Point-by-point response

Dear Editors and Reviewers,

We would like to express our sincerest gratitude to you for your editorial review and to the reviewers for their reviews on our manuscript 86850 "Fatty acid binding protein 5 is a novel therapeutic target for hepatocellular carcinoma". The insights and queries provided by the reviewers and editors have helped us to significantly strengthen our manuscript.

The manuscript has been extensively revised based on the reviews and we now hope you find it suitable for publication in *World Journal of Clinical Oncology*. A letter of point-by-point responses to reviewers' comments is enclosed below.

Reviewer #1:

1. Please add detailed methods and figure legends.

Thanks for the suggestion. We have added more details in the method session and in the figure legends for Figures 1-6 and supplementary Figures S1-S3 as highlighted in yellow in the revised manuscript.

2. It would be nice to validate the in-silico data in human samples or a greater number of cell lines compared with normal cell lines. What is the mechanism of low expression of FABP5 in HepG2 and high expression in Huh7.

Thanks for the great suggestion. Ohata T et al, performed immunohistochemical staining of FABP5 for 243 paired HCC and adjacent non-tumor liver tissue samples. The study confirmed that all normal liver cells were stained negatively, while liver tumor cells can be divided into two groups, FABP5 positive (57.2%) and FABP5 negative (42.8%). Therefore, this data supports that FABP5 is overexpressed in 57.2% of patients with liver tumors. Our data of FABP5 expression in HepG2 and Huh7 cells is consistent with published data in this study as well. This study showed a positive correlation of high FABP5 expression with distant metastasis and invasion. However, Huh7 and HepG2 are both considered to be low metastatic. Our data showed that high expression of *FABP5* mRNA correlated well with G2M checkpoint ($p = 1.1e-10$, $R = 0.33$) and TP53 signaling in liver cancer cells ($p = 1.7e-5$, $R = 0.22$) (**Figure 4-5**). We validated some of these gene expression differences involved in these two signaling networks including CDK1, CDK2, CDK4, and BIRC5 by western blotting in FABP5 low expressing HepG2 cells and FABP5-high expressing Huh7 cells (**Figure 7A**). Therefore, it is possible that the *TP53* genetic status affects the cell proliferation and expression of FABP5, which requires further validation. We have included this in the discussion in the revised manuscript.

Reference: Ohata, T., et al., *Fatty acid-binding protein 5 function in hepatocellular carcinoma through induction of epithelial-mesenchymal transition.* Cancer Medicine, 2017. **6**(5): p. 1049-1061.

3. No mechanism has been discussed. Is it due to variation in E2F1?

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4. What is the IC50 value of SFBI-26 for Huh7 and HepG2? What is the rational of using concentration 1uM, 10uM. I would rather use 75, 100, 125, 150 μM to see the effect of the inhibitor.

Thanks for the constructive suggestion. Based on the 8-dose cell viability in Figure 7C, we calculated IC_{50} for Huh7 and HepG2 (89 and 145 μM, respectively) at 6 days post treatment with SBFI-26 (**Figure 7C**). In addition, the IC_{50} values for Huh7 cells at 3 days and 6 days were 749 and 89 μM, respectively (**Figure 7E**). We have included this data in the revised manuscript. Based on this data, we chose to use 50 and 100 μM for 3-dose cell viability assays in **Figure 7B and 7D**. We agree with the reviewer that 1 and 10 μM are too low to see the anti-tumor effect of the inhibitor. In fact, neither of the concentrations at 1 μM and ten μM were ever used in this study. Instead, we performed cell viability assay with higher concentrations (50 and 100 μM) in **Figure 7B and 7D** and serial doses up to 100 μM in **Figure 7C and 7E**. To clarify this, we added the dose concentrations in the figure legends in the revised manuscript.

5. What is the explanation of differential sensitivity of Huh7 and HepG2 at 3d and 6d?

Thanks for this question. We observed stronger anti-tumor effect at 6d than at 3d upon SBFI-26 treatment in both Huh7 cells and HepG2 cells (**Figure 7D-E**). FABP5 and a few other FABP family members have been reported to contribute to lipid metabolism. Therefore, like many other compounds targeting regulators of cellular mechanism, the anti-tumor effect of FABP5 inhibitor SBFI-26 is a slow process, which requires time to show the anti-tumor effect. We have included this in the revised manuscript.

6. Authors have mentioned about Tp53 and Y220C mutation in huh7 cell line which has reduced DNA binding ability than wild type p53. Please add your comment on it.

Thanks for the comments. The hotspot mutation Y220C of *TP53* gene results in its decreased DNA binding, and reduced p53 tumor suppressor function, leading to cancer progression [1-3]. This may explain that Huh7 cells carrying *TP53* Y220C mutation grow much faster than hepG2 cells with wild type *TP53* (cell doubling time, 24 and 48 hours, respectively). We have included this in the discussion in the revised manuscript.

References:

1. Boettcher, S., et al., *A dominant-negative effect drives selection of TP53 missense mutations in myeloid malignancies.* Science, 2019. **365**(6453): p. 599-604.
2. Dearth, L.R., et al., *Inactive full-length p53 mutants lacking dominant wild-type p53 inhibition highlight loss of heterozygosity as an important aspect of p53 status in human cancers.* Carcinogenesis, 2007. **28**(2): p. 289-98.
3. Liu, X., et al., *Small molecule induced reactivation of mutant p53 in cancer cells.* Nucleic Acids Res, 2013. **41**(12): p. 6034-44.

7. Discussion needs to be improved.

Thanks for this suggestion. As stated above, we have updated the discussion in multiple aspects in the revised manuscript.

(1) Science editor:

(1) Please add detailed methods and figure legends.

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4 Specific comments:

(1) The manuscript structure is incomplete, lacking the content of “author's contributions”, and “Article Highlights”;

Thank you so much for this comment. We have added the content of “author's contributions”, and “Article Highlights” in the revised manuscript.

(2) The author needs to provide a “Institutional animal care and use committee statement”, “Informed consent statement”, “Institutional review board statement”, “ARRIVE guidelines”.

Thank you so much for this comment. We have added the content of “Institutional animal care and use committee statement”, “Informed consent statement”, “Institutional review board statement”, “ARRIVE guidelines”.

(2) Company editor-in-chief:

I recommend the manuscript to be published in the World Journal of Clinical Oncology. The 2023 Edition of Journal Citation Reports® cites the 2022 impact factor (IF) for WJCO as 2.8. When revising the manuscript, it is recommended that the author supplement and improve the highlights of the latest cutting-edge research results, thereby further improving the content of the manuscript. To this end, authors are advised to apply PubMed, or a new tool, the RCA, of which data source is PubMed. RCA is a unique artificial intelligence system for citation index evaluation of medical science and life science literature. In it, upon obtaining search results from the keywords entered by the author, "Impact Index Per Article" under "Ranked by" should be selected to find the latest highlight articles, which can then be used to further improve an article under preparation/peer-review/revision. Please visit our RCA database for more information at: https://www.referencecitationanalysis.com/, or visit PubMed at: https://pubmed.ncbi.nlm.nih.gov/.

Thank you so much for your recommendation and the suggestions for using PubMed or RCA system to improve the manuscript. To this end, we have included additional references (**reference #14-17**, see below) and added more discussion to interpret the data in our revised manuscript.

Reference #14: Ohata, T., et al., *Fatty acid-binding protein 5 function in hepatocellular carcinoma through induction of epithelial-mesenchymal transition.* Cancer Medicine, 2017. **6**(5): p. 1049-1061.

Reference #15: Boettcher, S., et al., *A dominant-negative effect drives selection of TP53 missense mutations in myeloid malignancies.* Science, 2019. **365**(6453): p. 599-604.
Reference #16: Dearth, L.R., et al., *Inactive full-length p53 mutants lacking dominant wild-type p53 inhibition highlight loss of heterozygosity as an important aspect of p53 status in human cancers.* Carcinogenesis, 2007. **28**(2): p. 289-98.
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