Peer Review File

Article information: https://dx.doi.org/10.21037/tcr-23-1236

Reviewer A

This work has merit but the following should be addressed before considering publication:

1. The analysis is confined to murine lung tumors. This places its relevance to human disease into question. Please justify why this mouse model is needed for these studies, when human samples have already been previously studied. This major limitation along with the other limitations of the study should be discussed in more detail the Discussion.

Reply 1: Thank you for your suggestion. We have explained the use of mouse models and stated the limitations in the Discussion section of the article.

"Furthermore, it should be noted that while human samples have been extensively studied in previous research, the mouse model provides us with a unique opportunity to explore the early stages of the disease, which is difficult to achieve with human samples. The use of murine lung tumor models, while not directly translatable to human disease, offers several advantages. Firstly, it allows for controlled studies in a genetically homogeneous population, eliminating many confounding factors present in human studies. Secondly, mouse models provide an opportunity to explore tumor development and progression in real-time, enabling a more comprehensive understanding of the disease. However, it is essential to note that findings from this study should be interpreted with caution when translating to human lung adenocarcinoma. We acknowledge this as a limitation, as mouse models may not fully reflect all nuances of human disease and further studies using human samples will be needed to validate our findings." (see Page 20, line 426-438)

2. Methods – please explain better the data pre-processing and handling of missing values

Reply 2: As suggested, we have added instructions on data preprocessing and missing values in the Methods-Statistical Analysis section.

"For data pre-processing, raw metabolomics data were subjected to normalization to account for differences in sample concentrations. Peaks were aligned across samples, and the intensities were scaled using a standard internal control. Handling of missing values was carried out using imputation techniques. For metabolites with less than 20% missing values, the missing data were imputed using the k-nearest neighbors algorithm. For metabolites with more than 20% missing values, they were removed from the dataset to ensure robust statistical analysis." (see Page 10, line 211-217)

3. The text within all the figures (except Figure 1) is unreadable – should be bigger.

Reply 3: As suggested, we have made the font size larger in the image.

4. Introduction – there has been extensive previous work looking at urine and plasma
samples from lung cancer patients, including in several review papers. This manuscript cites only two papers (references [25] and [26]). Please update so that this work can be placed in better context.

Reply 4: Thank you for your suggestion. We have added relevant content to the introduction to enrich the background of metabolomic analysis of metabolite differences in lung cancer patients. "In addition to the studies mentioned above, many other studies have also investigated metabolite changes in lung cancer patients in urine and plasma samples. For example, Kawamoto et al. (31163629) discovered specific metabolite markers associated with lung cancer progression, while Han et al. (33414999) used a metabolomic approach to reveal mutated genes that affect metabolic differences between lung cancer and healthy individuals." (see Page 7, line 133-138)

5. Please discuss how the key metabolites found in this study compare to those in previous studies, keeping in mind that this a mouse model while many other studies used human samples.

Reply 5: Thanks for your suggestion, we have discussed the key metabolites in the article in conjunction with previous literature. However, due to the limited number of studies currently targeting our differential metabolites in lung cancer patients/cells, less discussion can be conducted.

"Unfortunately, 12(R)-HETE and hippuric acid have been less studied in lung cancer, both in cell/mouse models and in lung cancer patients. 12(R)-HETE, is a metabolite derived from arachidonic acid. 12(R)-HETE is formed by the metabolism of arachidonic acid by enzymes such as 12- and 15-lipoxygenase in human platelets and polymorphonuclear leukocytes and has several biological activities that can be assessed in models of inflammation. Hippuric acid has been described as the intestinal microbial mammalian co-metabolite of benzoic acid. This means that both gut microbes and mammalian cells (especially liver cells) are involved in its formation. However, no studies have found its role in lung adenocarcinoma. Our study is the first to propose the effect of IDO inhibitors on 12(R)-HETE and hippuric acid, and its mechanism needs further study." (see Page 19-20, line 415-425)

Reviewer B

1. Please add a brief introduction to the study background in the "Background" section of the abstract.

Author's response: As suggested, we have supplemented the background section of the abstract.

2. You refer to "studies" with only one literature citation several times in the main text. Please check and revise.

Author's response: As suggested, we have modified our use of "studies". (Page 5, line 106; page 20, line 437)

3. Please indicate where to cite Figure 4, 5, and S3 in the main text.

Author's response: Due to an oversight, we omitted a section in the submitted version regarding 'Differential Metabolite Analysis in Early-Stage Lung Adenocarcinoma Mice at Day 28.' This section describes the results depicted in Figures 4 and 5, as well as Supplementary Figure 3. We have now rectified this and included the omitted results in the revised version.

4. Please add the title/unit of the X-axis in every subfigure of Figure 2 and 6

Author's response: As suggested, we have added the X-axis in every subfigure of Figure 2 and 6 .

5. Words in Figure 3D are upside down.

Author's response: As suggested, we have modified Figure 3D.

6. Please provide Figure S5.

Author's response: As suggested, we have uploaded Figure S5 in the submission system.

7. The caption of Figure 3 is exactly the same as Figure 2. Please check and revise. So do Figure 5 and Figure 4.

Author's response: Thanks for your suggestion. We have changed the titles of Figures 2, 3, 4, and 5.

8. There is no blue box in Figure 3 and 5.

686 Differential metabolites with a green box indicate significant differences (OPLS-
687 DA VIP > 1 and P value < 0.05) used for subsequent analyses. Differential
688 metabolites with a blue box indicate differences (OPLS-DA VIP > 1 and P value <
689 0.1) not involved in subsequent analyses.↵

Author's response: Thanks for your suggestion. We have revised the legends of Figures 3 and 5.

9. Provide a caption for Figure S5.

Author's response: As suggested, we have added the legend of Figure S5.