Author’s response to reviews

Title: CD147 increases mucus secretion induced by cigarette smoke in COPD

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Author’s response to reviews:

Sunil K. Nooti, MBBS, Ph.D (Reviewer 2):

1. The authors did not answer specifically when the diagnosis of COPD was made for the third group. While CD147 and MUC5AC are increased in smokers with COPD, over and above the levels in smokers without COPD it would only be a correlative evidence. But if CD147 and MUC5AC levels varied with the onset of COPD in the third group, it would be interesting to note whether increase in CD147 preceded the increase in MUC5AC. I don't see why the authors should not show the actual data and discuss at least in brief when they most probably have the clinical data for COPD onset and history.

Answer: We reanalyzed all the data actually and made a more detailed table, including the smoke years of all the subjects and the GOLD stage. We found that although there is no statistical significance of the pack-years of smoking history between smokers with COPD and smokers without COPD, the actual pack-years of smoking history is higher in smokers with COPD, what’s more we analyzed the years of smoking and we found that the years of smoking in smokers with COPD is higher than smokers without COPD and there was statistical significance.

It is hard to know the CD147 and MUC5AC levels with the onset of COPD since patients are engaged because of pulmonary nodules, when can only know the clinical symptoms and when they were diagnosed as having COPD. For all the COPD patients, the main clinical symptoms are cough and sputum.

It is really a great idea to explore the levels of CD147 and MUC5AC with the onset of COPD, but it is hard to get enough lung samples since we don’t know whether they will have pulmonary nodules in the future. As your wonderful suggestions we plan to collect the sputum and blood of different stages of COPD patients and to further learn whether increase in CD147 preceded the increase in MUC5AC and the relationship among CD147, MUC5AC and COPD exacerbation.
2. It is indeed interesting and commendable that the authors were able to get COPD lung tissue, which is a salient feature of this manuscript. However, though the authors reply that "specimens from lung cancer may have influenced the responses", it is not clear why they should not present the data of the diagnosis and reasons for surgical resection of the pulmonary nodule for each patient in the form of a table or supplementary info. All the clinical data summarized in the methods section of "subjects" could be shown in the form of a table for clarity and let the reader decide on their effect.

Answer: These specimens were collected from the department of cardiothoracic surgeon, they only supplied and shared a part of the clinical data of these patients. We don't have the methods section of these “subjects”. It is a normal phenomenon to share a part of clinical data since the doctors in the department of cardiothoracic surgeon also need these data for their own researches. We are sorry that we cannot show all the clinical data.

3. Smoking and Vaping has been shown to increase MUC5AC secretion (PMID: 29481290). Increase in CD147 and consequent MMP9 regulation is also known (ref 19 in the manuscript). CD147 regulating MUC5AC through MMP9 and p38 MAPK is the main result of this study. However the author's use of non-specific chemical inhibitors is a weak point as I had mentioned earlier. SB-3CT is not only MMP9 inhibitor but also inhibits MMP2 (PMID: 25150065). If the authors cannot use more specific inhibitors they should at least dissect the role of MMP2 and include the same in the discussion about mechanism.

Answer: We have taken into consideration the specificity of the inhibitors, we also detected MMP2 expression after we transfected the CD147 siRNA, and we found that there was no difference of MMP2 expression between CD147 siRNA transfection and control transfection. We will add the MMP2 expression results in the revised manuscript.

4. Similarly SB203580 though is commonly used to inhibit p38 MAPK catalytic activity, it is also known not to inhibit the phosphorylation of p38 MAPK by upstream kinases (PMID: 10512765). 10µM SB203580 concentration as used in this study is also known to inhibit phosphorylation and activation of PKB (AKT) by inhibiting the PKB kinase, phosphoinositide-dependent protein kinase 1. Extreme caution has been advised when interpreting data where SB203580 has been used at concentrations above 1-2 µM (PMID: 10702313). Hence the authors should try using lower concentrations for SB203580 or use a more specific inhibition and elaborate mechanistic discussion in light of the above.

Answer: Same as the above answer. We also detected phosphorylation of Akt after we transfected the CD147 siRNA, and we found that there was no difference of phosphorylation of Akt between CD147 siRNA transfection and control transfection. We will add the results of phosphorylation of Akt in the revised manuscript.

Minor concerns:
1. The significance notation (* and #) in figure 6 is confusing. It is not obvious and clear unlike in other figures, as to what they are significant to.

Answer: We have revised our manuscript.
2. The authors have indeed addressed most of the English language concerns of mine.

3. The authors should also include referring to more recent articles published which are relevant in
the role of CD147 (PMID: 30005623) and MUC5AC production (PMID: 29906464).

Answer: We have added some recent articles in the revised manuscript as your suggestion.