatory networks of the above-mentioned immune cytokines in the development of lung injury, which provided research direction and important theoretical basis for further exploring the molecular mechanism of immune and inflammation of acute lung injury and its clinical diagnosis and treatment.

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2. Material method

2.1. Meta analysis

The first is the literature search work related to the study of acute lung injury. We searched for the keywords “acute lung injury”, “inflammation-related factors”, and “immune-related factors” in CNKI (the China Integrated Knowledge Resources Database) and the pubmed public database. According to conditions such as the year of research, completeness of result data and similarity of mechanism pathways were read and screened for the articles needed for this study.

Among them, the literature screening criteria were: (1) the content of the study was about the immune factors or inflammatory factors related to acute lung injury and should be in English or Chinese; (2) the type of literature was retrospective study; (3) in all the included literature, the standard method was ELISA test of plasma samples, the experimental group was the group with acute lung injury induced by lipopolysaccharide, the control group was in normal condition; (4) According to the literature results, the number of samples and the expression levels of the relevant factors of the experimental group and control group were obtained from the experiment, they were expressed in the form of mean ± standard deviation; (5) the research method was rigorous, the idea was clear and standard; (6) the literatures that were selected all were full text, and the language was in Chinese or English, only published literature were selected, all the data were obtained from original articles.

Exclusion criteria: (1) literature data is not examined by using standard methods; (2) reviews were excluded; (3) data cannot copy from the literature searched in previous step.

Meta-analysis of selected literature was performed by RevMan 5.3. Heterogeneity was analyzed by using the I² test of Q statistic. P > 0.05 was considered to have no significant heterogeneity between studies, the data was combined through a fixed effect model; if there was significant heterogeneity between studies (P < 0.05), the data was combined by a random effect model. For the measurement data, the SMD value was used as the effect statistic, and the effect index was expressed by the combined SMD value and the 95% confidence interval (95%CI). Z-test was performed on the combined statistic, P > 0.05 showed that the combined statistic of several studies was not statistically significant, P < 0.05 represented that the combined statistic was statistically significant.

2.2. GO analysis

The Gene Ontology Consortium is an online site for GO analysis. We performed GO ontology analysis on encoding related factors’ gene. On the home page of the Gene Ontology Consortium website, the searching keywords were “gene name” and “Homo sapiens”, then GO annotation analysis was conducted. After that, we focused on the genetic annotation information involved in the regulatory network of acute lung injury based on the correlation between the annotation information of GO and acute lung injury.

2.3. KEGG network analysis

The online website “KEGG PATHWAY Database” can analyze the regulatory network of genes. After entering the KEGG PATHWAY’s home page, the search conditions were: organism was “hsa”, key words was “gene name” (Wilby and Nasr, 2016). By searching the network pathway of the gene in inflammation and immune response, the regulatory network of the gene was regarded as a large network, we collected and organized the pathways and nodes involved in the regulation of each factor, and there was a cross between the pathways, so it was integrated into a primary regulatory network involving related factors of acute lung injury.

3. Results

3.1. Meta-analysis of factors involved in acute lung injury

3.1.1. Situation of literature screening

A total of 210 articles on acute lung injury-related factors were searched in the database, the methods section and results section of the literatures focused on the study of acute lung injury caused by lipopolysaccharide, and the research methods were consistent, the same measurement method was adopted to obtain the result data. Based on the above three aspects, we screened the articles for meta-analysis, including 6 articles on immune factor CD14 and acute lung injury; 7 articles on inflammatory factor TNF-α and acute lung injury; 5 articles on inflammatory factor IL-6 and acute lung injury. The results of the literature screening are shown in Tables 1, 2, and 3.

3.1.2. Meta-analysis results of CD14 correlation with acute lung injury

In this study, a meta-analysis of six literatures related to CD14 and acute lung injury was performed (Srivalli and Mishra, 2016). The results are shown in Fig. 1. It can be seen that the heterogeneity test result I² = 0%, indicating that there was no heterogeneity between the literatures, and a random effect model was adopted; the combined effect SMD value was 3.81, 95%CI was 2.87–4.74, the upper and lower limits of combined SMD value and 95%CI were all greater than 1, indicating that the correlation between CD14 gene and acute lung injury was statistically significant.

3.1.3. Meta-analysis results of the correlation between TNF-α and acute lung injury

We performed a meta-analysis of seven articles related to TNF-α and acute lung injury. The results of the analysis are shown in
Fig. 2. Among them, the heterogeneity test results was $P < 0.05$, indicating that there was heterogeneity among the literatures, the random effects model was used; combined effect SMD value was 4.36, 95%CI was 1.87–6.84, the upper and lower limits of combined SMD value and 95%CI all were higher than 1, representing that the correlation between TNF-$\alpha$ gene and acute lung injury was statistically significant.

3.1.4 Meta-analysis results of correlation between IL-6 and acute lung injury

This paper conducted a meta-analysis of five articles related to acute lung injury caused by IL-6. The results of the analysis are shown in Fig. 3. It showed that from the figure that the heterogeneity test results was $P < 0.05$, demonstrating that there is heterogeneity between the articles, a random effect model was
employed; the combined effect SMD value was 22.28, 95% CI was 12.2–32.36, and the upper and lower limits of combined SMD value and 95% CI were all greater than 1, indicating that the correlation between IL-6 gene and acute lung injury was statistically significant.

3.2. GO analysis of factors involved in acute lung injury

Functional annotation information of CD14, TNF-α and IL-6 was obtained by using GO online database (Rajiah et al., 2016). As shown in Table 4, the molecular function of the CD14 gene involves the binding of lipopolysaccharide and the activation of the opsin receptor, while biological processes include the toll-like receptor signaling pathway, the MyD88-dependent toll-like receptor pathway, and the positive regulation of interleukin-8 secretion. These functions are all involved in the regulation of immune response and are closely related to the occurrence of acute lung injury.

The functional annotation results of the TNF-α gene are shown in Table 5. The biological processes include lipopolysaccharide-regulated signaling pathway, ILk kinase or NF-kb signaling pathway, positive and negative regulation of interleukin-6, and the biosynthesis process of positive regulation of interleukin-8.

Table 4
GO function analysis of CD14 gene.

| Gene Accession | GO class                  | Ontology                  | Reference        |
|----------------|---------------------------|---------------------------|------------------|
| CD14 GO:0001530 | Lipopolysaccharide binding | molecular_function        | PMID:12594207    |
| GO:0022244     | Toll-like receptor signaling pathway | biological_process Reactome:R-HSA-168898 |
| GO:0028947     | Ossin receptor activity   | molecular_function        | PMID:2402637     |
| GO:002755      | MyD88-dependent toll-like receptor signaling pathway | biological_process Reactome:R-HSA-166058 |
| GO:002756      | MyD88-independent toll-like receptor signaling pathway | biological_process Reactome:R-HSA-166166 |
| GO:0007249     | I-kappaB kinase/NF-kappaB signaling | biological_process Reactome:R-HSA-937072 |
| GO:2000484     | Positive regulation of interleukin-8 secretion | biological_process | PMID:15039339 |

Table 5
GO function analysis of TNF-α gene.

| Gene Accession | GO class                  | Ontology                  | Reference        |
|----------------|---------------------------|---------------------------|------------------|
| TNF-α GO:0043123 | I-kappaB kinase/NF-kappaB signaling | biological_process        | PMID:21873635   |
| GO:0031663     | Lipopolysaccharide-mediated signaling pathway | biological_process        | PMID:21147091   |
| GO:0027155     | Negative regulation of interleukin-6 production | biological_process        | PMID:10436808   |
| GO:0045416     | Positive regulation of interleukin-8 biosynthetic process | biological_process        | PMID:20551324   |
| GO:2000778     | Positive regulation of interleukin-6 secretion | biological_process        | PMID:29702085   |

Table 6
GO function analysis of IL-6 gene.

| Gene Accession | GO class                  | Ontology                  | Reference        |
|----------------|---------------------------|---------------------------|------------------|
| IL-6 GO:0002675 | Positive regulation of acute inflammatory response | biological_process        | PMID:2444978    |
| GO:0005138     | Interleukin-6 receptor binding | molecular_function        | PMID:12829785   |
| GO:0065006     | Interleukin-6 receptor complex | cellular_component        | PMID:12829785   |
| GO:0071222     | Celluar response to lipopolysaccharide | biological_process        | PMID:23776175   |
| GO:0031002     | Positive regulation of NF-kappaB transcription factor activity | biological_process        | PMID:12419823   |
| GO:2000660     | Negative regulation of interleukin-1-mediated signaling pathway | biological_process        | GO_REF:0000024   |
which is a common disease with high mortality rate in medicine. Animal studies have shown that after one hour of lipopolysaccharide injection or organ reperfusion after blood loss, the experimental animals will show a variety of morbidity of lung function and organ, such as dyspnea, decrease of arterial oxygen partial pressure, lung enlargement, increase of the coefficient of pulmonary edema, lobar hemorrhage; alveolar edema thickening, bronchial epithelial cell death, pulmonary interstitial and alveolar hemorrhage accompanied by edema, a large number of inflammatory cell infiltration (Wu, 2015), this phenomenon changes with time obviously. Numerous studies have shown that lipopolysaccharide is one of the leading causes of ALI and ARDS and ultimately lead to death caused by infection. The mechanism of lipopolysaccharide-induced lung injury is most commonly caused by induction of uncontrolled inflammatory response, which causes activated inflammatory cells and effector cells to release a large amount of inflammatory mediators or cellular mediators. Excessive or uncontrolled inflammatory factors can lead to complications, then resulting in lung injury (Li et al., 2010). Lipopolysaccharide can induce the activation of monocytes/macrophages to generate inflammatory factors such as tumor necrosis factor TNF-α, interleukin IL-1, IL-6, IL-8 and IL-12, etc. (Gouda and Bhandary, 2019). When generated inflammatory factors are excessive or are out of control, they can cause a variety of complications, such as microcirculatory disorders, tissue damage, septic shock, and multiple organ damage. Lipopolysaccharide first stimulates effector cells to induce transduction factors to generate a large number of inflammatory factors and inflammatory mediators, including cell nucleus factor kappaB (NF-kB) and activated protein 1 (AP-1). LPS requires the recognition of LPS receptor complex and combines effector cells to transduce signals (Zheng et al., 2018). The LPS receptor complex consists of three receptor proteins: the CD14 receptor, the TLR4 receptor, and the MD-2 receptor. A variety of lipopolysaccharide receptor complexes are present on the surface of both monocytes and macrophages, which are essential for the body to recognize and initiate inflammatory responses (Wang, 2005). The CD14 receptor is also an important receptor mediating LPS-activated cells and has high affinity and sensitivity to LPS receptors.

Numerous studies have shown that LPS-induced lung tissue neutrophil aggregation and pulmonary microvascular endothelial cell response in mice are CD14-dependent. LPS stimulates inflammatory effector cells to bind to LPS receptor CD14 as a LPS complex, thereby activating intracellular cascade signaling and cell nucleus factor NF-kB, then nuclear translocation occurs. It specifically binds to the promoter of the target gene and the enhancer region, which in turn initiates regulation of a series of inflammatory factor responses, such as the expression and release of TNF-α. In addition, they are further involved in the activation of NF-kB factor in effector cells with LPS, and subsequently initiate the expression of more cytokines (e.g., interleukin IL-1, IL-6, etc.) (Gouda and Bhandary, 2019). NF-kB is a kind of eukaryotic transcription factor, of which p50/p65 was founded earliest, and it has the widest distribution and effect (Pan et al., 2012). Studies have shown that proteins of the NF-kB family often bind to the IκB family of inhibitory proteins in the form of homodimer or heterodimers and exist in inactive forms within the cell. Increasing studies have found proteins related to immunity and inflammation such as TNF-α, IL-1, IL-6, IL-8, monocyte chemotactic protein 1 (MCP-1), ICAM-1, iNOS, etc., they all contain a binding site for NF-kB (Chepurnova et al., 2018). In the case of trauma, infection, etc., NF-kB in the cytoplasm of inflammatory cells is induced to activate, causing excessive production of inflammatory mediators such as inflammatory cytokines, adhesion molecules, chemotactic molecules and biologically active enzymes, thereby triggering the systemic inflammatory response characterized by cell self-destruction. Interaction between cytokines forms an extremely complex cytokine regulatory networks that are involved in mediating and regulating immune as well as inflammatory processes. Due to the interaction and synergy between the cytokines, a cascade of amplification of the inflammatory mediators will be triggered, resulting in a large number of mediators involved in the induction of tissue cell damage, ultimately leading to acute lung injury.

In this paper, 210 related literatures such as LPS, CD14, TNF-α and IL-6 were included, and the pathway regulatory network of LPS-induced cytokine cascade involved in lung injury was initially screened and constructed by meta-analysis, GO and KEGG. The effect of LPS on the expression of inflammatory factors such as CD14, TNF-α and IL-6 in the pathway was confirmed. According to the results of meta-analysis, the correlation between CD14, TNF-α, IL-6 gene and acute lung injury was statistically significant. GO analysis and KEGG analysis showed that the process of acute lung injury induced by LPS contained CD14, TNF-α, IL-6 and other factors, these inflammatory factors and immune proteins collectively regulate the process of disease occurrence.

5. Conclusion

In summary, this paper adopted Meta, GO and KEGG analysis to identify three important cytokines involved in the process of acute lung injury, and to construct a network of regulatory pathways for LPS-induced ALI. However, there are still some shortcomings in this study. Among them, the number of high-cited literature and top journal articles is relatively small, and most of the selected literatures are animal test sample data, and there are fewer experi-
mental samples, which may cause difference in clinical symptoms and therapeutic effects to some extent. At the same time, most of the relevant researches at home and abroad lack clinical sample data, or there is a small number of observational cohort studies, in addition, the complete research system has not been established yet, so the support strength of this study is not enough. It is hoped that the results of this paper could guide the development of similar research and provide reference for researchers.

The establishment of immune system related factors involved in the regulation of acute lung injury network provides a new theoretical basis and research ideas for clinical acute lung injury inflammatory factors and immune proteins to jointly regulate the process of disease occurrence and early clinical screening. In the follow-up work, we will conduct more in-depth and detailed studies, as well as experimental science and clinical science on the signaling pathway of immune inflammatory response to lung injury.

Declaration of Competing Interest

The author states that there is no conflict of interest in the content of this article.

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