Peer Review File

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Reviewer A

1) I was surprised that the authors state that they measured the concentration of WNT5a by ELISA from R&D systems. To my knowledge this company never sold an ELISA for this cytokine. Yet it is not so easy to find a good ELISA test system for WNT5a on the market. Thus the authors should clearly indicate which ELISA from which company was used.

Reply 1：We clearly indicate that ELISA of WNT5a is ELK5544, Elkbiotech, China in the chapter of ELISA line 175.

2) An ethical statement for the animal experiments is missing.

Reply 2：We have added an ethical statement for the animal experiments in the chapter on animal experiments line 131: Animal experiments were performed according to the Chinese Association for Laboratory Animal Science Policy, and were approved by the Institutional Animal Care and Use Committee of Guangzhou Medical University.

3) Line 145: Which manufacturer for which kit you are referring to?

Reply 3：We added the of SABC-POD (Mouse/Rabbit IgG) Kit(SA1020, BOSTER, China).

4) Line 150: please cite the previous report

Reply 4：We cited the previous report(5).

5) Line 303: form of citation is wrong

Reply 5：We have removed the incorrect form.

6) Line 366: What is meant with enhancement of WNT5a? Increased production? Please improve

Reply 6：Changes in the text: increase the production of Wnt5a.

7) Last sentence of conclusion: “These findings provide new insights into potential COPD therapeutics…” the authors did not show data for COPD therapeutics… please stick to the things that were really shown.

Reply 7：Suppressing the Wnt5a/JNK pathway provides new insights into potential COPD therapeutics targeting the Wnt5a/JNK pathway.

8) Line 337: “We further demonstrated that PM2.5 phosphorylated JNK and NF-κB…” please improve the sentence PM2.5 does not directly phosphorylate proteins.

Reply 8：Changes in the text: PM2.5 increased the levels of phosphorylated JNK and NF-κB

9) Line 73: “Wnt5a, a noncanonical Wnt pathway, can trigger…” please improve sentence, WNT5a by itself is not a pathway…

Reply 9：Changes in the text: which is a member of the noncanonical Wnt glycoprotein family,

10) Proof-reading should be done by a native speaker.

Reply 10：The article has been revised by a native speaker.

Reviewer B

This is an interesting manuscript looking at the BAL levels of Wnt5a in healthy and COPD subjects and linking it to a high or low PM2.5 exposure. The authors then conducted experiments using an animal model and cell culture of airway smooth muscle cells to investigate a potential mechanism. Here PM2.5 exposure was linked to activation of NFkB together with Wnt5a/JNK pathways with increased production of actin and collagen and increased inflammation. All of this could be blocked using inhibitors or Wnt5a siRNA.

My main points are:

1 The authors mention numerous times the correlation between PM2.5 exposure and Wnt5a BAL levels in healthy and COPD subjects. Figure 1 just shows a bar graph and not a correlation. Do the authors have the individual subjects PM2.5 exposure levels which they could plot against the Wnt5a levels? This would give a much better view of a correlation and should be shown instead of the bar graph. If this is not available then the data in the bar graph needs to be shown as individual data points (dot plot).

Reply 1：Thanks for the editor's suggestion. We cannot obtain the individual subjects PM2.5 exposure levels.
Changes in the text: the data in the bar graph be shown as individual data points (dot plot).

It could be that the subjects living in the higher polluted areas have a different life style which could account for the differences. For example smoking is known to upregulate Wnt5a, is the smoking history of the subjects known? Also it was not clear if COPD disease severity was the same in the 2 areas (low & high PM2.5). Would a correlation between Wnt5a and PM2.5 exposure be seen when looking at the different GOLD stages, since no difference is seen in healthy subjects?

Reply 1：We have these description in 2.1 Clinical specimens and patients line 104 : excluding standards for patient enrollment: 1) They had smoked five packs of cigarettes every year at least in their life.
We have these description in discussion line 356:There are not enough cases in this study and COPD patients (stage I or II) is not specifically differentiated. People's living environments are influenced by many uncontrollable factors, such as climate and time spent outdoors.Therefore a larger sample size may be needed to demonstrate the association of PM2.5 concentration with Wnt5a in COPD.

2 Regarding the animal model: The PM2.5 was injected into the trachea. This is not the normal way an individual would be exposed to PM. Can the authors explain why they chose this model and did not use an aerosol method? Would the results be the same?

Reply 2：We added explain in discussion line 360: we should use an aerosol method, which is the normal way an individual is exposed to PM2.5. However, PM2.5 was injected into the trachea of animals in this study due to limitations in the amount of PM2.5 and laboratory conditions. We attempted to ensure that PM2.5 was uniformly injected into each trachea during the modeling process, and the results of the two models were similar.

3 The study used healthy mice and then injected PM2.5 twice a week for a month. The authors should also have used another group with established COPD to look at the effect of PM2.5 on these. Does it make the COPD (lung function etc) worse. Is more inflammation seen?

Reply 3：Thanks for the editor's suggestion. Changes in discussion line 360: Moreover, in future experiments, we will add another COPD models to observe the effects of PM2.5 on lung function and inflammation.

4 The authors should comment on the level of PM2.5 administered to their animals, 100ug/20ul. Is the amount used a reflection of the low or high levels of PM used to characterise the human subjects? This amount seems very high.

Reply 4：Changes in the results of section 3.2: Based on the structural changes in alveoli, lung function and survival rates of mouse, the level of PM2.5(100μg/20μL) administered to animals according to the results of our previous animal model(5).

5. Very few details given regarding the cell culture experiments. Were the ASMCs from healthy subjects? A n=3 is stated, is this 3 experiments using the same donor cells or are these from 3 different donors? What was the concentration of PM2.5 used in these cell experiments? Only one concentration used? A dose response of increasing PM2.5 concentration on Wnt5a would have been useful. Was the PM2.5 characterised at all? Do

you know the chemistry? e.g. different metal content.

Reply 5：Changes in the results of section 3.4: Based on our previous results, A 24 h exposure to PM2.5 (3 μg/mL) promoted ASMC proliferation (5), and this concentration of PM2.5 was selected for follow-up experiments.

Changes in discussion line 360: it would be ideal to use primary human bronchial smooth muscle cells for experiments, but due to technical limitations, we used a human bronchial smooth muscle cell line, and we cannot exclude the possibility of differences.

Changes in the section of PM2.5 Preparation line 124: The mean concentrations of polynuclear aromatic hydrocarbons (PAHs) and n-alkanes in PM2.5 were 108.453 μg/g and 18,670.883 μg/g, respectively, with a final PAH recovery of 44.62%; the mean concentrations of these DMSO extracts were 48.392 μg/g and 164.675 μg/g, respectively. The collections and analysis methods of the PM2.5 samples were based on our previous research(5).

6. Have you done the same experiments in ASMCs from COPD patients? Do they respond the same regardless of disease severity?

Reply 6：Changes in discussion line 360: it would be ideal to use primary human bronchial smooth muscle cells for experiments, but due to technical limitations, we used a human bronchial smooth muscle cell line, and we cannot exclude the possibility of differences.

7. Did the PM2.5 affect the ASMC viability? Did you measure this? How?

Reply 7：Changes in the results of section 3.4: Based on our previous results, A 24 h exposure to PM2.5(3 μg/mL) promoted ASMCs proliferation (5), the concentration of PM2.5 were selected for follow-up experiments.

8. The English is poor, in the abstract line 31 it reads as if the siRNA and inhibitors were given after the PM2.5..."following PM2.5 treatment" and then in section 2.2 line 116 it states "pretreated with BOX5 etc then subsequently exposed to PM2.5 in medium for 24h". I am assuming cells were pretreated with the inhibitors and siRNA? Do the inhibitors still suppress the effects of PM2.5 if given after the PM2.5? Would this be a useful therapy for established COPD patients?

Reply 8：Changes in the text: “before PM2.5 stimulation” in the abstract line 31. Our subsequent experiments will validate this idea which the inhibitors still suppress the effects of PM2.5 if given after the PM2.5 in vitro.There is still a lot of work to be done to truly use these inhibitors for COPD patients. And we also added description in discussion line 360: Moreover, in future experiments, we will add another COPD models to observe the effects of PM2.5 on lung function and inflammation.

9. Did you look at any other timepoints other than 24hrs in the cell culture experiments? This is quite a late time for phosphorylation as this often happens in the first 8 hours. A later time point would also be interesting to see what happens to the IL-6, L-8, TNFa, Collagen and alpha-SM actin.

Reply 9：Based on our extensive preliminary experiments, we observed changes in inflammatory markers and fibrosis after 24 hours of PM2.5 stimulation, meanwhile, the changes in the molecular mechanisms related to the Wnt5a/JNK pathway were observed.We have added these description in the results of section 3.4: Based on our previous results, A 24 h exposure to PM2.5(3 μg/mL) promoted ASMCs proliferation (5).

10. The English is very poor and difficult to follow in places. The reader is left to look at the figures to fully understand the text in the results and discussion. English needs improving.

Reply 10：The article has been revised by a native speaker.