Peer Review File

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Reviewer A

The authors investigated about the mechanism of Slfn5 in pneumonia.

Overall the topic could be interesting but some details could be improved.

I recommend that the paper be accepted with minor revision:

Comment 1: a) The authors should not use acronyms in the title.

Reply 1: We have replaced all acronyms in the title as advised (see Page 1, line 1-3).

Changes in the text: Knockdown of schlafen family member 5 alleviates lipopolysaccharide-induced pneumonia by regulating Janus kinase/signal transduction and activator of transcription pathway

Comment 2: b) The authors should mention in the abstract more details about model used.

Reply 2: We have mentioned more details about model used in the abstract as advised (see Page 3, line 57-59).

Changes in the text: Mice were intratracheally administered 5 mg/kg LPS to construct pneumonia model. *In vitro*, A549 cells were treated with 10 μg/ml LPS to construct cellular pneumonia model.

Comment 3: c) In the introduction section, little previous evidence is provided about the importance of pulmonary diseases in daily life. Incorporating comparisons with other studies would increase the strength of the paper. Please refer to doi: 10.3390/biom12091308.

Reply 3: Thanks for your comments. In discussion section, we made comparison with other studies, including the above literature (doi: 10.3390/biom12091308) (see Page 17, line 359-361).

Changes in the text: Animal experiments have shown that acute inflammatory

reactions can be stimulated by LPS, and early histopathological changes appeared in the lung tissues [46,47].

Comment 4: d) The authors should mention JAK/STAT pathway also in the introduction.

Reply 4: We have mentioned JAK/STAT pathway in the introduction as advised (see Page 7, line 131-138).

Changes in the text: Janus kinase/signal transduction and activator of transcription (JAK/STAT) pathway is related to diverse biological processes, such as cell growth, immune response, apoptosis, and inflammatory response [19,20]. Various growth factors, ligands, and cytokines can trigger the JAK pathway [21]. The tyrosine of STAT can be phosphorylated by activated JAK, the phosphorylated STAT transfers to the nucleus and bind to specific promotor, resulting in target mRNA expression [22]. At present, more and more evidences have confirmed that JAK/STAT pathway is involved in lipopolysaccharide (LPS)-induced lung injury in mouse [23-25].

Comment 5: e) Is there a dose/concentration for in vivo and in vitro administration of Slfn5?

Reply 5: We provided the dose for in vivo and in vitro administration of Slfn5 (see Page 8, line 168-170; Page 11, line 220-222).

Changes in the text: mice were injected with adenoviral vectors containing sh-NC (1×10^9 pfu/100 μL) and sh-Slfn5 (1×10^9 pfu/100 μL) via the tail vein.

The pcDNA-Slfn5 (200 ng) and sh-Slfn5 (2 μg) and their respective negative controls [pcDNA-NC (200 ng) and sh-NC (2 μg)] were transfected into the cells using Lipofectamine 2000 (Life Technologies, USA).

Comment 6: f) The authors should better emphasize the conclusions

Reply 6: We revised the conclusions as advised (see Page 19, line 394-399).

Changes in the text: Collectively, our results indicated that Slfn5 expression was upregulated in the LPS-induced pneumonia model. Knockdown of Slfn5 reduced lung

injury, apoptosis, and inflammatory response, which might be related to the inhibition of the JAK/STAT pathway. Our study identified a promising therapeutic target for the treatment of pneumonia.

Comment 7: g) There are some minor grammar issues that should be fixed in order to aid the accessibility of the results to the reader.

Reply 7: To aid the accessibility of the results to the reader, our manuscript was revised by language editing company (see Page 1-25, line 1-608).

Changes in the text: Detailed changes can be found in the text.

Reviewer B

The aim of this study is to evaluate the role and underlying mechanism of Slfn5 in LPS- induced pneumonia model. The results are interesting and within the scope of the Journal of Thoracic Disease, however, important points should be improved and clarified before recommending for publication.

Comment 1: Abstract: In the "introduction" section, more information should be included. What is described is insufficient.

Reply 1: We have added more information in this section as advised (see Page 3, line 48-55).

Changes in the text: In recent years, the incidence of pneumonia has been increasing, which is the main cause of death and morbidity of children and the elderly in the world. Schlafen family member 5 (Slfn5) is implicated in multiple cancers, and Slfn5 promotes EMT and metastasis in lung cancer. However, the influences of Slfn5 in pneumonia have not yet been completely cleared. Herein, we aimed to explore the underlying effect and regulatory mechanism of Slfn5 in lipopolysaccharide (LPS)-induced pneumonia in mice and A549 cells.

Comment 2: Highlight Box

Please, review the sentence of the “Key Findings”, the sentence is not a "Highlight". Please, summarize the key findings of the study.

Reply 2: We have revised and summarized the key findings of the study (see Page 5, line 87-94).

Changes in the text:

Key findings

1. Slfn5 expression was increased in LPS-induced pneumonia model.
2. Knockdown of Slfn5 mitigated LPS-induced mice lung injury and inflammation
3. Overexpression of Slfn5 enhanced apoptosis and inflammatory cytokines in LPS-treated A549 cells.
4. Knockdown of Slfn5 weakened lung injury, apoptosis, and inflammatory response through the inhibition of JAK/STAT pathway.

Comment 3: Introduction

Please review the sentence included in lines 93 and 94. Include more information about the identification of Slfn.

Reply 3: We are sorry for our unclear description. We have added more information about the identification of Slfn (see Page 6, line 119-123).

Changes in the text: The family of schlafen (Slfn) genes is originally identified in mice, they are preferentially expressed in lymphoid tissue[12]. During thymocyte maturation and T cell activation, the genes of Slfn family are differentially regulated. Moreover, in fibroblasts and thymoma cells, cell growth is inhibited by the expression of Slfn1.

Comment 4: In the paragraph starting at line 101, please include more information about the mechanism and pathway of LPS in inducing an inflammatory response in pneumonia. Include information about the inflammatory immune response that occurs

in pneumonia.

Reply 4: According to your suggestion, we added these information (see Page 7, line 140-149).

Changes in the text: LPS is produced by gram-negative bacteria, which is essential for the inflammatory reaction related to pneumonia [26-28]. Toll-like receptor 4 (TLR4) is a key receptor for LPS induction [29]. The activated TLR4 can activate myeloid differentiation factor 88, extracellular signal-related kinases 1 and 2, JAK2, and interleukin-1 receptor-associated kinase 1, further promoting the nuclear translocation of nuclear factor kappa B and the phosphorylation of STAT3, consequently, upregulating the levels of inflammation related factors, such as tumor necrosis factor (TNF) and interleukins (IL-6, IL-8, IL-1β) [30-34]. Excessive accumulation of inflammatory cytokines can cause the dysfunction of immune system and, thereby damaging to multiple tissues, including lung tissue [35].

Comment 5: Methods

Include information about the gene analysis conducted using the GSE111241 database.

Reply 5: We added it as advised (see Page 8, line 153-155).

Changes in the text: ***Bioinformatics analysis***

Slfn5 expression in rat bronchoalveolar lavage fluid (BALF) was analyzed through Gene Expression Omnibus database (GSE111241).

Comment 6: In Figure 2, cytokines were measured by ELISA in rats, not in a cell line as described in the methodology. Please include in the ELISA methodology which sample was used for the analysis (BALF, plasma, or tissue homogenate).

Reply 6: We are sorry for any misunderstanding for you. In Figure 2, cytokines (TNF-α, IL-1β, and IL-6) in lung tissues were measured by western blot. In Figure 4 and 6, cytokines (TNF-α, IL-1β, and IL-6) in A549 cells were measured by ELISA.

Changes in the text: no changes.

Comment 7: Please include information about the GSEA analyses.

Reply 7: We added it as advised (see Page 8, line 155-156).

Changes in the text: Gene Set Enrichment Analysis (GSEA) was used to analyze the pathways enriched by Slfn5.

Comment 8: Results

In line 201, it was mentioned that there was a significant increase in the expression of Slfn5 in LPS-treated animals compared to the controls, but the statistical significance of this increase was not mentioned. It is important to provide the statistical significance values, such as p-values, to validate the observed difference.

Reply 8: We have provided p-value as advised (see Page 13, line 268-269).

Changes in the text: An increase in Slfn5 expression was observed in LPS-treated rats (Figure 1A, P = 0.0043).

Comment 9: Figure 2: Please enlarge the size of the microscopy images in figures 2 and 2D for better visibility.

Reply 9: We have enlarged the size of the microscopy images in figures 2C and 2D (see Figure 2 revised).

Changes in the text: no changes.

Comment 10: In the text describing the results "In LPS-induced pneumonia mice, knockdown of Slfn5 inhibited JAK/STAT pathway," include information about the specific groups that were compared and relevant statistical results if available. Additionally, increase the size of the images in figure 3A for better visibility.

Reply 10: We have added information about the specific groups that were compared and relevant statistical results and increased the size of the images in figure 3A (see Page 14, line 298-302; Figure 3 revised).

Changes in the text: As shown in Figure 3B, LPS increased p-JAK2, p-JAK3, and

p-STAT3 expression compared with control group (P<0.01), whereas knockdown of Slfn5 decreased p-JAK2, p-JAK3, and p-STAT3 expression compared with LPS+sh-NC group (P<0.01).

Comment 11: Provide more information about the A549 cell line, including the cell type and why it was chosen for this work.

Reply 11: We have added more information about the A549 cell line (see Page 15, line 306-308).

Changes in the text: A549 cell is a human alveolar epithelial type II cell line. For various studies associated with respiratory diseases including pneumonia, A549 cells treated with LPS have been used as a cellular model [36-38].

Comment 12: In figures 4C, 4I, and 5C, include information about the markers used on the X and Y axes.

Reply 12: We have added the markers used on the X and Y axes (see Figure 4C, Figure 4I, and Figure 6C).

Changes in the text: no changes.

Comment 13: Also, increase the font size indicating the percentages in the quadrants for easier visualization.

Reply 13: We have increased the font size indicating the percentages in the quadrants (see Figure 4C, Figure 4I, and Figure 6C).

Changes in the text: no changes.

Comment 14: Figure Legends

Figure 1: Please include in the caption the methodology for inducing pneumonia with LPS, the number of animals used per experiment, the number of experiments conducted to create the figure, and the significance of the asterisks in Figure 1C (** p value).

Reply 14: We have added these information as advised (see Page 23, line 558-562).

Changes in the text: Mice were intratracheally administered 5 mg/kg LPS to construct pneumonia model. Immunohistochemistry (B) and western blot (C) were used to assess Slfn5 expression in mice lung tissues (N = 5). The data was from three independent experiments. Compared to control group, **P < 0.01.

Comment 15: Figure 2: Please include in the caption the methodology for inducing pneumonia with LPS and silencing the Slfn5 gene, the number of animals used per experiment, the number of experiments conducted to create the figure, and the significance of the asterisks in the figure (** p value).

Reply 15: We have added these information as advised (see Page 23 and 24, line 565-568, 574-575).

Changes in the text: To construct pneumonia-infected lung injury, mice were intratracheally administered 5 mg/kg LPS. One week before the construction of the pneumonia mouse model, in the LPS+sh-NC and LPS+sh-Slfn5 group, mice were injected with adenoviral vectors containing sh-NC and sh-Slfn5 via the tail vein. N = 5. The data was from three independent experiments. Compared to control or LPS+sh-NC group, **P < 0.01.

Comment 16: Figure 3: Please include in the caption the parameters used for GSEA analysis, the number of animals used per experiment, the number of experiments conducted to create the figure, and the significance of the asterisks in the figure (** p value).

Reply 16: We have added these information as advised (see Page 24, line 577-582).

Changes in the text: (A) GSEA analysis (permutation = geneset, metric = Diff_of_classes, metric = weighted, #permutation = 2500) showed that Slfn5 activated JAK/STAT signaling pathway.

N = 5. The data was from three independent experiments. Compared to control or

LPS+sh-NC group, **P < 0.01.

Comment 17: Figure 4: Please include in the caption information about cell culture and the methodology used in the experiments shown in the figure, the number of experiments conducted to create the figure, and the significance of the asterisks in the figure (** p value).

Reply 17: We have added these information as advised (see Page 24, line 585-586, 590-592).

Changes in the text: A549 cells were transfected with sh-NC, sh-Slfn5, pcDNA-NC, or pcDNA-Slfn5 followed by LPS stimulation.

The data was from three independent experiments. Compared to control, LPS+sh-NC, or LPS+pcDNA-NC group, **P < 0.01.

Comment 18: Figure 5: Please include in the caption information about cell culture and the methodology used in the experiments shown in the figure, the number of experiments conducted to create the figure, and the significance of the asterisks in the figure (** p value).

Reply 18: We have added these information as advised (see Page 25, line 595-599).

Changes in the text: A549 cells were transfected with sh-NC, sh-Slfn5, pcDNA-NC, or pcDNA-Slfn5 followed by LPS stimulation.

The data was from three independent experiments. Compared to control, LPS+sh-NC, or LPS+pcDNA-NC group, **P < 0.01.

Comment 19: Figure 6: Please include in the caption information about cell culture and the methodology used in the experiments shown in the figure, the number of experiments conducted to create the figure, and the significance of the asterisks in the figure (** p value).

Reply 19: We have added these information as advised (see Page 25, line 602-603,

607-608).

Changes in the text: A549 cells were transfected with pcDNA-NC or pcDNA-Slfn5 followed by AG490 and LPS stimulation.

The data was from three independent experiments. Compared to control, LPS+sh-NC, or LPS+pcDNA-NC group, **P < 0.01.

Comment 20: Discussion:

Certainly, here are your requests translated into English:

Please make it clear:

In lines 263 and 264, it is stated that the expression of Slfn5 is downregulated in the LPS-induced pneumonia model. However, Figure 1A appears to indicate the opposite. Can you provide clarification regarding this apparent discrepancy in this part of the discussion?

Reply 20: We are sorry for our mistakes. In the LPS-induced pneumonia model, Slfn5 expression was upregulated. We have corrected it (see Page 17, line 354-356).

Changes in the text: Here, in a LPS-induced pneumonia model, we found that Slfn5 expression was upregulated.

Comment 21: In line 288, please include the sample used to measure the mentioned cytokines (plasma, serum, BALF, etc.). Depending on where the cytokines are measured, we can assess whether the effect is systemic or local.

Reply 21: We have mentioned it (see Page 17, line 369-371).

Changes in the text: In the plasma of pneumonia patients, IL-6 level is increased; and high IL-6 level in circulating leukocytes increase incidence of secondary infection in pneumonia patients [50].

Comment 22: Additionally, cite studies linking the JAK/STAT pathway to the Slfn5 gene, beyond LPS, to strengthen the basis for this connection in your discussion.

Reply 22: Thanks for your comments. At present, there are no reports for the direct

linking of JAK/STAT pathway and the Slfn5 gene. We are sorry for not being able to cite studies linking the JAK/STAT pathway to the Slfn5 gene. Thanks for your understand.

Changes in the text: no changes.