Author’s response to reviews

Title: TRAIL signals through the ubiquitin ligase MID1 to promote pulmonary fibrosis

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Author’s response to reviews:

We are grateful for the opportunity to address the comments from our reviewers and the improvements these suggestions have facilitated in our manuscript. We are hopeful that our most recent revision is suitable for publication in BMC Pulmonary Medicine. Please see below a detailed response to each of the items raised by the reviewers outlining the improvements we have made in our attached manuscript in light of the issues raised.

Reviewer 1:

Thank you very much for offering me the opportunity to review the revised version of the interesting manuscript by Adam Collison and co-authors. I have still some questions regarding the methods, especially the diagnostic procedure of the patients whose lung biopsies were used in this study. Methods (pages 4-5): More detailed representation of the patients who had undergone the transbronchial lung biopsy operation would be informative since these 8 cases constituted the human lung biopsy material of the present study. It would important to state in
the manuscript that the histological confirmation for UIP/IPF of the patients was performed by analyzing the transbronchial lung biopsy samples, but not surgical lung biopsies, which causes some uncertainty in the diagnoses of IPF. In both ATS/ERS guideline (Raghu G et al Am J Respir Crit Care Med 2011) and the more recent suggestion ("White paper", Lynch DA et al Lancet Respir Med 2018) it has been straightforwardly informed that the transbronchial lung biopsy samples are not large enough for the diagnosis of IPF. Thus, the diagnostic procedure of the patients suspected for IPF was not performed according to the current international guidelines for the diagnosis of IPF, which fact would be appropriate to inform in the manuscript. Were the HRCTs of all biopsied patients categorized as possible UIP, and not definite UIP? Fig E1: Higher magnification and arrows would be demonstrative. Hyperplastic pneumocytes are not visible in the Fig E1 C. Moreover, intra-alveolar fibrotic lesions in the Fig E1 E may as well represent organizing pneumonia (OP) pattern, and not UIP.

Thank you for your considered comments. We have taken steps to clarify in the text precisely how our groups of IPF have been diagnosed and controls selected to exclude the possibility of selecting IPF patients as controls (Lines 95-109). We have also added a substantial section to the discussion addressing the implications of these limitations (Lines 345-368). We believe that this is now transparent for the reader including identifying the uncertainty introduced by this approach. It is worth noting that at the time this study was conducted these classifications were informed by the Raghu et al. ATS/ERS/JRS/ALAT guidelines as the more recent "White paper", Lynch DA et al Lancet Respir Med 2017 was not yet published though we now direct our readers to it in the discussion.

Furthermore, we believe that the uncertainty created by using transbronchial biopsies is reduced by using control tissues from tissues distal to resected lung cancer in patients with no clinical evidence of IPF or other fibrotic lung disease. Therefore the diagnostic limitation of using transbronchial biopsies regarding unknown sensitivity for IPF diagnosis is not relevant in this study. In regards to unknown specificity using transbronchial biopsies for the diagnosis of IPF this approach could have resulted -if anything- in a smaller difference in Midline 1 between IPF patients and controls in our study. However, despite this limitation, we found a significant difference in serum TRAIL, tissue Midline 1 and a trend in PP2A between groups which suggests consistency and robustness of our data. Alternatively, it is possible that using transbronchial biopsies may have impaired our ability to distinguish between the fibrotic pattern seen in IPF versus a pattern seen in organizing pneumonia. This may not however have affected the difference in expression of TRAIL, MID1 and PP2A between patients and controls in this study as long as the TRAIL-MID1-PP2A pathway promotes fibrogenesis in a manner that is not disease-specific. This hypothesis is supported by our earlier studies showing a profibrotic role of TRAIL, MID1 and PP2A in a range of very diverse diseases including eosinophilic esophagitis, COPD and asthma.
Additionally, we have aimed at extensively adjusting Figure E1 to include higher resolution images and a representative HRCT image demonstrating the image features on the basis of which the indication for performing a transbronchial biopsy made by the treating physician.

Reviewer 2:

Figure 2E is not efficient quality to show TUNEL positive cells. This figure's back ground is too high. Better figure is required.

We have now included arrows to highlight the TUNEL positive cells for the reader. The proportion of TUNEL positive cells is low, peaking at 4% in the highest groups and at <0.5% in control groups. The high exposure allowed certainty that all TUNEL positive cells were correctly identified. Unfortunately, these stains have now faded somewhat as they were conducted several years ago and it is not possible to further improve the image quality at this time and we no longer have access to tissue samples to repeat these stains.

Due to these limitations we suggest that the most appropriate solution may be to omit the supplement figure E2.