TRANSLATIONAL CANCER RESEARCH

Peer Review File

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Responses to the Reviewer:

The authors investigated the 8-genes methylation signatures of HCC patients using bioinformatics analyses. The TNFRSF12A methylation was validated in HCC patients for confirmation as the prognosis biomarker. Predicting HCC patients' outcomes is important, as HCC remains one of the cancers with a high mortality rate. The authors have done some extensive works.
However, a few issues need some clarifications:

Comment 1: Please improve Table 1, by including the discovery cohort of TCGA, side-by-side with the validation cohort of HCC patients. Is there any exclusion of the TCGA data? If there is, please describe that in the method section.
Reply 1: Thank you for your careful and constructive comments and we have added the discovery cohort of TCGA in Table 1. The original datasets were downloaded from the TCGA database as described in the method section, there is no exclusion of the TCGA data (Page 17, Table 1).

Comment 2: The AUC = 0.725, Figure 5 A, was this value refers to the set of prognosis methylation signatures (8 genes)?
Reply 2: Thank you for your comments. I am regret that we did not describe the Figure 5A and corresponding legends more clearly in the manuscript. The AUC=0.725 refers to the risk score (based on 8-gene prognosis signature). We have modified our manuscript according to your suggestions (Page 7, Line 193-194).

Comment 3: The AUC is a bit lower than the AUC of the Tumor stage to predict the patients' worse outcomes. Please discuss this more.
Reply 3: Thank you for your comments. In this present study, Univariate and Multivariate analysis indicated that risk score was the only independent prognostic factor for patients with HCC (hazard ratio=4.527, P<0.001). To assess the potential prognostic role of risk score which based on the 8-gene model, ROC analysis was performed. It showed that the AUC of risk score could effectively predict the prognosis of HCC patients. However, the AUC of risk score is a bit lower than that of tumor stage. In oncology, the standard classification of cancer is based on the TNM staging (J Hepatol; 2018 July; 182-236; Oral Oncology; Oct 2018; 82-86). To date, there are two widely accepted pathological staging systems for HCC, both of them use T (tumor: tumor size, number and nearby invasion), N (node: regional lymph node involvement) and M (metastasis: distant metastasis) as parameters to stage the disease (HPB 2013;15;439-448). For a long time, TNM staging has been effective in

guiding the treatment of patients with cancer. However, in HCC, TNM staging has several limitations, such as pathological information is required to assess microvascular invasion, which is only available in patients treated by surgery; it does not capture information regarding liver functional status or health status. These leads to that its prognostic value in non-early tumors is limited (HPB 2013;15;439-448). Our findings combined with the traditional tumor staging system might bring new and reliable ideas for clinical judgment of the prognosis of HCC patients. Related content has been added to the discussion (Page 11, Line 297-314).

Comment 4: Can the authors re-analyzed the ROC curve analysis, to check the best combination among the eight genes, for the highest AUC?
Reply 4: Thank you for your suggestion and we fully agree with you that re-analyzed is necessary to get the best combination for the highest AUC. LASSO analysis using glmnet package was applied to prognosis-related signatures to generate prognostic model. After running the program repeatedly, the current 8-gene prognosis model is the best combination we can get at present. However, the clinical value and exact mechanism of the prognostic model should be carefully studied in the future.

Comment 5: TNFRSF12A validation is an important part of the manuscript, yet the discussion of this loci/gene is minimal. Please discuss and hypothesize the TNFRSF12A role in HCC.
Reply 5: Thanks for your careful and constructive comments and we have added the discussion on the role of TNFRSF12A in HCC in the revised manuscript (Page12-13, Line339-360).

Comment 6: There are some grammatical errors throughout the manuscript.
Reply 6: Thank you for your comments. We re-checked the manuscript and revised the grammatical errors in the text, changes were marked in red.

Comment 7: Please revise the title to include TNFRSF12A, as it is one of the main findings.
Reply 7: Thank you for your suggestion, we have revised the title as your suggested (Page 1, Line 1-3).