Peer Review File

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Review Comments and Reply

Comment 1: What is the molecular classification and its prognostic value in hepatocellular carcinoma based on immune signature? It is recommended to add relevant contents.

Reply 1: Thank you for your suggestion. The immune infiltration and prognostic value analysis of the five immune-related candidate genes have been provided in Figure S11 (see Page 10, line 280-282). The clinical significance (including prognostic value) of the three HCC patient clusters classified according to the expression of candidate genes has been provided in Figure 7 (see Page 11, line 299-306). Immune and molecular characteristics of three HCC clusters have been provided in Figure 9 (see Page 12, line 330-334).

Comment 2: The tumor heterogeneous make-up of immune cell infiltrates is a key factor for the therapy response and prognosis of HCC. How to comprehensively understand the tumor immune microenvironment at the gene and cell levels? Please answer according to the content of this study.

Reply 2: We analyzed the relationship between the expression of candidate genes and the level of immune cells through the TIMER database to show the level of immune cell infiltration in HCC samples (Figure S11, see Page 10, line 280-282). Furthermore, the relationship between the tumor immune microenvironment and different HCC patient clusters was also explored through the analyses of immune cell infiltration (Figure 9A-D, see Page 12, line 329-331) and immune checkpoint gene expression levels (Figure 7G, see Page 11, line 303-306). The cluster 2 samples were found to have significantly higher quantities of $CD8^+$ T cells, macrophages, and $CD4^+$ T cells than samples from the other clusters, which suggested they were more likely to respond to immune checkpoint inhibitors. The abnormal upregulation of immune checkpoint genes in patients in cluster 2 supported this finding, suggesting that immune escape was the cause of the poor prognosis for patients in cluster 2.

Comment 3: It may be more meaningful to add functional research on key genes.

Reply3: Thank you for your suggestion. This is a limitation of our current work. We have pointed it out in the discussion part (see Page 15, line 422-426). The further vitro and in vivo functional experiments to validate the five key genes would be a part of our future work.

Comment 4: This study is based on bioinformatics analysis. It is recommended to increase in vivo and in vitro experimental studies, which may be more meaningful.
Reply4: We agree with your suggestion. This is a limitation of our current work. We have pointed it out in the discussion part (see Page 15, line 422-426). The further vitro and in vivo experimental studies would be a part of our future work.

Comment 5: It is suggested to increase the research progress of molecular and immunophenotyping and combined immunotargeting therapy for hepatocellular carcinoma.
Reply 5: Thank you for your suggestion. The research progress of tumor immunotherapy for HCC has been provided in the introduction part (see Page 2, line 56-59) and discussed in the discussion part (see Page 15, line 426-432). The research progress of five selected molecules can be seen in the discussion part (see Page 13-14, line 363-404).

Comment 6: What is the correlation between tumor heterogeneity and immunosuppressive microenvironment in hepatocellular carcinoma? It is recommended to add relevant contents.
Reply 6: Thank you for your suggestion. We have provided relevant content in the discussion part (see in Page 15, line 414-421).

Comment 7: What are the predictors of efficacy of immunotherapy? It is recommended to add relevant contents.
Reply 7: Thank you for your suggestion. Tumor mutation burden (TMB) and the expression level of immune checkpoint molecules are the predictors of efficacy of immunotherapy. We have provided relevant contents in the method part (see in Page 7, line 177-193) and result part (Figure 7E, 7G, see in Page 11, line 302-306).