Peer Review File

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Review comments

The paper titled “TMEM43 promotes the development of hepatocellular carcinoma by activating VDAC1 through USP7 deubiquitination” is interesting. The study demonstrated that USP7 participates in the growth of hepatocellular carcinoma (HCC) tumors through TMEM43/VDAC1. The results suggest that USP7/TMEM43/VDAC1 may have predictive value and represent a new treatment strategy for hepatocellular carcinoma. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) It is necessary to clearly indicate the relationship between TMEM43 and tumor-infiltrating immune cells and the role of TMEM43 play in prognosis in HCC in the manuscript.
2)The language used in this study is not rigorous, for example, sometimes described as "hepatocellular carcinoma", and sometimes described as "hepatocellular carcinoma". Please carefully check and make corrections.
3)All fonts need to be enlarged in Figures.
4)There are a variety of genes that can regulate HCC. Why did the author choose USP7/TMEM43/VDAC1 axis for research? Please describe the reason.
5)It is recommended to increase the relationship between protein ubiquitination, deubiquitination and tumorigenesis in the discussion.
6)All figures are not clear enough. It is recommended to provide clearer figures again.
7)The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Bioinformatics analysis and single-cell RNA sequencing: elucidating the ubiquitination pathways and key enzymes in lung adenocarcinoma, PMID: 37559628”. It is recommended to quote this article.
8)What is the effect of this study on further HCC treatment and prognosis? Please add relevant content to the discussion.

Comment 1:It is necessary to clearly indicate the relationship between TMEM43 and tumor-infiltrating immune cells and the role of TMEM43 play in prognosis in HCC in the manuscript.
Reply 1:In this study, we have not yet found any correlation between TMEM43 and tumor-infiltrating immune cells. In addition, we discovered through the TCGA database that TMEM43 is highly expressed in hepatocellular carcinoma tissues. TMEM43 is closely related to the prognosis of hepatocellular carcinoma, and high expression of TMEM43 can lead to poor prognosis

Comment 2：The language used in this study is not rigorous, for example, sometimes described as "hepatocellular carcinoma", and sometimes described as "hepatocellular carcinoma". Please carefully check and make corrections.
Reply 2：We have made modifications in the article

Comment 3：All fonts need to be enlarged in Figures
Reply 3：We have made modifications in the figures.

Comment 4：There are a variety of genes that can regulate HCC. Why did the author choose USP7/TMEM43/VDAC1 axis for research? Please describe the reason.
Reply 4：Firstly, we identified TMEM43 through RNA seq and predicted that TMEM43 may affect the occurrence and development of liver cancer. After knocking down TMEM43, we detected downstream proteins that may be regulated by TMEM43 through WB analysis. We found a decrease in the expression of vdac1 and developed protein interactions between the two through experiments such as coip. In previous studies, USP7 has been shown to regulate the expression of multiple downstream genes through ubiquitination, So we also explored whether there is a connection between USP7 and TMEM43. Progressive research has found that USP7 can regulate the expression of TMEM43 by deubiquitination of TMEM43.

Comment 5：It is recommended to increase the relationship between protein ubiquitination, deubiquitination and tumorigenesis in the discussion.
Comment 5:“The post-translational modification of proteins known as ubiquitination is a key process that has been observed to be dysregulated in several types of cancer, including HCC(31). DUBs play an important role in tumor development by removing ubiquitin from substrate proteins through the deubiquitination process(32). Several families of DUBs exist in the human proteome, including UCH, USPs, OTUs and MINDYs(33).”

Comment 6：All figures are not clear enough. It is recommended to provide clearer figures again.
Reply 6：We have made modifications in the figures.

Comment 7：The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Bioinformatics analysis and single-cell RNA sequencing: elucidating the ubiquitination pathways and key enzymes in lung adenocarcinoma, PMID: 37559628”. It is recommended to quote this article.
Reply 7：We have made modifications in the article

Comment 8：What is the effect of this study on further HCC treatment and prognosis? Please add relevant content to the discussion.

Reply 8：These findings provide a new understanding of the pathology and treatment of HCC, and demonstrate the potential of USP7/TMEM43/VDAC1 axis as a new biomarker for monitoring and treating the disease, laying a solid foundation for the development of targeted therapies for invasive cancer.