Reviewer's report

Title: Genes Associated with MUC5AC Expression in the Human Small Airway Epithelium

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Reviewer: Burton Dickey

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Mucus hypersecretion is a cardinal feature of many lung diseases, but little is known about changes in gene expression that might be associated with variations in mucin gene expression in human airway epithelial cells in vivo. This manuscript provides an extensive gene expression analysis from peripheral airway brushed specimens in relation to the levels of MUC5AC expression, in normal subjects and in smokers. In general, this is a valuable addition to the literature, with only minor flaws as follows.

1) It might be helpful to add a few words to the title to better specify the populations studied, such as “… Expression in Small Airway Epithelium of Human Smokers and Non-Smokers”.

2) In the Abstract, first paragraph, it is stated that “MUC5AC is the major component of airway mucus”, which could be improved on two counts. First, it is a “major macromolecular component” (water accounts for a far greater fraction of the mass of mucus, and salts a several-fold greater fraction). The word “macromolecular” should also be inserted in the first sentence of the Discussion. Second, MUC5B is present at similar or greater levels (see many articles by John Sheehan and colleagues). Nonetheless, the authors are justified in focusing on MUC5AC since its expression is far more variable than MUC5B, and they should simply rephrase the sentence slightly. Similarly, on page 9, second paragraph, it is not necessarily true that MUC5AC is the dominant secretory mucin in surface airway epithelial cells, rather it depends upon how much MUC5AC is upregulated relative to MUC5B which is constitutively expressed at high levels. In peripheral airways, in particular, MUC5B is generally more strongly expressed than MUC5AC.

3) It would be helpful if the authors would make a brief statement about the contamination of their brushed specimens with epithelial cells from central airways. This information is probably provided in one of the group’s previous papers, but it would be helpful to have this information without retrieving those papers.

4) In Table I, how were secretory cells identified? Morphology? Immunohistochemical staining?

5) In Table II, it would be more elegant to identify the first two categories as “secreted” since the next two are “tethered”. In Supplemental Table I, the CLCA2
summary should read “involved in mucus hypersecretion”.

6) It is surprising that the manuscript does not cite and discuss the airway epithelial gene expression studies of Prescott Woodruff and John Fahy. Either this reviewer missed it in the manuscript, or the authors should provide a reason for not addressing, or they should cite and discuss.

7) It might be helpful to end the Discussion with a short summary paragraph. This could include a statement of the limitations of the study, one of which is that the authors have focused on a set of “MUC5AC Core Genes” that were selected in part based upon their annotation “making sense” in the context of differences in MUC5AC expression. This obviously is akin to looking for lost keys under the lamplight because that is where one can see, and runs the risk of not identifying the most important genes because clues to their possible role in mucus hypersecretion do not yet exist. Nonetheless the study is valuable in determining changes in gene expression in the light of currently known biology. Other limitations should also be addressed. Such a paragraph might end with a short take-home message.

**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'