Peer Review File

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Review Comments:

The paper titled "Elevated NDC1 expression predicts poor prognosis and correlates with immunity in hepatocellular carcinoma" is interesting. The results demonstrated that NDC1 might serve as a valuable predictor in the prognosis and immunotherapy of HCC. NDC1 played an oncogenic role in HCC. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) What are the correlations between NDC1 and the tumor microenvironment? How valuable are NDC1 in predicting survival and drug sensitivity in HCC patients? It is recommended to add relevant content

Reply: (1) In this study, we did not find any significant correlation between NDC1 and tumor microenvironment.

(2) As shown in Figures 1E and F, we discovered that patients with high expression of NDC1 had lower overall survival vs low expression groups via utilizing K-M survival analysis and ROC curves. That is, high expression of NDC1 was associated with a poor prognosis. High expression of NDC1 could be a useful diagnostic and prognostic biomarker.

(3) We briefly predicted the association between NDC1 and drug sensitivity (BI-2536) from CTRP database (http://portals.broadinstitute.org/ctrp.v2.1/?featureName=TMEM48), but did not explore it in depth, which can be further investigated in future studies.

2) Please analyze the potential molecular mechanism and pathobiology of NDC1 in HCC based on the existing results and literature.

Reply: Based on bioinformatics prediction and previous basic experiments, we can boldly speculate that NDC1 plays a game with immune cells during HCC process, promoting tumor cell proliferation and invasion through cell cycle, ERBB, INSULIN, MTOR, NOTCH, P53, WNT and other signaling pathways, thus promoting the development of HCC.

3) It is recommended to add in vivo experiments to study the biological function of NDC1.

Reply: We appreciate the reviewer's insightful suggestion and agree that it would be useful to add in vivo experiments to study the biological function of NDC1. However, such an analysis is beyond the scope of our paper, which aims only to preliminarily explore whether there might be a present correlation between NDC1 and HCC. Nevertheless, we recognize this limitation should be mentioned in the paper, so we added this deficiency to the limitation section (the last paragraph of the Discussion section), and we would further add additional in vivo experiments to verify the biological function of NDC1 in HCC in future in-depth analyses, so as to provide strong support for the fact that NDC1 can indeed promote HCC progression.

4) It is suggested that the internal mechanism of NDC1 and immune cells should be added to the discussion.

Reply: We agree with the reviewer that further discussion on this point would be helpful. So we added the possible internal mechanism of NDC1 and immune cells at the end of the fourth paragraph of the discussion section.

5) It is recommended to increase the study of lncRNA or miRNA to regulate NDC1, which may make the whole study more complete.

Reply: The study of lncRNA or miRNA to regulate NDC1was not the scope of this study. Keeping the reviewer's comment in mind, we prefer to search for potential lncRNA or miRNA and that might exist in NCD1 to further improve the mechanism of NDC1 in our following works.

6) There have been many studies on hepatocellular carcinoma. What is the difference between this study and previous studies? What is the innovation? These need to be described in the introduction.

Reply: First of all, we screened many genes that were significant for the study of HCC development in the database, from which we further reviewed the literature and selected NDC1 after in-depth analysis, because we found that NDC1 was closely related to immune cells in liver cancer, and the application of immunotherapy in liver cancer has attracted more and more attention. Immune checkpoint inhibitors have brought revolutionary changes to the treatment of hepatocellular carcinoma. Our study on NDC1 found that NDC1 was closely related to tumor invasion of immune cells and the signaling pathway of immune cell invasion, which was why we chose NDC1 for further study.

7) There are many genes that regulate HCC. Why did the author choose NDC1 for research? Please describe the reason.

Reply: More and more studies have shown that the occurrence, development, recurrence and metastasis of HCC is not an isolated event, but the result of constant competition between tumor cells and their surrounding immune microenvironment. We selected NDC1 from many genes, and innovatively found that NDC1 was closely associated with immune cell infiltration, immune checkpoint molecules, and immune cell pathways in HCC, which could better guide the clinical application of HCC immunotherapy.