Reviewer’s report

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

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Reviewer: Megan Ballinger

Reviewer's report:

This manuscript investigates the role of peroxiredoxin (PRDX)4 as a diagnostic marker of acute exacerbations in IPF. The authors have previously published that overexpression of PRDX4 protected against nonalcoholic fatty liver disease and atherosclerosis by protecting against oxidative injury. This paper examines the expression of PRDX4 in serum of both stable IPF patients and in IPF patients suffering from an acute exacerbation. In addition, WT mice and mice overexpressing human PRDX4 were challenged with bleomycin and then development of fibrosis, expression of PRDX4, and cytokine profiled in lungs were measured.

Major:

1. The authors examined serum biomarkers from IPF and AE-IPF patients and found elevated PRDX4 (Figure 1). This raises the question as to whether there are differences between PRDX4 in IPF and normal donor controls. A recent publication (Elko et al) suggests that there is differences in mRNA expression of PRDX4 between IPF and normal donor controls, but they do not measure protein expression. Addition of this important control in necessary to understand the implications of PRDX4 as a biomarker of pulmonary fibrosis.

2. Since PRDX4 is both intracellular and secreted, what is the correlation between the amount secreted and the amount retained in the cells? The authors demonstrate elevated PRDX4 in the serum of AI-IPF patients (Figure 1) and in the serum and BALF fluid of bleomycin challenged mice (Figure 5). However, the immunofluorescence staining of the lung epithelial cells and macrophages in Figure 5B does not seem to be different between the saline and bleomycin challenged Tg mice. Understanding the relative amount of intracellular and secreted PRDX4 in specific lung cell types is important in understanding the role of PRDX4 in mediating pulmonary fibrosis. In addition, isolated cell (rather than lung sections) or use of light field images would be helpful in distinguishing the location of PRDX4 via IF.
3. The authors state in line 163 that they measured PRDX4 levels in serum and BALF from humans and mice, but Figures 1 and 2 are both serum measurements (Line 626 and 630). How do levels of PRDX4 differ between BALF and serum of IPF and AE-IPF patients? In addition, the authors state that some of the S-IPF patients also had an acute exacerbation. Additional information regarding the timing of sample collection and the sequence of samples are needed to understand Figure 2. In every case, was the S-IPF sample collected before the AE-IPF sample?

4. In Figure 5c, d, e, the authors state that they are using WT and Tg, but the results say Tg-BLM and WT-BLM. Does the ELISA pick up human and mouse PRDX4? Otherwise, since this is a human Tg overexpressing mouse, shouldn't the control be a Saline-Tg rather than a WT-BLM which doesn't have the human transgene overexpressed? Is the system leaky, and how much PRDX4 is expressed at baseline?

5. PRDX4 has a regulatory role in the activation of NF-kB; however there is little to no difference in expression of NF-kB regulated cytokines in the WT and Tg mice after saline or bleomycin challenge. One possibility is that the cytokine signal is diluted in the whole lung homogenate. Additional experimentation using the BALF, serum samples, or isolated cells are needed to characterize the role of PRDX4 in regulating cytokine and chemokine production after bleomycin challenge.

Minor:

1. According to the methods, the authors use only male mice for the experiments. Limitation on the interpretation of the data need to be included since only male mice were used for the experiments.

2. Figure 3 is difficult to read because of similarities between the lines. The use of color or other symbols would greatly improve understanding.

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

Yes
Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

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

No

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