Author's response to reviews

Title: Genes Associated with MUC5AC Expression in Small Airway Epithelium of Human Smokers and Non-smokers

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Author's response to reviews: see over
April 9, 2012

Dr. Paul Boutros  
Associate Editor  
*BMC Medical Genomics*

**Re: MS 1209127642679658, Wang et al**

Dear Dr. Boutros:

Enclosed please find our revised manuscript entitled “Genes Associated with MUC5AC Expression in Small Airway Epithelium of Human Smokers and Non-smokers,” along with a point-by-point response to all of the comments by the reviewers and in comments of the Associate Editor.

We appreciate the careful review and believe the paper is much improved with the changes made in response to this review.

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We look forward to hearing from you.

Sincerely yours,

Ronald G. Crystal, MD
Associate Editor's Comments

Associate Editor: “The authors need to integrate and cross-analyze their data in the context of the Woodruff and Fahy studies. How many genes are common?”

Response: We agree this would be useful. We analyzed data from the Woodruff and Fahy asthma studies and found 6 out 73 MUC5AC-core genes were up-regulated and 1 MUC5AC-core gene was down-regulated by asthma. We have amended the Methods (page 20, paragraph 2), Results (page 7, paragraph 1) and added supplementary Table VI, VII to incorporate these new data.

Associate Editor: “Are they in synchronous directions? Is the overlap more/less than expected by chance alone?”

Response: Our MUC5AC-core genes were significantly ($p<10^{-4}$) enriched in MUC5AC correlated genes in the Woodruff and Fahy asthma studies (see revised manuscript, supplementary Table VII). Considering the variables between our study and Woodruff study (different microarray processing, different sampling location, different study population), the similarities and differences reflected by the data are expected, but also very interesting. We have incorporated these finding in the Results (see revised manuscript, Results, page 7, paragraph 1).

Associate Editor: “Additionally they should investigate the behaviour of Her2/neu and add supplementary data showing it explicitly and possibly discussing why the results are/are-not unexpected.”

Response: In response to the comments regarding Her2, we performed correlation analysis of Her2 (ERBB2). Unlike in primary lung mucinous adenocarcinomas, we did not find a close correlation between Her2 and MUC5AC gene expression in the airway epithelium from healthy individuals. The results are expected, as tumor cells might activate MUC5AC gene expression in a different fashion. These data are included in the revised manuscript (see revised manuscript, Results, page 8, paragraph 2; Discussion, page 11, paragraph 3 through page 12, paragraph 1, and supplementary Table VIII).
Response to Reviewer # 1

Reviewer: Burton Dickey

Reviewer: “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’.

Response: We agree with the reviewer’s comment and have changed the title accordingly.

Reviewer: “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.”

Response: We agree with the reviewer’s comment and have changed the text accordingly.

(Revised manuscript, Abstract, paragraph 1, line 6).

Reviewer: 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.

Response: We agree with the reviewer’s comment and have changed the text accordingly.

Reviewer: 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.”

Response: We agree with the reviewer’s comment and have changed the text to “one of the major secretory mucins” (revised manuscript, Discussion, page 10, paragraph 2, line 1).

Reviewer: “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.”

Response: We agree with the reviewer’s comment and have added the text accordingly (revised manuscript, Method, page 16, paragraph 3, line 2 and 3)
Reviewer: “4) In Table I, how were secretory cells identified? Morphology? Immunohistochemical staining?”

Response: The secretory cells were identified by morphology after DiffQuick staining. We have added footnotes to the Table I to clarify this (revised manuscript, Table I, footnotes 4).

Reviewer: “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’.”

Response: We agree with the reviewer’s comment and have changed the text accordingly (revised manuscript, Table II; Supplemental Table I, page 5).

Reviewer: “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.”

Response: We agree with the reviewer’s suggestion and analyzed the data from Woodruff and Fahy study. We found 6 out 73 MUC5AC-core genes were up-regulated and 1 MUC5AC-core gene was down-regulated by asthma. And interestingly, MUC5AC-core genes were significantly enriched in the MUC5AC correlated genes from this asthma study. These data have been incorporated into the revised manuscript (revised manuscript, Supplementary Table VI and Supplementary Table VII; Methods, page 20, paragraph 2; Results, page 7, paragraph 1).

Reviewer: “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.”

Response: We agree with the reviewer’s comment and have changed the text accordingly (revised manuscript, Discussion, page 15, paragraph 2).

Reviewer:

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’
Response to Reviewer # 2

Reviewer: Giulio Rossi

Reviewer: “In this work, the authors identified by gene expression profiling the genes significantly up-regulated in healthy nonsmokers subdivided in two groups: high MUC5AC expressors and low MUC5AC expressors. At the end, the authors identified a list of 73 MUC5AC-associated genes with different roles in mucin composition and functions. Identification of these genes could be helpful in selecting therapeutic targets in inhibiting mucus hypersecretion.

I have just a minor comment/curiosity about this paper.

Among up-regulated genes, the authors did not identify HER2/neu. In fact, some works have highlighted overexpression of HER2/neu in primary lung mucinous adenocarcinomas (so-called mucinous bronchioloalveolar carcinoma) (see Casali C et al J Thorac Oncol 2010; Zhang Y et al Clin Cancer Res 2012). These tumor entity is characterized by KRAS activating mutations as well as very high levels of MUC5AC production.

In light of the possible recognition of MUC5AC-related target genes, have the authors the possibility to comment this fact in the discussion section of the paper?”

Response: We agree with the reviewer that HER2 related pathway might be involved in the regulation of MUC5AC expression. In response to the reviewer’s comment regarding the relation between HER2 (ERBB2) and MUC5AC expression, we did genome-wide correlation analysis for HER2. Based on pathway analysis, ERBB2 or EGFR related pathways, but not mucus production related genes or pathways, are among the top 5 pathways enriched in HER2 correlated genes. This data suggests that the activation status of HER2 (e.g., phosphorylation) might be more important in normal airway epithelium. On the other hand, in lung mucinous adenocarcinomas, persistent activation of HER2 by consistent up-regulation of HER2 or HER2 mutations might contribute to the abnormal MUC5AC expression. These data and a more detailed explanation of the results can be found in the revised manuscript (Supplementary Table VIII; Results, page 8, paragraph 2; Discussion, page 11, paragraph 3 through page 12, paragraph 1).

Reviewer:

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
'I declare that I have no competing interests'