Peer Review File

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Reviewer A

Major Comment

Comment 1: As this study focused primarily on HCC, I do not see the necessity of performing pan-cancer analyses such as analyze the RACGAP1 expressions through the TIMER database on other cancer types, such as bladder cancer and breast cancer, as described in pages 7-8. The authors should expand on this.
Reply 1: Thank you for your constructive feedback. Your comments are very reasonable. We have deleted the content of pan-cancer.
Changes in the text: We have modified our text as advised (see Page 7, line 22 to Page 8, line 3).

Comment 2: There is no panel D in figure 2.
Reply 2: Thanks for your careful checks. We are sorry for our carelessness. We lost a panel in figure 2, and we have added this panel in the revised figures.
Changes in the text: We have added the panel in the revised figures.

Comment 3: The IHC analysis is hard to interpret. I recommend authors provide a zoomed in version for images in Figure 2B.
Reply 3: Thank you for your constructive feedback. We have provided a zoomed of this figure.
Changes in the text: We have modified the figure in the revised figures.

Comment 4: Perform quantitative analysis on IHC images to assess the differential expressions on RACGAP1 will be preferable.
Reply 4: Thank you for your constructive feedback. We have added quantitative analysis of IHC images. The IHC scores were referred as the value of the staining intensity (0–3) and the portion of stained cells (1–4).
Changes in the text: We have added a quantitative analysis of IHC images in the revised figures. Moreover, we have added this content in the part of Methods. (see Page 6, line 20)

Comment 5: The authors showed that RACGAP1 was correlated with immune cell infiltrations especially CD8+ and CD4+ T cells. This seems contradictory since RACGAP1 was also shown to be correlated with poor survival. The authors should provide insights on this phenomenon.
Reply 5: Thanks for your kindly suggestions. Generally, CD8+ and CD4+ T cells play an antitumor immunity role. In 2017, the Cell journal published an article showed both CD8+ and CD4+ T cells have different subsets (DOI: 10.1016/j.cell.2017.05.035). And only certain subsets could have antitumor immunity. CD8+ T cells have 5 different subsets (Tc 1, Tc 2, Tc 9, Tc 17, Tc 22). Of which, Tc 9 and Tc 17 produce less IFN-γ, which didn't have the function of antitumor immunity (DOI: 10.1016/j.tcb.2020.06.003). This study revealed HCC

patients have high exhausted CD8+ T cells (a kind of CD8+ T cells) counts, and then inhibit the killing effect of CD8+ T cells on tumor. Similarly, CD4+ T cell has these subsets as follows: Th1, Th2 and Treg cells. As described previously, Treg cells could suppress tumor immunity and promote the occurrence and development of tumors. Importantly, this will be the focus of our next research.
Changes in the text: Thanks for your kindly suggestions. We didn't change in the manuscript.

Minor comment
Comment 6: The texts in figures are too small and hard to interpret, please increase the text size.
Reply 6: Your suggestion really means a lot to us. Yes, it would be more understandable. We have tried our best to increase the size to let reader understand clearly.
Changes in the text: We have increased all figures size to let reader understand clearly.

Comment 7: Grammar check
Reply 7: Thanks for your suggestions. We feel sorry for our grammar mistakes and poor writings. Then, we invite a friend of us who is a native English speaker from Bangladesh help polish our article. Due to our friend's help, the article was edited extensively. And we hope the revised manuscript could be acceptable for you.
Changes in the text: A native English speaker of my friends help us polish our article. The article was edited in several places.

Comment 8: Add relevant refs on HCC TME analysis on the immune infiltration:
Mi, Haoyang, et al. "Multi-scale spatial analysis of the tumor microenvironment reveals features of cabozantinib and nivolumab efficacy in hepatocellular carcinoma." Frontiers in immunology 13 (2022): 892250.
Ho, Won Jin, et al. "Neoadjuvant cabozantinib and nivolumab convert locally advanced hepatocellular carcinoma into resectable disease with enhanced antitumor immunity." Nature Cancer 2.9 (2021): 891-903.
Zhang, Shuming, et al. "Informing virtual clinical trials of hepatocellular carcinoma with spatial multi-omics analysis of a human neoadjuvant immunotherapy clinical trial." bioRxiv (2023): 2023-08.
Reply 8: According to your suggestion, we have added the mentioned references.
Changes in the text: We have added these references in the HCC TME part. (Reference 34-36)

Reviewer B
Comment 1: A brief introduction to the study background must be added in the "Background" section.
Comment 2: You refer to "studies" but have only one citation in the sentence: "And previous studies reported immune infiltration was closely associated with the prognosis of cancers[37]."

Comment 3: Please add the scale bars and staining methods of Figure 2C and 2D.
Comment 4: Each subfigure should have its own Y-axis. Please revise Figure 6A.
Comment 5: Incorporate "Lay summary" into the abstract or main text, within the structure. Otherwise, please delete it.
Reply 1-5: Thanks for your suggestions. We have modified the paper carefully according to your kindly suggestions.

Comment 6: Some numbers seem to be missing in the legends of Figure 5C and S2.
Reply 6: We have revised this figure

Comment 7: Please swap the order of these two to match Figure S3.

> **Supplementary Figure 3** The proportion of tumor-infiltrating lymphocytes in HCC and normal tissues. (A-D) the proportion of macrophages M0, resting mast cell, monocyte and activated mast cell in HCC and normal tissues. **p <0.01, *** p<0.001.

Reply 7: Thanks for your careful checking. We have changed the order of these to match Figure S3.

Comment 8: Explain what the number 204 represents in Table 1.

Gender	204	1.81(1.31-2.81)	**0.00068**	204	1.81(1.31-2.81)	**0.00068**
Male	246	4.04(1.94-8.40)	**5.3e-05**	246	1.92(1.33-2.76)	**0.00038**
Female	118	1.98(1.08-3.62)	**0.024**	120	2.63(1.49-4.66)	**0.00055**

Reply 8: Thanks for your careful checking. It is a mistake in the Table. We have deleted the number 204 in the revised manuscript.

Comment 9: Numbers in Table 1 do not add up.
Reply 9: Your suggestion was very constructive. The correlation of Rac GTPase Activating Protein 1 (RACGAP1) mRNA expression and clinical prognosis in HCC with different clinicopathological factors was explored by Kaplan-Meier plotter. KM-plotter is the most sophisticated online survival analysis tool, and the gene expression data, clinical data and the overall survival information are downloaded from GEO, EGA and TCGA. Since the clinicopathologic information for some patients is unknown, we could only list these patients for whom explicit information is available. Thus, numbers in Table 1 do not add up.

Comment 10: The full name of MF in Figure S2 is incorrect.

> **Supplementary Figure 2** Functional enrichment analysis of RACGAP1-interacting genes and proteins. (A-D) biological process (BP), biological process (MF), cellular component (CC) and kyoto encyclopedia of genes and genomes (KEGG) pathways of the genes related to RACGAP1.

Reply 10: Thanks for your kindly reminding. It is our mistake. We have modified this mistake in the revise manuscript.