Reviewer’s report

Title: The overexpression of peroxiredoxin-4 affects the progression of idiopathic pulmonary fibrosis

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Reviewer: Michelle Armstrong

Reviewer's report:

The manuscript submitted by Hanaka et al. investigates the potential role of peroxiredoxin-4 (PRDX4) as an early biomarker for early diagnosis of acute exacerbation (AE) in idiopathic pulmonary fibrosis (IPF). In brief, they investigated the levels of PRDX4 in serum of stable IPF patients (S-IPF; n=51) versus patients who were undergoing AE-IPF (n=38). They also look at the role of PRDX4 in transgenic mice which overexpressed this protein in a murine bleomycin model of pulmonary fibrosis. This is a novel and interesting study. I recommend that it is accepted after minor essential revisions.

My comments to the authors are as follows:

(1) In the Abstract and Background/Discussion sections of the manuscript (MS), the authors have summated that serum PDRX4 "is" or "can be" a useful biomarker in distinguishing S-IPF from AE-IPF. This is a premature observation, further validation of the role of PRDX4 is required. This text should be rephrased in the respective sections.

(2) In the Abstract and Background/Discussion sections of the MS, the authors report that other members of the PRDX have been investigated in IPF (namely, PRDX1 and PRDX6). The authors should note that PRDX1 was investigated in a murine bleomycin model of IPF and has not been investigated in IPF patients (Ref 18 in MS). PRDX6 has been investigated previously in a paraquat-induced model of lung injury (PRDX6-KO mice died at 4 days post-treatment; the bleomycin model is routinely a 21-day model). This murine study (see Ref 19 in MS) has no relevance to IPF. The authors should address these anomalies regarding Refs 18 and 19 within the MS. Furthermore, a study in 2008 by Vuorinen et al. (10.1369/jhc.2008.951806) investigated the role of PRDX2 in lung tissue biopsies from IPF patients, which showed that results suggest that Prx II oxidation does not relate to the pathogenesis of IPF/UIP and that Prx II, PDGFRs, and proliferating cells colocalize in the IPF/UIP lung. The MS should be updated to include this reference also.
(3) In the Methods/Human Study section (page 9 of MS), the authors state that they collected serum from IPF patients from April 2010 to December 2016. On page 15, the authors state that they analyse blood samples from n=51 S-IPF and n=38 AE-IPF patients (Figure 1). Did the authors just collect serum at diagnosis or do they have follow-up serum?

(4) On page 16, the MS, the authors state that "during the observation period" 9 patients with S-IPF developed AE-IPF (Figure 2). What was the duration of the observation up period? This should be clarified in the MS.

(5) In Table 1, the authors give the baseline characteristics for the S-IPF and AE-IPF patients. The MS would be improved if they also included values for FVC and DLco. If the authors have these PFT values, they should include decline in FVC over the duration of the study in the MS. Also, was there any correlation between decline in FVC and serum levels of PRDX4?

(6) In the murine lung homogenates from the bleomycin model, only CCL2 and IL-17A are increased in PRDX4-Tg mice+Bleomycin compared with PRDX4-Tg mice+saline. No other significant differences were found in cytokine/growth/pro-fibrotic factors. The authors quantitated levels of IL-1b, IL-6 and TNF-a transcription, as per serum analyses, and which were not significantly different. Why did the authors not quantitate mRNA levels of Th2 markers such as IL-4 and IL-13? It would be interesting to see if overexpression of PRDX4 in the Tg mice increases their in murine lungs expression compared with WT mice, as the levels of TGF-b mRNA in lung homogenates in these mice are similar. This would help to address the question as to how PRDX4 worsens lung fibrosis when overexpressed in the bleomycin model.

(7) In this study, overexpression of PRDX4 in Tg mice drives pulmonary fibrosis in the bleomycin model. In contrast, in Ref 18 of the MS, pulmonary fibrosis is worse in PRDX1-knockout mice compared with WT mice. The authors should comment on this in the Discussion section.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No
Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.
Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.
Yes

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