Peer Review File

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Replies to reviewer #1

1. Comment

The study is of interest and provides novel findings. However, in my opinion, the manuscript should be improved by adding and discussing the potential therapeutic impact of the study results. In mu opinion, two important issues should be recalled and discussed based on the study findings: 1) in the current, evolving, scenario of systemic treatments for HCC patients, we urgently need prognostic markers to better select patients with highr chance of treatment response. In this regard, the authors should recall the recent advamcements of systemic treatments of HCC based on the combination treatment approaches based on immune checkpoint inhibitors (ICI) plus tyrosine kinase inhibitors or anti-VEGF inhibitors, as well-described in a recent very comprehensive review (TKIs in combination with immunotherapy for hepatocellular carcinoma. Expert Rev Anticancer Ther. 2023;23(3):279-291. doi: 10.1080/14737140.2023.2181162.).

Answer:

We feel great thanks for your professional review work and constructive comment on our article. We have added this content in the Discussion section in the revised manuscript. **(see Paragraph 6 of Discussion, Page 18, line 347-354)**

2. Comment

2) the authors should recall and discuss the problem of more fragile HCC patients who, due to suboptimal liver function, cannot receive most approved systemic treatments due to the risk of liver function deterioration, with the exception of metronomic treatment approach, as showed in two cohort studies demonstrating the very good safety and efficacy profiles of metronomic capecitabine in HCC patients resistant or intolerant to first-line sorafenib, as previously demonstrated (Dig Liver Dis. 2015;47(6):518-22. doi: 10.1016/j.dld.2015.03.010; J Cancer Res Clin Oncol. 2018;144(2):403-414. doi: 10.1007/s00432-017-2556-6.).

Since the present study shows that RBM39 is functionally involved in pathways associated with the cell cycle, a target of capecitabine, this safe and inexpensive treatment approach should be

investigated in randomized clinical trials to expand the chance of systemic treatment of HCC patients currently excluded from approved systemic therapies.

Answer:

We feel great thanks for your professional review work and constructive comment on our article. As you suggested, we have discussed this issue in the revised manuscript, which greatly increases the depth of our article. **(see Paragraph 7 of Discussion, Page 18-19, line 363-368)**

Replies to reviewer #2

1. Comment

Introduction:

The introduction could be more concise by avoiding repetition, simplifying complex concepts, and directly engaging with existing literature. A brief overview of research methods and acknowledgment of other factors contributing to HCC development would enhance clarity.

Answer:

We greatly appreciate your valuable comment and constructive suggestion, which is very helpful to improve the quality of our article. As you suggested, We have rewritten the Introduction and added a brief overview of HCC pathogenesis and current therapies. (**see Paragraph 1 of Introduction, Page 4, line 51-59)**

2. Comment

Methods:

The methods section needs clarity in data selection criteria, rationale for gene set choice in GSEA, details on immune cell analysis, IHC scoring criteria, cell culture specifics, transfection conditions, array selection rationale in qRT-PCR, and parameters measured in the wound-healing assay. Including explicit details, justifications, and clarifications would improve transparency.

Answer:

We feel great thanks for your professional review work and constructive comment on our article. As you suggest, we added some details of the methods to improve the clarity. (**see Paragraph 1, 2, 3, 5, 6 and 10 of Methods, Page 6, 7, 8 and 10, line 99-100, 108-113, 118-121, 138-139, 144-147 and 177-179**)

3. Comment

Statistical Analysis:

The statistical analysis has strengths in patient categorization and various tests but lacks a detailed rationale for chosen methods. Acknowledging the potential for overfitting, considering adjustments for multiple comparisons, providing clarity on variables in multivariate Cox regression, details on ROC analysis, and addressing small sample size limitations in validation would enhance reliability and interpretability.

Answer:

Thanks for your constructive suggestion. We have rewritten the Statistical Analysis section as you suggested, and added more details of the multivariate Cox regression, ROC analysis, and multiple comparisons. We hope this may enhance the reliability and interpretability of our manuscript. (**See the last Paragraph of Methods, Page 10-11, line 181-197**)

4. Comment

Discussion:

Address repetitiveness in RBM39's association with poor prognosis, provide better contextualization for RBM39's correlation with Th2 cells, and establish a clearer connection between splicing modulation, neoantigen generation, and HCC development. Integrate GSEA results for a more coherent interpretation, qualify RBM39 as a promising target with clear criteria, and elaborate on how RBM39 influences specific tumor pathways in HCC development for added depth.

Answer:

We greatly appreciate your valuable comment and constructive suggestion, which is very helpful to improve the quality of our article. Following your suggestion, we have added relevant content to the discussion section. (**See Paragraph 4 of Discussion, Page 16, line 312-316; Paragraph 5 of Discussion, Page 17, line 339-345;**)