Peer Review File

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Reviewer A

In this article, Zhu et al. hypothesized that the expression of membrane tension-related genes could predict the prognosis of lung adenocarcinoma (LUAD). The authors analyzed the TCGA LUAD dataset and identified five membrane tension-related genes associated with prognosis. Based on these five genes, the authors constructed a prognostic model. They explored the relationship between the prognostic value calculated using this model and the estimated immune cell compositions for the LUAD tumors in the TCGA database. The authors conclude that their model could predict the prognosis of LUAD and suggest that the five membrane tension-related genes are potential therapeutic targets.

Several major issues in study design and data interpretation negatively affect the scientific merits of this study.

Major critiques:

1. The clinical utility of this prognostic model is unclear. As shown in Figure 4A-B, the risk scores calculated based on the model had a negligible impact (HR: 1.060) on the prognosis of LUAD, despite a good statistical significance ($p<0.001$). In contrast, the tumor stage had a much bigger impact (HR: 1.633) on the prognosis. The author may consider integrating tumor staging in their prognosis-predicting model.

Reply1: Thank you for your comments. Regarding the prognostic model developed in this study, there is some uncertainty regarding its clinical usability, but our goal is to develop and validate a prognostic model for a specific clinical condition, and to provide more possibilities for further research. We will incorporate tumor staging into our prediction model to improve its predictive performance, as TNM staging is a common consensus in which patients with later stage have poorer prognosis. Therefore, this study did not explore tumor staging extensively. However, as risk scores are the main focus of this study, we will supplement the article with a figure showing the relationship between tumor staging and risk scores.

Change in the text: We have modified our text as advised (see Page 10，line 1-8).

2. The correlations identified in Figure 6 E-G were driven by 1-3 data points. If these points (which should be considered outliers) were excluded, the remaining data points do not appear to show any correlations.

Reply2: Thank you for your comments. After carefully reviewing the data and considering your comments, we agree that there were some data points that may be considered outliers and may have influenced the correlation analysis. So we decided to delete the Figure 6 E-G and upload the reformatted Figure 6.

Change in the text: We have modified our text as advised (see Page 11，line 8-18), (see Page 24，line 3-4)

3. The authors did not include an independent dataset (a non-TCGA dataset) to validate their findings.

Reply3: Thank you for your comments. We agree that an independent dataset would be valuable in validating our results, and we appreciate your suggestion. Unfortunately, we did not have access to an appropriate independent dataset during the course of our study. However, in order to reduce the potential impact of individual data sets on our findings, the TCGA cohort has been divided into Training, Testing and Whole sets for internal validation and the results of the three groups can be validated against each other with good biological significance. Meanwhile, TCGA database is the largest database of cancer genetic information, the most abundant resources and the most authoritative data database. We will also explore combining the analysis with other datasets in the future to further validate our own research. Thank you again for your valuable comments.

4. The study only evaluated the prognostic values of these MRGs and provided no data to show that these MRGs are important for tumor growth. Therefore, it is not accurate to conclude that 'targeting MRGs at the cellular level provides a new idea to promote personalized cancer therapy'.

Reply 4: Thank you for your comments. We agree that our study focused on evaluating the prognostic values of the MRGs, and we did not explore the biological mechanisms underlying their roles in tumor growth. We understand that this limitation may affect the significance of our conclusions and have revised the manuscript accordingly. Although we did not provide data to demonstrate that targeting MRGs can directly affect tumor growth, our findings suggest that these MRGs have the potential to serve as therapeutic targets for personalized cancer therapy. This is because the expression levels of these MRGs were significantly associated with patient prognosis, indicating their functional relevance in disease progression.

We appreciate your constructive feedback, and we have modified our conclusion to be more precise.

Change in the text: We have modified our text as advised (see Page 18, line 9-10)

Minor critiques:

1. Line 196-197 UCSC should be 'the University of California Santa Cruz'.

Reply1: We agree with you and revise it in the manuscript.

Change in the text: We have modified our text as advised (see Page 7, line 15-16)

2. Hazard ratio should be shown in Figures 2D-E, and 3G-3I.

Reply 2: Thank you for your comment suggesting the inclusion of hazard ratios in Figures 2D-E and 3G-3I. We apologize for the confusion and would like to explain that we did not include the hazard ratios in these figures because they were not computed in our study. Since we read a large amount of literature showing that after survival difference analysis, significant p-values were obtained that could represent important biological implications. Thank you for your understanding.

3. Figure 5C-D. What are the biological significances of these pathways? The Discussion did not provide much insight.

Reply3：Regarding the pathway in Figure 5C-D, both pathways were derived from enrichment of membrane tension genes with differential expression, indicating an intrinsic connection between membrane tension genes and their associated pathways. However, due to the complexity of the pathways, related research is scarce, and we are unable to further elaborate on their significant biological implications. If there are further studies on these two pathways in the future, we will continue to pay attention." Thank you for your understanding.

4. Line 338-339: aDCs stands for 'activated dendritic cells' instead of 'antibody-drug conjugates'.

Reply4：Thank you very much for the valuable feedback from the reviewer. I appreciate it greatly and am grateful for pointing out my mistake.

Change in the text: We have modified our text as advised (see Page 12, line 3)

Reviewer B

Here, Prof. Pu and collaborators developed a novel prognostic model based on membrane tension and combined with immune status. The study is a good example of exploring the predictive ability of membrane tension on the prognosis of LUAD patients.

1. English needs careful revision.

Reply1：Thank you for your valuable comments on our manuscript. We have worked hard to revise this English manuscript carefully before submitting it. We appreciate your comments and will devote more time to ensuring the quality of future English manuscripts.

2. How can authors be certain that these five genes (FLG, SLK, CFL1, PECAM1, and ITGB1) are membrane tension genes?

Reply2：Firstly, our research team reviewed the literature to find the pathways related to membrane tension (which can be reflected in the references), and then we checked the GSEA/MSigDB (https://www.gsea-msigdb.org/gsea/msigdb/human/search.jsp), KEGG (https://www. kegg.jp/kegg/pathway.html) to review relevant genes about membrane tension pathways, and composed a gene set of each pathway gene for subsequent analysis, because this gene set was collected by ourselves and not an official gene set, so it was not mentioned or cited in this manuscript. The relevant analyses in this manuscript are based on this gene set, so these five genes were screened through a series of analyses, so they are considered as membrane tension-related genes.

3. It is not clear why it is necessary to use single-cell sequencing to explore the predictive ability of membrane tension on the prognosis of LUAD patients.

Reply 3：We used single-cell sequencing to identify cell subtypes and explore the expression of prognostic membrane tension genes at the cellular level. This approach enabled us to identify the potential roles of membrane tension genes in the pathogenesis of LUAD at the cellular level.

Due to limited technical capabilities of the personnel involved in this study, we could only explore the correlation between membrane tension genes and single cells, providing a new avenue of research for other investigators. We will continue to explore it later with our enhanced analytical capabilities. Thank you for your valuable comments.

Reviewer C

The study aimed to construct a prognostic model associated with membrane tension-related genes (MRGs) and explore its prognostic value in LUAD patients. Discovered that patients in the low-risk group had a better prognosis than those in the high-risk group, and confirmed that the model had a better predictive value for LUAD patients. GO and KEGG analyses of differential genes in the high- and low-risk groups were significantly enriched in immune-related pathways. I hope the author can continue to apply this study to prospective studies of clinical patients and further validate the clinical significance of the study.

Reply：Thank you very much for your suggestion. We also hope to further validate the clinical significance of our study in prospective studies involving clinical patients. Due to the multiple issues involved in clinical patient data collection and the busy clinical schedule of the investigators, a corresponding prospective clinical study has not been conducted. We will take it into consideration for our future research. Thanks again for your valuable feedback.

Reviewer D

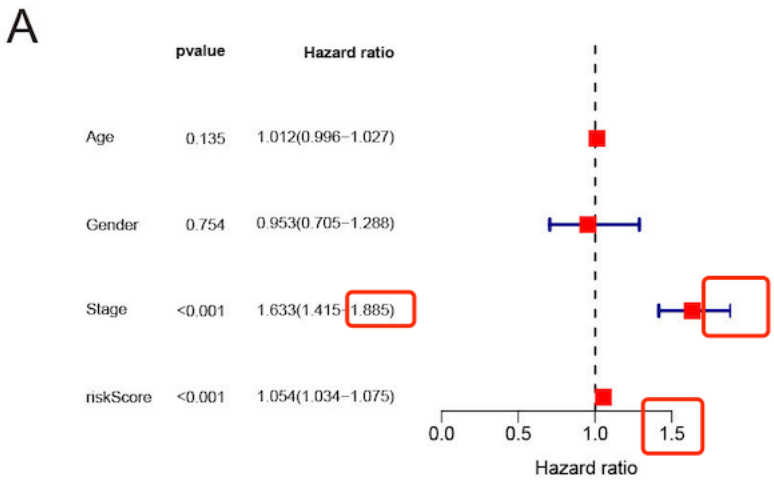
The Authors provide a very deep analysis of a new panel for potential prognostic factors in primary lung adenocarcinoma. The paper is a really interesting piece of work in applied genetics and might add consistent knowledge to the current scince. Besides the paper has several limitations in its current form. The body of text is pretty wordy and really difficult to follow in its development. I think the paper needs a gross cut and represented in a more suitable form. At the moment, a fast reading is impossible and to take out a simple message/info from a specific paramenter is almost impossible. English revision needed. tables and figures good but too many.

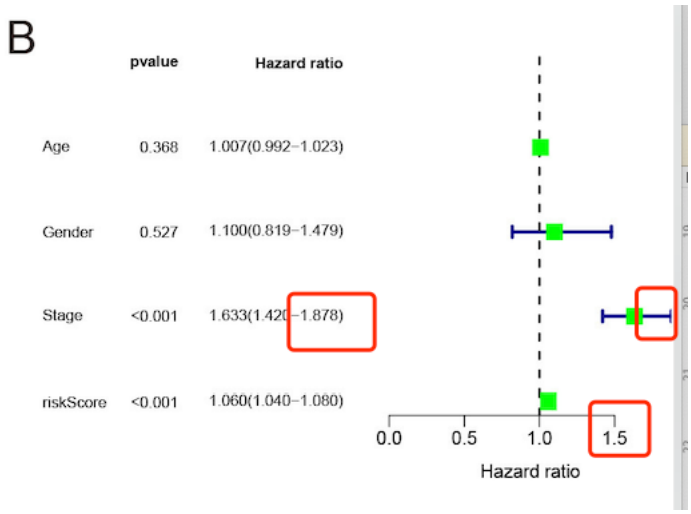
Reply：Thank you for bringing this concern to our attention. We understand that our manuscript covers a broad range of topics, which may have led to some lack of continuity in the text. Nonetheless, we believe that all the content is relevant and necessary to support the main arguments of our study. We will take your feedback into consideration and pay attention to the structure and readability of the manuscript in future studies, while also maintaining its comprehensiveness. Thanks again for your valuable feedback.

Reviewer E

1. Figure 4

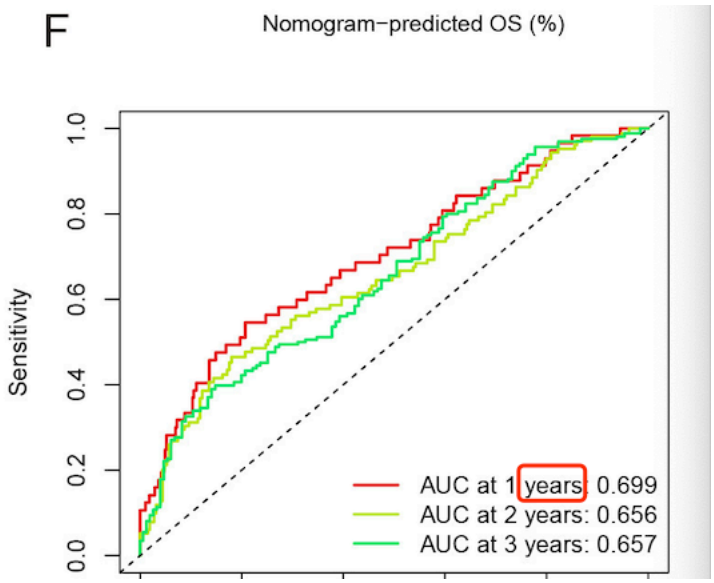
a) To standardize the results, the part that exceeds the horizontal coordinates should be indicated by arrows.





Reply(a): I have increased the horizontal coordinates to 2.

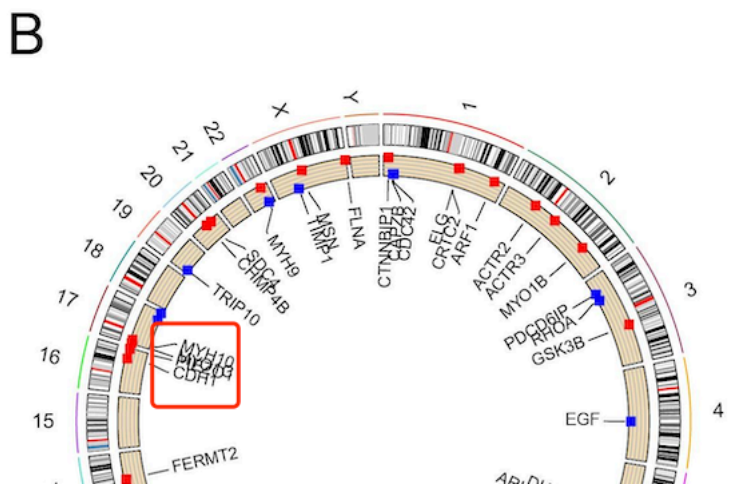
b) Here should be “1 year”, please revise.



Reply(b): I have revised.

2. Figure 6

Some words are covered, please revise.



Reply: I have shown the covered words.

3. Figure 8

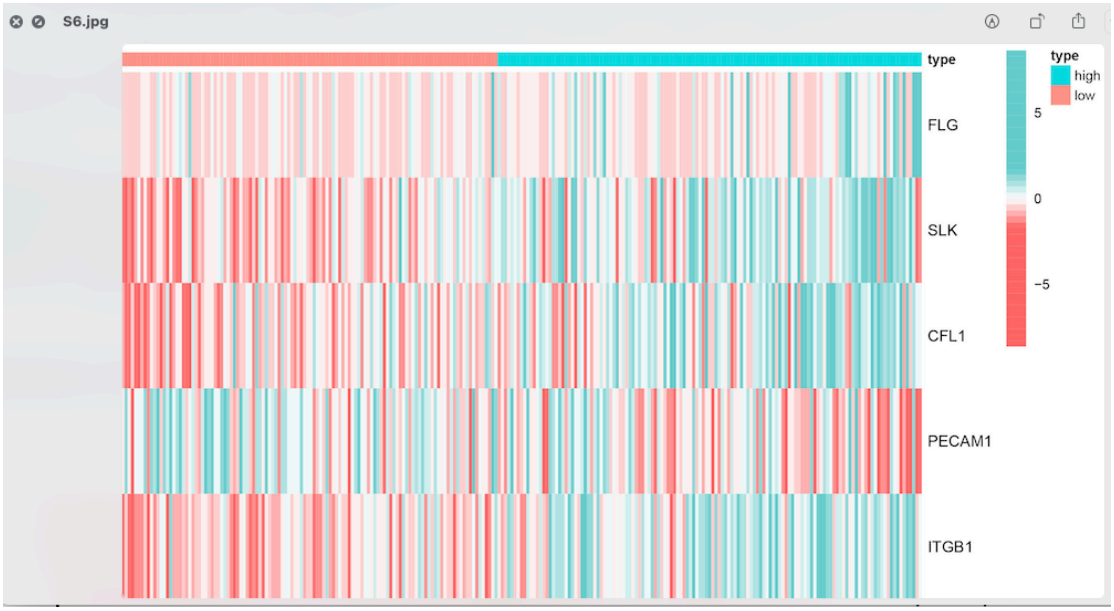
Please explain UMAP in the legend.

Reply: I have provided a detailed explanation of UMAP in the legend.

4. Figure S6

It seems that figure S6 is not a KM survival curves, please double check.

Figure S6 Kaplan-Meier survival curves for high and low expression levels of prognosis-related membrane tension genes.



Reply: I have modified the legend for FigureS6.

5. References/Citations

a) Please double-check if citations should be added as you mentioned “studies”.

Previous studies have shown that TMB is a valuable predictor of tumor immune response, and high TMB can benefit from ICIs. ~~A positive correlation between the risk~~

prognosis of LUAD patients. Previous studies have found that ERM proteins, which maintain membrane-actin adhesion in cancer cells, can be dissociated from the cell membrane, thereby making the cancer cell membrane more flexible, while cancer cells

pathway, Afadin is positioned at the adhesion junctions (34). Studies evaluating the effect of Afadin reorientation on intercellular adhesion by immunofluorescence-measured E-cadherin staining showed that Afadin exhibited definite intercellular adhesion, and that Afadin knockout with a specific shRNA reduced cell migration. The

Reply(a): We have added the corresponding literature and made corrections. Due to the revised format of the document, the citations are shown at the end of the text.

b) Please double-check if more studies should be cited as you mentioned “studies”.

and may also be involved in tumorigenesis. Studies have shown that shRNA downregulation of AIMP2-DX2 expression inhibits the EGFR/MAPK signaling pathway, thereby suppressing glucose uptake and cancer cell growth (32). Together, it

metastasis, proliferation, and prognosis of NSCLC tumors. Studies have reported that the expression of *CD276* in NSCLC tissues is significantly up-regulated, and its expression is positively correlated with the tumor stage of NSCLC. Silencing *CD276* inhibits cell invasion and migration by reducing integrin-related protein expression (53).

Reply(b): There is no need to cite more studies, I have changed the word "studies" to "study".