Peer Review File

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Reviewer A

Comment 1: My major concern with the study is about the experimental model. The Bleomycin-induced pulmonary fibrosis (BLM) model does not reproduce the classic histological pattern of IPF, that is, peripheral acinar fibrosis with areas of temporal heterogeneity (fibroblastic foci, honeicombing, mural fibrosis, alongside areas of normal lung parenchyma. O model of fibrosis induced by intratracheal injection of MBB is centered in the airways, that is, bronchocentric and therefore more consistent with an inhalational disease. To mimic IPF, the appropriate experimental model would be that induced by 3-5-di-tert- butyl-4-hydroxytoluene (BHT) that mimics the histological pattern of human Usual Interstitial Pneumonia (UIP) (see references Lab. Invest. 1977;36:26–32; Int J Exp Pathol. 2008 Oct; 89(5): 350 –357; Braz J Med Biol Res. 2014 Jul;47(7):567-75).

Reply 1: Thank you for your comment. The bleomycin model is the most widely used and well-characterised mouse model, and bleomycin-induced pulmonary fibrosis models can be readily induced in a short period of time with high reproducibility. Although the pathological manifestations of the bleomycin model are more similar to interstitial pneumonitis, i.e., the airway centre is impaired, than those of the BHT model, there is no literature available to indicate whether the molecular mechanisms of signalling in the different models of pulmonary fibrosis differ in the way they ultimately lead to pulmonary fibrosis. I appreciate your comment that although the BHT model pathology is more similar to human idiopathic pulmonary fibrosis, there is less relevant literature and this was not taken into account in the design of the preliminary experiment.

Changes in the text: We have modified our text as advised (see Page 13, line 242)

Comment 2: Another limitation of BLM-induced fibrosis would be the reabsorption of fibrosis after 30 days of evolution in mice. In this context, the authors should have included a group with more than 30 days of evolution.

Reply 2: Regarding the reabsorption of the injury after 30 days in the bleomycin model, the changes of lung fibrosis markers in the alveolar epithelial cells of mice after 30 days were not sufficiently taken into account and further explored in this experiment, which is exactly the defect of this experiment. We are sorry that due to experimental limitations, it is not possible to continue to explore the fibrotic changes and signalling pathway mechanisms in the lungs after 30 days for the time being. I am very grateful to you for this comment, which has greatly helped me to refine my experimental design in the furture. Although the model still suffers from resorption of pulmonary fibrosis in mice after 30 days of evolution, the model system provides an opportunity to study the regression of fibrosis at these later time points.

Changes in the text: We have modified our text as advised (see Page 13, line 251)

Comment 3: How was the dose of antifibrotics for intratracheal injection determined?
Reply 3: In this study, drug concentrations of pirfenidone and nintedanib determined in pre-designed experiments by reading Kurita et al. and Wollin et al. In response to your question, we have added references in the appropriate sections of the article.
Changes in the text: We have modified our text as advised (see Page 7, line 108)

Comment 4:Analyzing the panel of photos in Figure 1B, other questions arise: a) were the lungs inflated with 4% buffered formalin under a pressure of 15 mmHg to avoid alveolar collapse? b) in the BLM panel, the distribution of fibrosis around the bronchovascular axis with slight extension to the adjacent alveoli is clear, the process doesn't involve the peripheral alveoli; c) the simple observation that the treated lungs had reduced fibrosis is not convincing only with Masson's trichrome staining of the lungs; d) could have better visualization under staining with Picro-Sirius under polarized light or immunohistochemistry for collagen fibers; a semiautomated quantification should be applied; e) In fact, by carefully observing the photo panel of the treated lungs, it is difficult to discern whether there was a drug effect or reabsorption. Especially because the mechanism of action of antifibrotics is to prevent the progression of inflammation and fibrosis to the normal parenchyma. Other markers should have been investigated such as metalloproteinases (MMPs) and TIMs.
Reply 4: In the methods, we mention the use of 4 per cent formalin, on page 7, line 116. And at your suggestion, we performed immunohistochemistry to measure the mean optical density values of SMA and C1 and found that α-SMA and collagen I were highly expressed in the interstitium in the BLM group, as seen by positive immunolocalised areas in lung sections. Expression of α-SMA and collagen I was significantly lower in the pharmacological intervention group compared with the BLM group.
Changes in the text: We have modified our text as advised (see Page 10, line 175)

Comment 5: The authors must address all these limitations in the Discussion section.
Reply 5: As answered above, I have added a new element to the discussion.
Changes in the text: We have modified our text as advised we have modified our text as advised (see Page 13, line 242)

Reviewer B

Comment 1: In animal model, pirfenidone group (20 mg/kg), and nintedanib group (60 mg/kg), How did you choose dosage of two drugs?
Reply 1: In this study, drug concentrations of pirfenidone and nintedanib determined in pre-designed experiments by reading Kurita et al. and Wollin et al. In response to your question, we have added references in the appropriate sections of the article.
Changes in the text: We have modified our text as advised (see Page 7, line 108)

Comment 2: In the Enzyme-linked immunosorbent assays (ELISAs) were used to detect TGF-β1, SP-A, SP-D and KL-6, authors described clearly and exactly methods for ELISA assay.

Reply 2: ELISA kits were purchased from Jingmei Biotechnology to complete the experiments. All experimental steps were carried out according to the instructions.
Changes in the text: We have modified our text as advised (see Page 7, line 114)

Comment 3: In the antibodies used in this study (Anti-TGF- β 1, anti- α -SMA, anti-Fn, 123 anti-GAPDH, anti-JAK2, anti-p-JAK2, anti-STAT3, and anti-p-STAT3 monoclonal antibodies, Anti-TGF-β-R2 antibodies), authors clearly added the dilution factors.
Reply 3: Yes, the antibody was appropriately diluted for this experiment when doing protein blotting experiments. The antibody was diluted using the appropriate dilution ratio according to the instructions of the antibody. Both primary and secondary antibody dilutions were used from Beyotime (P0023A、P0023D)

Changes in the text: We have modified our text as advised (see Page 8, line 139)

Comment 4: Authors indicated expression and location of p-JAK2 in mouse lung tissue, However, these figure is not poor resolution.
Reply 4: I'm sorry I didn't get your point, is it possible that you are asking me about the resolution of the image, is it that the image is displayed too blurry.

Comment 5: Authors should be measured the fibrosis biomarkers in lung tissues of mice.
Reply 5: Following your recommendation, we performed immunohistochemistry to measure the mean optical density values of SMA and C1 and found that α-SMA and collagen I were highly expressed in the lung interstitium of the BLM group, with positive immunolocalised areas in the lung sections. The expression of α-SMA and collagen I was significantly lower in the drug intervention group compared with the BLM group.
Changes in the text: We have modified our text as advised (see Page 10, line 175)

Comment 6: In vitro cell system, authors should be compared using JAK2-siRNA system.
Reply 6: I apologize, as the frozen MLE12 cells have all been used up and it was not possible to complete this experiment at short notice. Thank you very much for your suggestion, I have modified Figure 3 again, changing the NC group to si-NC group, who is the negative control group.NC is a negative control small interfering RNA.When designing the experiment, I felt it was very necessary to design the NC group to compare with si-JAK2 to exclude confounding factors.

Reviewer C

Comment 1: In Figure 1B, the Control looks very fibrotic, and the Bleomycin looks less fibrotic.
Reply 1: In order to show the extent of pulmonary fibrosis more visually, I added

immunohistochemistry experiments of mouse lung tissue. And the immunohistochemical experiments were statistically analysed. As can be seen in Figure 1C, the positive areas of α-SMA and collagen 1 were significantly expressed in the bleomycin group compared to the control group.
Changes in the text: We have modified our text as advised (see Page 10, line 175)

Comment 2: The Abstract line 22 has a description of animal work in the methods line 29 but in the results there is no description of the results of the animal work.
Reply 2: Since most journals can't have too many words in their abstracts, I've streamlined them a bit. I have now refined this in the abstracts as per your suggestion.
Changes in the text: We have modified our text as advised (see Page 2, line 30)

Comment 3: It is also the main pathway involved in ILD - - > It is also a pathway involved in ILD
Reply 3: I have revised this statement in the article.
Changes in the text: We have modified our text as advised (see Page 5, line 75)

Comment 4: please explain what SP-A, SP-D and KL-6 are.
Reply 4: SP-A and SP-D are produced by type II alveolar epithelial cells, and together, the two participate in the formation of alveolar surfactants and in the formation and metabolism of alveolar hyaline membranesKL-6 is a mucin-like glycoprotein expressed on the extracellular surfaces of alveolar type II cells and bronchiolar epithelial cells. As a chemokine, KL-6 promotes the migration, proliferation and survival of lung fibroblasts. I have also explained their significance and added references in the discussion of the article.
Changes in the text: We have modified our text as advised (see Page 14, line 258)

Comment 5: please say what the samples were (serum? lung tissue?) If tissue, in the Methods, explain how the tissue was prepared, and how tissue amounts were normalized
Reply 5: I am very sorry for not being rigorous enough to see the problem here when writing the paper in the early stages. The samples for this ELISA experiment were taken from mouse peripheral serum, not plasma, and not mouse lung tissue.
Changes in the text: We have modified our text as advised (see Page 7, line 112)

Comment 6: but also enter the nucleus through direct phosphorylation' you need to tone this down as you have no proof that its due to direct phosphorylation
Reply 6: Through your suggestion I have modified the issue here in the article.
Changes in the text: We have modified our text as advised (see Page 11, line 194)

Comment 7: carefully explain what MLE12 cells are.
Reply 7: MLE-12 is a widely used mouse capillary alveolar epithelial cell line, which is mainly used to study the biological properties of alveolar epithelial cells and the pathogenesis of related diseases. I will include this section in the discussion of the

article.
Changes in the text: We have modified our text as advised (see Page 13, line 239)

Comment 8: please add references for these statements.
Reply 8: I have put references to the answers to the relevant questions in my paper.

Comment 9: were these experiments done with or without TGF-beta added to the cells?
Reply 9: Whether or not to intervene with TGF-β1 in all cellular experiments is explained in my legend.
Changes in the text: We have modified our text as advised (see Page 22, line 438)

Comment 10: All micrographs need size bars – photograph a microruler and then use this information for size bars.
Reply 10: I have added rulers in all the places in the text where they were needed.

Comment 11: In all figure legends, for each experiment, give the n value.
Reply 11: I've added n value to all of the legends.

Comment 12: Fig 3 all the graph Y axes say '..expression of GAPDH', this needs to be fixed, X axes on graphs need labels (TGF-beta1, ng/ ml).
Reply 12: I have made the changes.

Comment 13: Fig 3C caption explain what NC is.
Reply 13: NC is a negative control small interfering RNA