

Peer Review File

Article information: <https://dx.doi.org/10.21037/tcr-24-499>

Reviewer A

The study focused on m⁵C/m⁶A/m⁷G-related non-apoptotic regulatory cell death (NARCD) genes in hepatocellular carcinoma (HCC). Using data from The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC), researchers applied LASSO regression to identify relevant genes and constructed a prognostic model through multivariate Cox regression. This model was validated externally with ICGC data. Additionally, the study involved functional enrichment analysis and examined immune cell infiltration, drug response, and cellular communication in HCC. The findings suggested that patients in the high-risk group, as defined by the prognostic model, showed increased immune cell infiltration and enhanced response to certain chemotherapeutic agents and immune checkpoint inhibitors. The communication between tumor and immune cells via specific signaling pathways was also highlighted. This study effectively outlines a comprehensive study that leverages advanced genomic analyses and bioinformatics to explore the role of specific RNA modifications in the prognosis of HCC. This is timely and relevant, considering the increasing interest in the molecular underpinnings of cancer and the implications for targeted therapies. The study's focus on both chemotherapeutic and immunotherapeutic responses based on genetic profiles is particularly insightful and aligns with current trends in precision medicine.

However, while this study is informative and detailed, it is crucial to ensure accuracy in the nomenclature of RNA modifications. I suggest minor corrections regarding the use of superscripts and subscripts in the chemical modifications of the RNA. For precise guidelines on these notations, the researcher should refer to the Biochemical Journal's publication from April 26, 2019 (Vol. 476, Issue 8), which provides detailed standards for RNA modification nomenclature. Ensuring consistency with established scientific terminology will enhance the clarity and credibility of the research.

Reply: We consulted the article in response to the comments and revised m⁵C/m⁶A/m⁷G to m⁵C/m⁶A/m⁷G throughout the text.

Reviewer B

1. Please provide the full names of “m⁵C/m⁶A/m⁷G” “IGF” “MIF” “BMP” “LIGHT” in the abstract and “lncRNA” “cDNA” “M-MLV” “GAPDH” “TMN” “IGF” “MIF” “BMP” “LIGHT” 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: We have made changes as suggested, in the abstract: m⁵C/m⁶A/m⁷G: see Page 1, line 23; IGF: see Page 2, line 43; MIF: see Page 2, line 44; BMP: see Page 2, line 45; LIGHT: see Page 2, lines 45-46.

In the main text: lncRNA: see Page 3, lines 95-96; cDNA: see Page 6, line 192; M-MLV: see Page 6, lines 193-194; GAPDH: see Page 6, line 195; TNM: see Page 8, line 259; IGF: see Page 9, line 311; MIF: see Page 10, line 312; BMP: see Page 10, line 317; LIGHT: see Page 10, line 317

Additionally, we checked the full text and filled out the entirety of the FC, see Page 4, line 130.

2. Figures

- **All abbreviations** in figures and legends should be explained (including the Supplementary figures). “m5C/m6A/m7G” “HCC” “CNV” in Figure 1 for example. Please check all abbreviations and provide the full names in the corresponding legends.

Reply: We check all abbreviations and provide the full names in the corresponding legends.

- Please indicate the meaning of “*” “**” “***” in Figure 1 legend.

Reply: We have added the meaning of “*” “” in Figure 1 legend.**

- The words are too close in Figure 1G-1I.



Reply: We have separated words in the image and the updated image has been put into the manuscript.

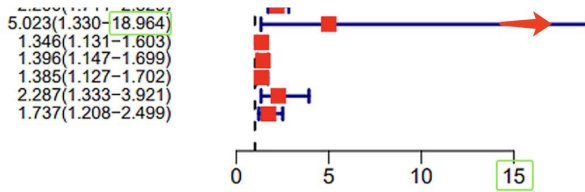
- Please unify “m5C/m6A/m7G” and “m⁵C/m⁶A/m⁷G” in Figures, legends and the text.

Reply: We have checked all the images and changed all m5C/m6A/m7G to m⁵C/m⁶A/m⁷G.

- Please revise “pvalue” to “P value” and “Hazard ratio” to “Hazard ratio (95% CI)” in Figure 2A, 4A, 4B.

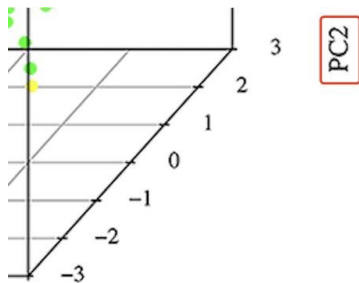
Reply: We have made changes as suggested and the updated image has been put into the manuscript.

- To standardize the results, the part that exceeds the horizontal coordinates should be indicated by **arrow** as below. Or you can add a scale bar 20 in the x-axis of Figure 2A. Please also check Figure 4A and 4B.



Reply: We have increased the scale of the x-axis in Figures 2A and Figure 4B-4D, and the updated images have been placed in the manuscript.

- It is suggested adjust the position of “PC2” in Figure 2E.



Reply: We have adjusted the position of ‘PC2’, which can be seen in Figure 2, and the updated image has been put into the manuscript.

- The description of the P value should be in the **uppercase** format, please revise “p” to “P” in Figure 2F. Please also check other figures for it.

$p < 0.001$

Reply: We have made changes as suggested and the updated image has been put into the manuscript.

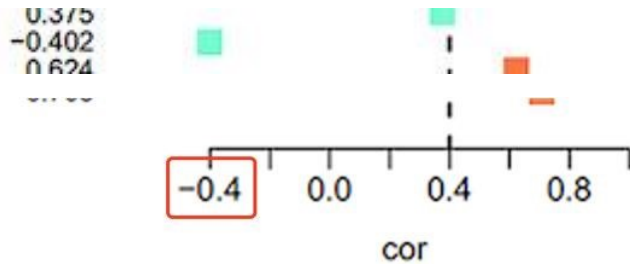
- Please double check the highlighted content in the following sentence.
 “Survival analysis demonstrated varied survival rates; cluster A had the **highest** survival rates compared to clusters B and C (**Figure 2F**).”

Reply: We have checked and amended the highlighted sections, see Page 7, line 216

- Please revise “pvalue” to “P value” in Figure 4C, 4D.

Reply: We have made changes as suggested and the updated image has been put into the manuscript.

- The data “-0.402” exceeds the horizontal coordinates, please also check and revise the scale bar.



Reply: We have increased the scale of the x-axis in Figure 4B-4D, and the updated images have been placed in the manuscript.

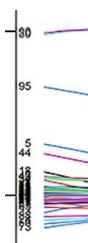
- Please double check Figure 4 legend.
“(A) Univariate Cox analysis of autophagy and **necroptosis** genes; (B) Univariate Cox analysis of ferroptosis and **pyroptosis** genes; (C) Co-expression relationships between autophagy and **necroptosis** genes and the most relevant m5C/m6A/m7G prognostic genes; (D) Co-expression relationships between ferroptosis and **pyroptosis** genes and the most relevant m5C/m6A/m7G prognostic genes.”

Reply: We checked the highlighted section, but after checking we did not find spelling errors in the four words in the highlighted section.

- Figures should be cited **consecutively** in the text and numbered in the order in which they are discussed. Therefore, Figure 4A, 4B should be cited before Figure 4C, 4D, unless Figure 4 is cited as a whole before. Please check through and revise.

Reply: We changed the order of Figure 4A, 4B, 4C, and 4D so that the citations in the article would follow the order.

- The numbers are not clear enough in Figure 5A.



Reply: Unfortunately, there is no way to separate the numbers due to their large number and dense distribution.

- Please revise “1 years” to “1 year” in Figure 5D, 6B.

— AUC at 1 years: 0.784

- There are spelling mistakes in Figure 5E and 5F, 6C and 6D.

Patients (increasing risk socre)

Reply: We have made changes as suggested and the updated image has been put into the manuscript.

- Please indicate the meaning of “**” “***” in Figure 7 legend.

Reply: The “*” in Figure 7 is of no real meaning, we have removed the “*” to avoid interference and the updated image has been put into the manuscript.

- Please add description for the x-axis in Figure 7B.

Reply: We have added a description of Nomogram-predicted OS on the x-axis, and the updated image has been put into the manuscript.

- Please check the unit “%” is correct or not in the y-axis of Figure 7B.



Reply: The “%” in figure 7B was in error and we have removed the “%”

- Please provide Figure 8, 9 in higher resolution in jpg/tiff format.

Reply: We offer higher resolution images to return.

- Please double check which group is 20 included in Figure 8.



Reply: We have checked and none of that one is located in the ≥ 20 group, so we have removed the legend for the ≥ 20 group and the updated image has been placed in the manuscript.

- Please indicate the meaning of “*” “**” “***” in Figure 9 legend.

Reply: We have added the meaning of “*” “” in Figure 9 legend.**

- Please double check the highlighted content in the following sentence.

“Within the realm of immune cell populations, several were significantly elevated in the high-risk group, including activated dendritic cells (aDCs), immature dendritic cells (iDCs), macrophages, follicular helper T cells (Tfh helper T cells 2 (Th2 cells) cells),, and regulatory T cells (Treg cells).”

Reply: We have checked and amended the highlighted sections, see Page 9, line 285

- Please check whether it should be “Figure 9G”.

“Subsequently, TIDE scores were calculated for the high-risk and low-risk groups, unveiling a higher TIDE score in the low-risk group (**Figure 10G**).”

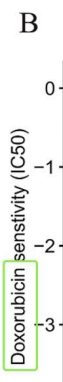
Reply: We have checked and amended the highlighted sections, see Page 9, line 302

- Please double check the following sentence.

“This dynamic interaction unfolded through the BMP and LIGHT signaling pathways, allowing tumor cells to accept signals from myeloid cells, NK cells, T cells, plasmacytoid dendritic cells (pDCs), T cells B cells, and plasmacytoid dendritic cells (pDCs) (Figure 10E-10F).”

Reply: We have checked and amended the highlighted sections, see Page 10, lines 318-319

- It is suggested to unify the description in Figure 11B, 11D and its legend and the text.



“(A-D) Sensitivity of high-risk and low-risk patients to cisplatin, paclitaxel, erlotinib, and mitomycin C”

“The results showed that the high-risk group demonstrated heightened sensitivity to cisplatin, docetaxel, and paclitaxel, while the low-risk group exhibited a greater susceptibility to erlotinib (Figure 11A-11D).”

Reply: We have checked and amended the highlighted sections, see Page 10, lines 323-324

- It is suggested to unify “Cmap” and “CMap” in Figure 11E legend and the text.

Reply: We have harmonized all Cmap and CMap into CMAP.

- Please indicate the meaning of “*” “**” in Figure 12 legend.

Reply: We have added the meaning of “*” “” in Figure 12 legend.**

3. Supplementary

- Please revise “1 years” to “1 year” in Supplementary Figure 1D-1F.

Reply: We have made changes as suggested and updated images have been placed in the Supplementary Images section.

- Please revise “roc” to “ROC” in Supplementary Figure 1 legend.

Reply: We have made changes as suggested.

- Please add unit for Age in Supplementary Figure 2A and 2E.

Reply: We have added the unit of age “year” to the image, and the updated image is in the Supplementary Images section.