

ANSWERING REVIEWERS

Jan 6, 2021

Dear Editor and Reviewer,

Name of Journal: **World Journal of Gastroenterology**

Manuscript ID: **61618**

Manuscript Title: **Oncogenic TUFT1 As A Potential Molecular-targeted For Inhibiting Hepatocellular Carcinoma Growth**

Author: **Meng-Na Wu, Wen-Jie Zheng, Wen-Xin Ye, Li Wang, Ying Chen, Jie Yang, Deng-Fu Yao, Min Yao**

We are truly grateful to your comments concerning our manuscript **MS-ID: 61618**.

Reviewer 1 comments:

Thanks to Reviewer 1 for your very kindly comments.

Comments to the Author

This is an interesting manuscript about the oncogenic role of TUFT1 in HCC. Experiments are well design and performed and results are properly described. However, there are several points that authors must clarify to achieve a clearer and more precise message for readers.

These minor points are:

1) Authors must describe in detail the method used to calculate the staining intensities and the rates of positive cells in the immunohistochemistry (count of cells, software used to calculate intensity, ...)

Thank you. They have been added in the manuscript.

2) Immunohistochemistry analysis was performed by two blinded independent pathologists. Results of statistical analysis of the agreement between observers must be described in the manuscript.

Yes, they have been added in the manuscript.

3) Overall survival and disease-free survival must be defined in the methods section.

Yes, the Kaplan–Meier method with log-rank test was used to calculate the curves of Overall survival (OS) and diseases-free survival (DFS).

4) In statistical analysis section - the statistical tests that have been used in the univariate and multivariate analyses remain to be detailed

Thank you. They have been added in the manuscript.

5) According to table 2 data, high TUFT1 expression is associated to absence of vascular invasion and ascites. This is in disagreement with the sentence “The level of high TUFT1 was associated with tumor size, vascular invasion, HBeAg, advanced TNM stage of HCC, and ascites of patients” (page 7).

Sorry, there were mistakes when registering the data.

6) It should be indicated at the bottom of the figures if the data are means or medians, standard deviation or standard error or interquartile range. It should also be indicated if a correction for multiple comparisons has been considered in comparisons between more than two groups.

Thank you. They have been added in the manuscript.

7) What statistical test was used in the table 1 to compare the TUFT1 score?

It has been added to compare the TUFT1 score by the Z test in Table 1.

8) HR values on table 3 are in disagreement with the order of categories in the column Group. For example, tumor size categories are indicated as <3 vs. >3 and HR = 3.680 indicating that the risk is higher in the category <3. All the table should be revised.

Thank you. They have been revised in the manuscript.

9) It must be indicated at the bottom of the figure 3 the number of patients at risk by time

Thank you. They have been added in the manuscript.

10) The last paragraph of result section is a speculation that is not supported by data in the manuscript. The speculation is based on genes and other components missing in this study. It would be better a simpler diagram mainly based in data obtained in this work.

Thanks to you. According to your suggestion, the Fig.6.C has been changed based on the data of this study.

11) English needs to be revised. There are both misspelled terms (“antigen retrievalling”, “HCC tissuess”, “Univarite analysis”, “flod-change” and more) and phrases with inappropriate syntax.

Thank you. They have been corrected

Reviewer 2 comments:

Thanks to Reviewer 2 for your very kindly comments.

Minor points:

1. In abstract section and core tip section, authors should describe the brief comment on the function of Tuftelin1 (TUFT1), such as, “It has been reported that Tuftelin1 (TUFT1) are regulated by hypoxia and involved in the Hedgehog signaling pathway.”
Thank you. It has been added in core tip section.

2. Insert space between 15.8 and y, like, “...the patients were 111 males and 21 females with 21 ~ 79 years old (average 60.04 ± 15.8 y).”
Thank you. It has been corrected.

3. Please make a correction from “Inhibiting TUFT1 plasmid” to “Plasmid coding short hairpin RNA against TUFT1”.
Thank you. It has been corrected.

4. Why did the authors choose MHCC-97H and Hep3B cells for transfection? Authors should explain the reasons clearly.
Based on the statues of TUFT1 expression in the MHCC-97H with high TUFT1 level and Hep3B cells with low TUFT1 level, they were chosen for further studied. The MHCC-97H cells were chosen to be used for interfering TUFT1 mRNA transcription with shRNA1~3 (Fig.4B Left) and the most significantly inhibiting effect was shRNA3 (sh-3) plasmid for TUFT1 (Fig.4B1 Left). The Hep3B cells were selected to over-express TUFT1 with constructed pEX-4 (pGCMV/MCS/T2A/EGFP/Neo) plasmid (Fig.4B Right) and showed markedly increasing of TUFT1 (Fig.4B1 Right).

We also appreciate the reviewer’ careful and thoughtful suggestions, since the comments are all valuable and helpful for improving our paper. We have studied comments and made modifications according to the reviewers’ comments.

Thank you again for publishing our manuscript in the *WJG*.

Sincerely yours,

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