Peer Review File

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Reviewer Comments

Comment 1: Technically, the work presented in this manuscript by Shao et al. is well-performed from a data analysis standpoint. However, I have several major reservations: What is the utility of this study? To me, it is that the ALDOB gene (and its protein product) may be involved in disease pathogenesis and progression given the differential expression between normal tissue and ccRCC; also the association between ALDOB and TNM staging.

Reply 1: Thanks for your comments. Regarding for the utility of this study, we think that there are the following points: First of all, we performed a comprehensive analysis of the expression level, prognostic value, functional enrichment, immune infiltration, and m6A modification of ALDOB in ccRCC. And we proposed a distinctive expression pattern of ALDOB in ccRCC patients. Furthermore, a decreasing trend of ALDOB expression with an upgrade of T stage, M stage, AJCC stage, and histologic grade of ccRCC patients, suggesting that ALDOB was strongly correlated with the clinicopathological factors of ccRCC patients and might strongly influence tumor development and progression in ccRCC. Secondly, this study discussed the prognostic value of ALDOB in ccRCC patients. And the results of multivariate Cox regression analysis showed that among the 24 DEGs, only ALDOB was an independent prognostic factor for OS, DSS, and PFS in ccRCC patients, and the ccRCC patients with higher expression level of ALDOB experienced a better prognosis. Therefore, ALDOB might be a novel prognostic biomarker for ccRCC patients. Thirdly, in this study, the nomograms with more prognostic predictive power were developed to predict the OS, DSS, and PFS of ccRCC patients by integrating T stage, N stage, M stage, histologic grade, and the expression level of ALDOB of ccRCC patients. Based on these nomograms, it would be helpful to more accurately assess the clinical outcome of ccRCC and provide more personalized prognostic assessment strategy for ccRCC patients. These are the utility of this work. Therefore, based on your professional comments, we revised the part of Discussion in the revised manuscript. At the end of some paragraphs in Discussion, we added the concluding statement to further emphasize the utility of our study. Thanks for your precious comments. Please review again, thank you very much.

Changes in the text: Page 19, line 409-411; Page 21, line 449-451; Page 22, line 468-470; Page 23, line 499-502. Please review again, thank you very much.

Comment 2: A portion of this study (e.g. AUC data) is dedicated to showing that ALDOB can help diagnose ccRCC vs. normal tissue. However, realize that this data is analyzing gene expression data from surgically obtained tumor tissue. Thus, there is no real diagnostic utility based on these study

results.

Reply 2: Thanks for your professional comments. Currently, the diagnosis of ccRCC mainly includes clinical diagnosis and histological diagnosis. In this study, we explored the diagnostic value of ALDOB in ccRCC. And the results of the diagnostic analyses indicated that ALDOB presented a high diagnostic ability to distinguish ccRCC tissues from normal kidney tissues. Therefore, ALDOB could be combined with the existing pathological diagnostic molecules and might contribute to improving the sensitivity and specificity of current postoperative diagnostic models. However, as your comments, the datasets analyzed in this study were mainly obtained from gene expression data from surgically obtained tumor tissues and normal tissues. Therefore, there is no real diagnostic utility based on the results of diagnostic analysis. Our initial intention was to construct a ALDOB-based diagnostic model. But the expression data that derived from postoperative tissues did not provide a real diagnostic utility. And the postoperative histopathology evaluation is still the gold standard for the diagnosis of ccRCC. Therefore, based on your professional comments, we deleted the sections of "Diagnostic analysis of ALDOB" in the part of Abstract, Methods, and Results in the revised manuscript. Furthermore, we also revised the part of Introduction and Discussion in the revised manuscript. Thanks for your precious comments. Please review again, thank you very much.

Changes in the text: Page 5, line 89-91; Page 19, line 403-405; Page 26, line 564-566. Please review again, thank you very much.

Comment 3: Regarding the prognostic signature, it is intriguing but is still based on one data set (TCGA) despite the bootstrapping methodology used. It would be much more convincing if it could be validated in a different data set (institutional or publicly available).

Reply 3: Thanks for your professional comments. In this study, we performed survival analysis mainly based on the clinical data from the TCGA-KIRC dataset. Therefore, based on your professional comments, we enrolled the E-MTAB-1980 dataset that downloaded from the ArrayExpress database as an independent validation cohort, which contained 101 ccRCC patients. Firstly, in the revised manuscript, we validated the prognostic value of ALDOB in ccRCC patients based on this independent cohort. The result showed that the expression level of ALDOB was closely related to OS (HR=0.157, 95% CI: 0.062 - 0.398, P<0.001) in ccRCC patients from validation cohort (Figure S3A). Secondly, in the revised manuscript, we validated the prognostic value of ALDOB in ccRCC patients by multivariate Cox regression analysis in the validation cohort. The result showed that ALDOB was the independent factor for prognosis of ccRCC patients (Figure S3D). Thirdly, in the revised manuscript, we also constructed the nomograms of 1-year, 3-year, and 5-year OS of ccRCC patients from the validation cohort (Figure S3B-C). Therefore, both in the training cohort (TCGA-KIRC dataset) and validation cohort (E-MTAB-1980 dataset), the results of this study showed that ALDOB was an independent prognostic factor in the prognosis of ccRCC patients.

Therefore, based on your professional comments, we added the validation cohort in the revised manuscript. Furthermore, we revised the number of ccRCC tissues and normal tissues enrolled in this study. Thanks for your precious comments. Please review again, thank you very much.
Changes in the text: Page 1, line 10-13; Page 2, line 25-26; Page 5, line 104-107; Page 12, line 248-250; Page 14, line 295-298; Figure S3 in Supplementary Materials. Please review again, thank you very much.

Comment 4: Regarding the calibration plots in Figure 7, they undoubtedly look great. However, there are so many known, important prognostic factors included (TNM staging, pathologic stage, histologic grade). Are the calibration plots significantly affected if you exclude the ALDOB low/high criterion?
Reply 4: Thanks for your professional comments. Based on your comments, we also constructed the nomograms to predict the OS, DSS, and PFS of ccRCC patients by integrating T stage, N stage, M stage, and histologic grade of ccRCC patients (see Figure below). The C-indices of the nomograms without the expression level of ALDOB were 0.747 (0.721-0.774), 0.836 (0.812-0.860), and 0.801 (0.776-0.825), respectively. In this study, the C-indices of the nomograms including the expression level of ALDOB were 0.764 (0.737-0.790), 0.860 (0.839-0.881), and 0.823 (0.801-0.846), respectively. By comparing the nomograms with or without ALDOB, we could observe that the C-indices of the nomograms with the expression level of ALDOB were higher than the C-indices of the nomograms without the expression level of ALDOB. Furthermore, we could observed that the calibration plots with the expression level of ALDOB were better than the calibration plots without the expression level of ALDOB. Therefore, in this study, the nomograms with more prognostic predictive power were developed to predict the OS, DSS, and PFS of ccRCC patients by integrating T stage, N stage, M stage, histologic grade, and the expression level of ALDOB of ccRCC patients. Based on these nomograms, it would be helpful to more accurately assess the clinical outcome of ccRCC and provide more personalized prognostic assessment strategy for ccRCC patients. Thanks for your precious comments. Please review again, thank you very much.
Changes in the text: Page 14, line 289-292; Figure 7; Please review again, thank you very much.

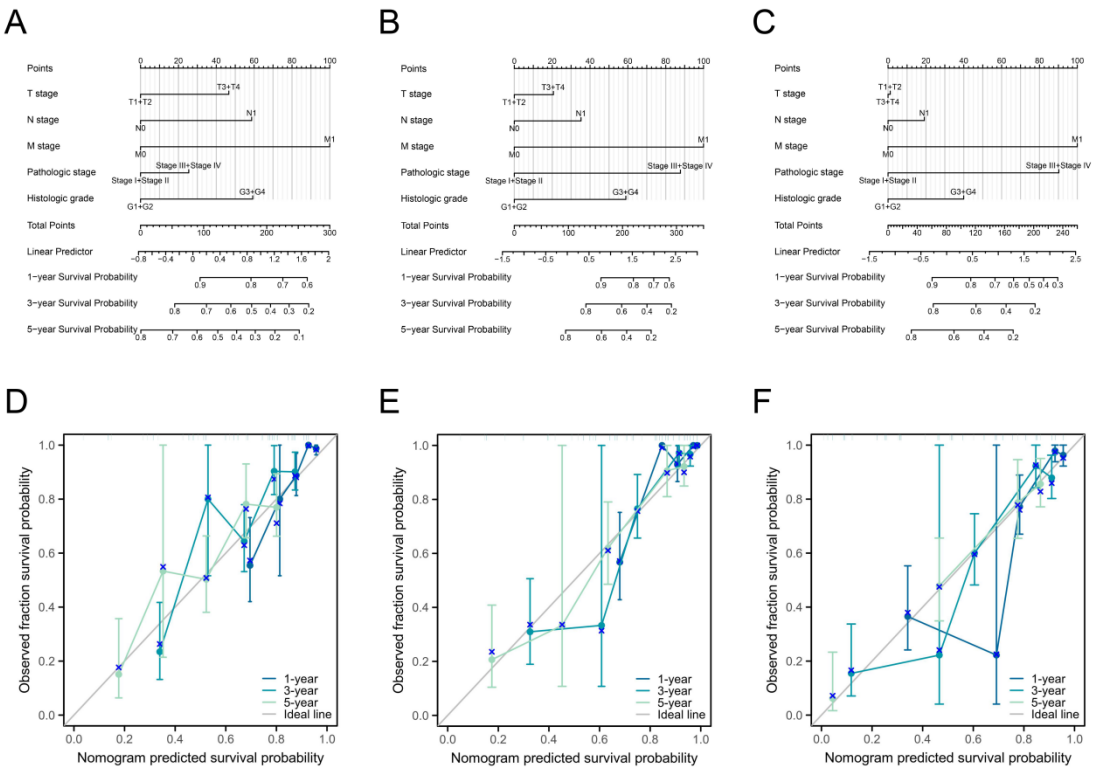

Figure: Nomograms and calibration plots without ALDOB for the prediction of the prognosis in ccRCC patients. (A) Nomogram for the prediction of overall survival (OS) in ccRCC patients; (B) Nomogram for the prediction of disease-specific survival (DSS) in ccRCC patients; (C) Nomogram for the prediction of progression free survival (PFS) in ccRCC patients; (D) Calibration plot of the nomogram for prediction of OS in ccRCC patients; (E) Calibration plot of the nomogram for prediction of DSS in ccRCC patients; (F) Calibration plot of the nomogram for prediction of PFS in ccRCC patients.

Comment 5: How was the cutoff for ALDOB low/high selected? Could you use the gene expression as a continuous rather than categorical variable?

Reply 5: Thanks for your suggestion and we were happy to revise the manuscript based on your helpful comments. In this study, we determined the ALDOB low/high group according to the median value of the expression level of ALDOB in ccRCC patients. Therefore, based on your professional comments, we used the expression level of ALDOB as a continuous variable. Firstly, we performed the multivariate Cox regression analyses to identify the independent predictors of OS, DSS, and PFS in ccRCC patients. Here, we used the expression level of ALDOB as a continuous variable to perform the multivariate Cox regression analyses. The forest plots showed that, the expression level of ALDOB (continuous variable) was closely related to OS (HR=0.866, 95% CI: 0.794-0.945, P=0.001), DSS (HR=0.830, 95% CI: 0.741-0.931, P=0.001), and PFS (HR=0.838, 95% CI: 0.764-0.919, P<0.001) in ccRCC patients (Figure 4). Secondly, the prognostic predictors, including T stage, N stage, M stage, AJCC stage, histologic grade, and ALDOB, were used to establish the nomograms of one-year, three-year, and

five-year OS, DSS, and PFS of ccRCC patients. Likewise, we also we used the expression level of ALDOB as a continuous variable to construct the nomograms (Figure 7). Thirdly, we also used the expression level of ALDOB as a continuous variable to validate the prognostic value of ALDOB in ccRCC patients from the independent validation cohort (Figure S3). Thanks for your precious comments. Please review again, thank you very much.

Changes in the text: Page 12, line 242-245; Page 12, line 248-250; Page 13-14, line 283-299; Figure 4; Figure 7; Figure S3. Please review again, thank you very much.

Comment 6: There are several typos and grammatical errors that will need to be addressed.

Reply 6: Thanks for your careful review. We apologize for the typos and grammatical errors of our manuscript. Therefore, we carefully checked the manuscript and tried our best to correct the typos and grammatical errors in the manuscript. And we really hope that the language level has been substantially improved. Thanks for your careful review. Please review again, thank you very much.

Changes in the text: We carefully checked the manuscript and tried our best to correct the typos and grammatical errors in the manuscript. Please review again, thank you very much.

Finally, please allow me to express my highest respect. I really appreciate your careful and professional comments. Your meticulous attitude towards academic will encourage me to keep improving. Thanks again.