Reviewer's report

Title: Reduced transient receptor potential vanilloid 2 expression in alveolar macrophages causes COPD in mice through impaired phagocytic activity

Version: 0 Date: 28 Sep 2018

Reviewer: Fabienne Gally

Reviewer's report:

In this manuscript by Masubuchi et al., the authors investigated the role of TRPV2 in the development of COPD. They used in vitro and in vivo approaches to show that (1) cigarette smoke exposure decreases TRPV2 expression, (2) siRNA-mediated TRPV2 knockdown in cells or knockout in mice decreases phagocytosis, and (3) TRPV2-deficient mice have increased alveolar space enlargement.

This study highlights an important question about the contribution of TRPV2 in COPD, however I still have some major concerns about the data presented in the manuscript as outlined below.

1. The major results (i.e. increased alveolar space enlargement in TRPV2 knockout mice) should be presented in a figure pertaining to the original manuscript rather than a supplementary figure.

2. Please provide more details for the morphometry analysis in the Results section. Did you use an analytical software to measure the airspace enlargement? Were the airways and blood vessels excluded from the analysis?

3. Figure 1: Please give a plot with mean numbers (+/- SEM) to quantify TRPV2+ F4/80+ double positive cells over F4/80+ cells with the different treatments.

4. Figure 2: Are cells starting to die after 24 hours of 10% CSE? Has a viability assay been performed? The B-actin band intensity is decreased after 24 hours.

5. Figure 2: the legend does not correspond to the data shown.

6. Figure 3: How can luminescence be detected in the absence of FITC-dextran? A better representation would be to compare the intensity in vehicle versus CSE-treated samples,
which would make the description of the data in the manuscript less confusing. Also, the legend does not correspond to the data shown.

7. Figure 4: Not sure what Figure 4 is supposed to show. TRPV2 has already been shown to be expressed in macrophages. This figure could certainly be moved to Supplementary Material. A quantification and comparison with air-exposed mouse lung staining would give more sense to this Figure. Are the samples from 2 or 6 months CS exposure?

8. Figure 5: The description in the body of the manuscript does not match the data shown in the Figure.

9. Figure 6: The comments for Fig 3 can be applied here as well. Fig 6B is not that convincing and should mention TRPV2KO mice in the legend.

10. Supplementary Figure 3: Panels A and C are interesting but somehow redundant with Panels B and D. Consider showing pictures of non-smoked lungs and the same time-course of CS exposure in WT animals. This Figure should be part of the main manuscript.

11. Discussion: TRPV2 mRNA expression is discussed but no data are showing mRNA results.

12. The authors state that "TRPV2 may provide a therapeutic target for COPD" but is it clinically druggable? Please discuss.

Minor comments:

13. The source, rather than the generation of TRPV2KO mice should be described before the Mice paragraph in the Methods section. It would allow the removal of the sentence "contents of the intervention was similar to wild-type mice" which is difficult to interpret as stated.

14. For all the Western blot presented, a molecular weight should be mentioned for each assay.

15. Several typos can be found throughout the manuscript and need your attention:
- Are the BAL cells really spun at 3,000 g?

- A red secondary antibody was used for the immunofluorescence of TRPV2, please describe in the Methods section. Is the same truncated TRPV2 antibody being used for immunofluorescence, immunohistochemistry and Western blot?

- "All mice were euthanized by sevoflurane before tissue harvest"

- Remove "respectively" in the first paragraph of the Results section

- "Figure 1. Changes in TRPV2 expression in MH-S cells by immunofluorescence".

**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.

No

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

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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