Peer Review File

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Reviewer A

The manuscript titled "Exploring the Impact of Centrosome-Related Gene Signatures on Uveal Melanoma Clinical Outcomes" by Yu et al. delves into the relationship between centrosome-related genes and clinical outcomes in uveal melanoma (UVM). Using data from TCGA-UVM and GSE22138, the study aimed to construct a centrosome-related gene signature composed of specific genes. The research findings suggested that this gene signature could effectively distinguish between high-risk and low-risk UVM groups in terms of cancer progression, inflammation, and immune response, shedding light on the significance of centrosome aberrations in UVM and the potential identification of valuable biomarkers.

However, several critical points require substantial revision:

1. Definition of Clinical Outcome: The manuscript discusses clinical outcomes without providing a clear definition. It is essential to specify what is meant by "clinical outcome." Does it refer to overall survival, disease-free survival, or another metric? Clarification is necessary.

Reply:

Thank you for helpful advice. This study focused on the overall survival of UVM. According to your helpful advice, we have reworded the title into “A centrosome-related gene signature for predicting the overall survival of uveal melanoma”. Moreover, we have replaced “clinical outcome” with “overall survival” throughout the manuscript.

2. Key Findings Clarification: In the "Key findings" section, stating "for UVM prediction" lacks specificity. It should explicitly state what aspect of UVM prediction the gene signature is related to. For example, "prediction of patient prognosis in UVM."

Reply:

Thank you for helpful advice. We have reworded the key findings clarification into "prediction of patient prognosis in UVM." (line number 44)

3. Methodology and Equation Presentation: In the Methods section, when describing the equation for risk score calculation, include a sigma sign (∑) to represent the addition of coefficients multiplied by gene expression values for the six markers. This will make the equation clearer.

Reply:

Thank you for helpful advice. According to your helpful advice, we have revised the Methods section and used a sigma sign (∑) to represent the addition of coefficients multiplied in the revised manuscript. (line number 84-85)

4. Fold Change Criteria Explanation: The manuscript mentions the criteria of FC>3/2 for uDEGs and FC<2/3 for dDEGs without explaining the rationale behind these specific cutoffs. The basis for selecting these criteria should be provided.

Reply:

Thank you for valuable advice. We used the criteria of FC>3/2 for uDEGs and FC<2/3 for dDEGs. The criteria were referred to previous studies. (ref 18-21 in the revised manuscript) The criteria can effectively distinguish genes with significant differential expression while avoiding excessive false positive and false negative results. We have added an explanation of the selection of the criteria in the manuscript and cited relevant literature (line number 89-91).

5. Statistical Analysis Packages: The manuscript should specify the statistical analysis packages or software used in the analysis. This information should be mentioned in each relevant results section to enhance transparency.

Reply:

Thank you for valuable advice. According to your advice, we specify the statistical analysis packages in the section of Statistical Analysis (line number 99-102) and the

relevant results section (line number 127, 142).

6. Results Clarity: All results sections need substantial revision to elaborate on how the bioinformatics analysis was conducted. For instance, in the "Construction of CA Gene Signature" section, clarify the criteria used to screen out 204 genes and how the final 16 genes were selected. Additionally, specify the sample sizes for the TCGA-UVM and GSE22138 cohorts in each analysis and detail which dataset was used for DEG identification.

Reply:

Thank you for valuable advice. We tried our best to explain how the bioinformatics analysis was conducted in the revised manuscript. Univariate cox regression analysis was used to screen out the 204 genes ($P < 0.05$) and LASSO regression was used to select 16 genes (1,000 iterations). We provided the more detailed criteria for the selection of genes (line number 112-115). TCGA-UVM contains 80 samples and GSE22138 contains 63 samples and TCGA-UVM cohort was used for DEG identification. We provided the information in the revised manuscript (line number 118, 128).

7. Clarification of Line 139-140: The statement "The existence of CA in a subtype of high-risk UVM has been confirmed, including M3 and BAP1 mutant" needs clarification. It's unclear whether M3 and BAP1 mutations are confirmed in high-risk UVM or if they are related to the existence of centrosome aberrations. Elaborate on this point for clarity.

Reply:

Thank you for valuable advice. We are sorry for the lack of clarity in the statement. M3 and BAP1 mutation have been recognized as two important predictors of poor prognosis in the UVM by numerous studies (ref 3,13, 15,23,24,25 and etc.). In our signature, high-risk group also had more frequent BAP1 mutations. The only previous study on centrosome in UVM confirmed that centrosome aberrations are more common in UVM in M3 (ref 13). In addition, their study also confirmed that MP46, a BAP1

mutant primary UVM cell line, has the highest level of centrosome aberration in primary UVM cell lines (ref 13). We have reworded the statement in the revised manuscript (line number 151-156).

8. Missing References: Add appropriate references for the statement made in lines 167-170 to support the claims made in the manuscript.

Reply: We are sorry for not citing the relevant references. We have correct the mistake in the revised manuscript (line number 177-180, ref. 37-41).

In summary, while the manuscript presents intriguing findings, it requires major revisions to address the aforementioned issues and improve overall clarity and transparency.

Reply:

We greatly appreciate the provided comments and suggestions, and we hope that the revised manuscript will be acceptable for publication.

Once again, thank you very much for your consideration.

Reviewer B

- Please provide the full names of "mRNA" "RNA-seq" "EMT" in the main text. Please also check through your article to make sure **all** the abbreviated terms have been defined when they **first** appear in the Abstract and the main text.

Reply:

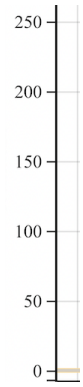
Thank you for valuable advice. We have provided the full names of "mRNA" "RNA-seq" "EMT" in the main text and checked our article to make sure all the abbreviated terms have been defined when they first appear in the Abstract and the main text

- Please recheck the full name of "GSE" and "M3".

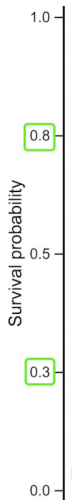
Reply:

Thank you for valuable advice. We have reworded the “monosomy 3” (M3) in the revised manuscript. However, we cannot make any change about “GSE” because “GSE” is a series of Gene expression omnibus (GEO) and the name of “GSE” is according to the principle of GEO and it cannot be changed.

- Please add description of the y-axis in Figure 1.



- Figures should be cited **consecutively** in the text and numbered in the order in which they are discussed. Therefore, Figure 2B should be cited before Figure 3A, unless Figure 2 is cited as a whole before. Please check through and revise.
- Please add unit for **Time** in Figure 2.
- Please recheck the scale bar of the y-axis in Figure 2B and Figure 3B.



- Please recheck the highlighted content in the following sentence since they are inconsistent with that in **Figure 3B**.

  “The high-risk group had worse survival compared to that of the low-risk group, HR (hazard ratio) was 4,942,441,957.67 and 2.9 respectively (Fig. 2B and 3B).”

- The citation of Figure 5D is missing in the text. Please add.
- Please recheck the citation of the following two sentences.

  “For cellular component (CC), DEGs were mainly enriched in extracellular matrix, collagen−containing extracellular matrix and plasma membrane protein complex. For molecular function (MF), DEGs were mainly enriched in cell adhesion molecule binding, actin binding and amide binding (Fig. 5C).”
- It is suggested to revise “pValue” to “P value” in Figure 5.
- It is suggested to unify the cell names in the figure and the text.

  “The results of CIBERSORT showed that CD4+ memory resting T cells, CD4+ memory activated T cells and monocytes were highly infiltrated in the high-risk group and CD8+ T cells, M0 macrophages and M1 macrophages were highly infiltrated in the low-risk group (Fig. 6A).”
- Please indicate the meaning of “*” “**” “***” “-” “.” in Figure 6 legend.
- Please recheck the number in the main text. There are only 15 genes in Figure 7.

  The top 20 frequent gene mutation frequency and patterns are shown in Fig.7 by Maftools R package.

Reply:

We have added the description of the y-axis in Figure 1.

We made some changes in the manuscript to make sure that Figures be cited consecutively in the text.

We are sorry for the mistake on the scale bar of the y-axis in Figure 2B and Figure 3B and we have corrected the mistake on the figures in the email.

We have corrected HR in the manuscript (line number 125).

We have added the citation of Fig.5D and corrected the mistake of the citation of Fig.5C (line number 134-136).

We have revised the “pValue” to “P value” in Figure 5 in the email.

We have reworded the cell names in the manuscript to unify the cell names in the figure and the text.

We have indicated the meaning of “*” “**” “***” “-” “.” in Figure 6 legend.

We have corrected the “20” to “15” in the manuscript (line number 144).