Peer Review File

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Reviewer A

This study by Ma et al., describes the role of PGC-1alpha in alveolar epithelial cell (AEC) senescence and pathology of IPF. The authors show that in lungs from human IPF and bleomycin-induced pulmonary fibrosis in mouse a decrease in PGC-1alpha and NRF-1 and an increase in p21 WAF1 expression. The decrease in PGC-1alpha was shown to be involved in epithelial cell senescence by regulating mitochondrial morphology and function. Further, ZLN005, an activator of PGC-1alpha alleviated hydrogen peroxide mediated cell senescence by increasing PGC-1alpha expression and improved mitochondrial morphology and function. Interestingly, deletion of PGC-1alpha weakened the effect of ZLN005 in AECs. There are several concerns related to the study.

General Comment:

1. Expression of PGC-1alpha is reduced in human IPF and bleomycin-induced flung fibrosis in mouse model. The in vitro experiments with reduced expression of PGC-1alpha suggest that ZLN005 efficacy is reduced in rescuing hydrogen peroxide mediated senescence in AEC. If this is true, then ZLN005 cannot be a potential drug for the treatment of IPF.

Specific Comments:

1. The effect of ZLN005 was studied only in A549 epithelial cell line and isolated AECs from mice. The efficacy of ZLN005 in bleomycin model of pulmonary fibrosis needs to be demonstrated and this in vivo study is lacking.

Reply1:Thank you for your suggestion and inspiration. Our article is about preliminary explores the role of mitochondrial function in the aging of alveolar epithelial cells. Your suggestion and inspiration, which will be the focus of our further research. Our team has found similar results in animal experiments.

Changes in the text: None

2. The mitochondrial morphology should include mitochondrial dynamics (fusion vs fission).

Reply2:Thanks for the editor's reminding. Considering the relatively long experimental time, our research focuses on mitochondrial function. The author has been away from the laboratory for 2 years and has entered clinical work. Due to busy tasks, I am unable to supplement experimental data in the short term. I have applied to the team for

relevant work. Our next work will adopt your valuable suggestions.
Changes in the text: None

3. In the bleomycin model, demonstrate the expression of PGC-1alpha and NRF-1 in AECs of lungs using IHC of the lung tissues.
Reply3:Thanks for the editor's reminding. Our next work will adopt your valuable suggestions.
Changes in the text: None

4. Data in Fig 3 suggest that exogenous hydrogen peroxide stimulates mitochondrial ROS. Several published studies have demonstrated a role of mitochondrial ROS in modulating mitochondrial morphology and function. What is the role of mitochondrial ROS in AEC senescence? Experiments with mitochondrial ROS scavengers such as Mito TEMPO or Mito Q can reveal the potential role of mito ROS in aging senescence.
Reply4:Thanks for the editor's reminding. Our study found that there is mitochondrial dysfunction in the aging of AEC cells, and an increase in ROS levels is a manifestation of mitochondrial dysfunction. Our next work will adopt your valuable suggestions.
Changes in the text: None

Reviewer B

1) First, the authors need to indicate the research design of this study in the title, i.e., in vitro and in vivo experiment.

Reply1): Thanks for the editor's comments. Our research focuses on the cell testing part, and further research and verification are needed in vivo experiments.
Changes in the text: None

2) Second, the abstract needs some revisions. The background did not describe the potential clinical significance of this research focus. In the methods, the authors need to briefly describe the hypotheses to be confirmed by these experimental procedures. The results need detailed statistical data such as the expression levels and accurate P values to support these findings. The conclusion remains vague and unclear. More detailed comments for the possible implications are needed.

Reply2): Thanks for the editor's comments. We have made corresponding modifications.
Changes in the text: None

3) Third, in the introduction of the main text, the first question is the significance of this research focus, which has not been clarified by the authors. The second question is why the mechanisms of AT2 depletion are understudied, what the knowledge gap

is, and why the proposed research pathways can fill this knowledge gap. The authors need to provide more arguments for supporting the proposed relationships.

Reply3): Thanks for the editor's comments.We have made corresponding modifications. This article mainly explores the role of mitochondrial function in the aging of alveolar epithelial cells. Other mechanisms such as research on the mechanism of AT2 exhaustion have not been addressed, so we didn't delve into it in-depth discussion.

Changes in the text: None

4) Fourth, in the methodology of the main text, please first have a brief overview of the research procedures and the questions or hypotheses to be examined. In statistics, please ensure P<0.05 is two-sided.

Reply4): Thanks for the editor's comments.We have an explanation of the experimental statistical methods in the supplementary explanation.

Changes in the text: None

Reviewer C

The paper titled "ZLN005 improves the protective effect of mitochondrial function on alveolar epithelial cell aging by upregulating PGC-1α" is interesting. These results provide preliminary insights into the potential clinical application of ZLN005 as a novel therapeutic agent for the treatment of IPF. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) There have been many studies on idiopathic pulmonary fibrosis. What is the difference between this study and previous studies? What is the innovation? These need to be described in the introduction.

Reply1): Thanks for the editor's reminding. This article mainly explores the role of mitochondrial function in the aging of alveolar epithelial cells. This study conducted in vitro and in vivo experiments to preliminarily explore the mechanism by which PGC-1α is involved in AEC senescence through regulating mitochondrial morphology and function. The protective effect of the PGC-1α agonist ZLN005 on senile AECs was also examined. Our findings may provide preliminary insights into the potential clinical application of ZLN005 in the treatment of IPF.

Changes in the text: None

2) What molecular mechanism does ZLN005 promote the mRNA levels of PGC- 1α and the expression of downstream genes that is primarily dependent on? Additional relevant discussion is suggested.

Reply2): Thanks for the editor's comments. Regarding ZLN005 promoting PGC-1 α

Exploration of mRNA levels (see lines 137 to 145 on page 5 of the article)
Changes in the text: None

3) What are the consequences of senescent epithelial cells? What changes in their ability to respond to normal activation and impact on local niches? It is recommended to add relevant content.
Reply3): Thanks for the editor's comments.The senescence of alveolar epithelial cells (AECs) is thought to be an important mechanism in the pathogenesis of IPF. A prevailing concept is that alveolar type 2 (AT2) cells can induce a fibrotic response in the lungs.
Changes in the text: None

4) How to provide candidate targets for the treatment of idiopathic pulmonary fibrosis based on the results of this study? It is recommended to include relevant descriptions in the discussion.
Reply4): Thanks for the editor's comments.This study conducted in vitro and in vivo experiments to preliminarily explore the mechanism by which PGC-1α is involved in AEC senescence through regulating mitochondrial morphology and function. The protective effect of the PGC-1α agonist ZLN005 on senile AECs was also examined. Our findings may provide preliminary insights into the potential clinical application of ZLN005 in the treatment of IPF.

5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “The oncogenic landscape of the idiopathic pulmonary fibrosis: a narrative review, Transl Lung Cancer Res, PMID: 35399571”. It is recommended to quote this article.
Reply5): Thank you for your suggestion. We will carefully review this literature and refer to it for future research.
Changes in the text: None

6) It is suggested to add the research progress of ZLN005 in respiratory diseases in the discussion.
Reply6): Thanks for the editor's comments.There are few literatures about ZLN005 in respiratory disease.

Reviewer D

1. Figure 2

As for the special symbol "ns" in Figure 2, please explain its meaning in the legend.

Reply: Thanks for the editor's comments. We have made corresponding modifications.

ns: no significance.

2. Figure 4

Should it be "**". Please check.

703 respiratory, ATP production, and proton leak. β-actin served as an internal parameter. *,

704 P<0.05; *, P<0.01; ***, P<0.001. PGC-1α, peroxisome proliferator-activated receptor-γ

Reply: Thanks for the editor's comments. We have made corresponding modifications.

3. Figure 6

Should it be "**". Please check.

749 ATP production, and proton leak. β-actin served as an internal parameter. *, P<0.05; *,

750 P<0.01; ***, P<0.001; ****, P<0.0001. PGC-1α, peroxisome proliferator-activated

Reply: Thanks for the editor's comments. We have made corresponding modifications.

4. Figure 7

Should it be "**". Please check.

773 respiration, maximal respiratory, ATP production, and proton leak. β-actin served as an

774 internal parameter. *, P<0.05; *, P<0.01; ***, P<0.001; ****, P<0.0001. PGC-1α,

775 peroxisome proliferator-activated receptor-γ coactivator-1 alpha; NRF-1, nuclear

Reply: Thanks for the editor's comments. We have made corresponding modifications.