Peer Review File

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Reviewer A

Comment 1: Please edit your conclusion, this line does not make sense- 'In conclusion, the immunotherapy of HCC using FOXP3 gene was not only for the FOXP3+ Treg cells but also for FOXP3+ 352 tumor cells'; are you trying to say treatment of HCC should be directed against FOXP3?

Reply 1: Thanks, we have modified our text as advised.

Changes in the text: See Page 17, line 319-320.

Comment 2: Would also be nice if there was a flowsheet or a figure explaining the role of FOXP3 in promoting metastasis.

Reply 2: This study suggested that FOXP3 in HCC cells promoted tumor cell migration and invasion via up-regulating MMP-1, the content involving specific mechanism research needs further research in the future.

Reviewer B

Minor changes:

Comment 1: Line 1, “promotes” is not correctly written in the tittle. The same for line 3, “hepatocellular” must be corrected.

Reply 1: Thanks, we have modified our text as advised.

Changes in the text: See Page 1, line 2-3.

Comment 2: Figures 1A and B, detection of FOXP3 by RT-PCR and Western blot. Both experiments should be conducted in the same cell lines to follow a more logical reasoning.

Reply 2: Thanks, we have modified our Figure as advised. In Figure 1A, B cell is used as a negative control (B cells hardly express FOXP3). In Figure 1A and B, CD4+ T cells are used as a positive control. In Figure 1B, PBMC is actually a positive control. At the beginning, the experimental design was not rigorous enough, and a few more cell lines were added for control. At present, the cell lines have been unified.

Changes in the text: See Figure 1 revised.

Comment 3: Fig 1C, the protocol and antibody used for flow cytometry is not indicated in Material and Methods.

Reply 3: Thanks, we have modified our Figure as advised. Consistent with the above, because the experimental design is not rigorous, the cell lines used are different, and PCR and WB experiments have been able to prove the expression of FOXP3 in HCC

cell lines, so we delete this part of the flow cytometry result graph.

Changes in the text: See Figure 1 revised.

Comment 4: Figure 2 must be revised. The figure mixes FOXP3 analysis in tumor, normal tissue and Tregs infiltration, which makes it difficult to understand. One easier approach could be a Figure 2A representing FOXP3 IHC in normal tissue, tumors from primary lesions and metastasis and Figure 2B with Treg infiltration in normal tissue, tumors and metastasis. Also, the legend should indicate the n analyzed for each IHC.

Reply 4: Thanks, we have modified our Figure as advised.

Changes in the text: See Figure 2 revised.

Comment 5: Figure 3A does not contribute anything with respect to the figure 1B.

Reply 5: Thanks. Huh7, HepG2, MHCC97L and MHCC97H, the invasion ability of these cell lines is getting stronger and stronger from left to right. So we selected these cell lines to see the expression trends of FOXP3 and MMP-1 in cell lines with different invasion capabilities.

Comment 6: Figure 4B, the full name for MHCC97H cell line must be indicated.

Reply 6: Thanks, we have modified our Figure as advised.

Changes in the text: See Figure 4 revised.

Comment 7: Figure 4B, p-Fsh seems to be ineffective to knock FOXP3 down.

Consequently, the quantification in histogram below does not seem very credible.

Reply 7: Thanks. The efficiency of this part of shRNA knocking down FOXP3 is indeed not high enough, but FOXP3 can still be knocked down. The value (histogram) calculated by the software for the grayscale image is such ($P<0.05$), which is the real data.

Comment 8: Figure 6B, the legend should indicate the day after tumor injection in which the image was performed. Also, a graph with lung metastasis quantification could help. And, why the experiment has been conducted with MHCC97L cells instead of MHCC97H?

Reply 8: Thanks, we have modified our text and Figure as advised. There are two liver cancer cell lines numbered by MHCC97. 97L is relatively easy to metastasize to the lung, and the tumor formation rate is higher, so MHCC97L was chosen for the *in vivo* experiment.

Changes in the text: See Figure 6 revised, Page 22, line 450, 452.

Comment 9: Figure 7B, the first histogram showed is not clear, neither the image nor the concept.

Reply 9: Thanks, we follow the reviewer's suggestion and delete the experimental content related to Figure 7 from the article to make our research more targeted.

Comment 10: Figure 7E, remove IL-4 analysis, the data is expendable.

Reply 10: Thanks, we follow the reviewer's suggestion and delete the experimental content related to Figure 7 from the article to make our research more targeted.

Major changes:

Comment 1: I am aware this is a hard work and may be this is not the main goal of the paper. Thus, it could be better focus the study in FOXP3 expression in tumor cells and leave the impact of FOXP3 expressing tumor cells on the immune system for a second study. Those two ideas are mixed throughout the article and make it confusing to me.

Reply 1: Thanks, we follow the reviewer's suggestion and delete the experimental content related to Figure 7 from the article to make our research more targeted, and expand discussion on existing experimental results

Changes in the text: See Page 15, line 279-320.