Reviewer's report

Title: Transgenically-expressed secretoglobin 3A2 accelerates resolution of bleomycin-induced pulmonary fibrosis in mice

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Reviewer: Robert Guzy

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General overview:
In this manuscript, Cai and co-authors describe a transgenic mouse that overexpresses Scgb3a2 under control of the human Surfactant Protein C promoter. While this mouse has no observed phenotype under homeostatic conditions, the authors show that Scgb3a2-overexpressing mice have an altered response to intratracheal bleomycin. At three weeks post-bleomycin, transgenic mice have increased inflammation and fibrosis, while at 6 and 9 weeks post-bleomycin the transgenic mice have decreased fibrosis and collagen deposition compared to wild type controls. Lastly, the authors use microarray analysis of transgenic mice to suggest that Scgb3a2 overexpression may alter cellular homeostasis and regulation of inflammation, thus making mice potentially more susceptible to the early effect of bleomycin treatment.

Overall, the experiments are thorough with appropriate controls. This is an interesting report with potentially useful therapeutic implications for pulmonary fibrosis. Specific comments and questions are stated below:

Major Compulsory Revisions

1. It is unclear why the authors chose a constitutively-active overexpression of Scgb3a2, compared to an inducible system. Additionally, it is unclear why the authors chose to overexpress Scgb3a2 in alveolar epithelial cells, rather than airway epithelium. Additional explanation in the text would be useful.

2. While it is clear that there is no observed homeostatic phenotype, the microarray analysis suggests that there are clearly cellular signaling alterations induced by continuous Scgb3a2 overexpression, in particular cellular metabolism and control of inflammation. The authors should discuss this apparent discrepancy, and also describe whether any metabolic or inflammatory abnormalities have been identified in transgenic mice to support the microarray data.

3. The authors describe previous reports of Scgb3a2 anti-fibrotic activity via STAT1 phosphorylation, SMAD7 expression, and inhibition of TGF-beta and SMAD2 phosphorylation. This data should be included in the current study, as it would provide some potential cellular mechanism for the observed anti-fibrotic phenotype. Furthermore, microarray data of transgenic (vs wild type) mice treated with bleomycin would potentially be useful to evaluate a mechanism for the accelerated reversal 6 weeks after bleomycin.
4. The authors should reconcile two apparently contradictory statements – 1) that Scgb3a2 overexpression predisposes mice to a more severe phenotype 3 weeks after bleomycin, and 2) that Scgb3a2 overexpression is anti-fibrotic and accelerates recovery from bleomycin at 6 weeks after injury.

5. For Figure 1F, the immunohistochemistry for both SP-C and Scgb3a2 is not impressive. For the DAB staining, images with improved signal relative to hematoxylin counter-stain is needed. Additionally, co-immunofluorescence for SPC and Scgb3a2 is needed to convincingly prove co-localization.

6. For Figure S1, it is not clear why ex vivo organ culture studies are appropriate for this study. Previous studies applied exogenous Scgb3a2 to cultured embryonic lungs, while this system utilizes overexpression. Analysis of branching of embryonic lungs at different stages are appropriate, but should be done immediately after dissection. Furthermore, given that the SP-C promoter is not active at E11 (and no measurement of Scgb3a2 expression was done at this time point), E11 is not an appropriate time point for this analysis. Analysis of lung development and branching morphogenesis in transgenic mice is appropriate, but at later stages.

7. For figure S1G, the immunohistochemistry of Scgb3a2 is not clearly demonstrated. Improved staining signal is needed, and immunofluorescence would be a good alternative. Additionally, co-IF of Scgb3a2 and SPC would be useful in this figure.

8. For bleomycin experiments, lower-magnification images of entire lung lobes would be useful to compare overall patterns of lung fibrosis.

9. For bleomycin experiments, survival data is needed (either added to Figure 2 or as a supplemental figure).

10. For Figure 4B, improved quality IHC is needed, as it is difficult to appreciate the colorimetric stain presented.

11. In the discussion (lines 485-487), the authors state that ectopic overexpression of Scgb3a2 may have perturbed lung development. Do the authors have data to support this – i.e. microarray data from pre-natal timepoints or other data to support abnormal development in transgenic mice?

12. In the discussion (lines 498-499), the authors state that Scgb3a2 expression is critical for maintenance of lung homeostasis. This statement should be clarified, as data presented in the supplemental figures argue that no abnormal phenotype exists in transgenic mice in the absence of injury.

13. In the discussion (lines 516-518), the authors state that overexpression of Scgb3a2 occurred in the “therapeutic phase” after bleomycin. While overexpression did recover in transgenic mice, this statement is confusing, given that the transgenic mice are non-inducible and have constitutive overexpression of Scgb3a2.

Minor Essential Revisions
1. Is the recovery of increased Scgb3a2 expression following bleomycin (Figure 4) related to recovery of SPC-expressing epithelium? Please comment on how
overexpression is lost initially after bleomycin, and then recovers at the 6- and 9-week time points.

2. Why does secretion of Scgb3a2 into BALF in transgenic mice decrease steadily as mice age?

3. N-values are needed for Figure 2 and Figure 4.

4. Figure S1: need better labeling of IHC – please state in the figure that this is IHC for Scgb3a2.

5. Do adult transgenic animals have normal numbers of ciliated cells?

6. For Figure 3, analysis of other genes involved in bleomycin-induced fibrosis would be useful. In particular, aSMA (Acta2) and CTGF in addition to matrix remodeling genes such as MMPs would be potentially helpful.

7. (Line 152) “as bovine serum” should be changed to “with bovine serum”

8. Figure legend for Figure 3: “monocygtes” is mis-spelled.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests