Dear Editor,

Thank you for your letter. We highly appreciate the valuable comments of the reviewers on our manuscript (title: LncRNA HAND2-AS1 ultrasound microbubbles suppress hepatocellular carcinoma progression by regulating miR-873-5p/TIMP2 axis). We have improved our manuscript according to their suggestions, and the amendments are highlighted in yellow in the revised manuscript. Point by point responses to the reviewer's comments are listed below. We would like to re-submit this manuscript for your kind consideration.

Yours sincerely,

Hui Gao

No.18 Tuanjie Street, Baotou, Inner Mongolia Autonomous Region 014030, China.

E-mail: gaohui145@163.com

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision

Specific Comments to Authors:

This is an interesting study investigating the role of UTMB-mediated HAND2-AS1 in hepatocellular carcinoma (HCC) progression. Qiang Zou and colleagues delivered HAND2-AS1 into HeGp2 HCC cells by UTMBs and found that UTMBs carrying HAND2-AS1 suppressed the cell invasion, proliferation and EMT. Their findings would indicate that HAND2-AS1 suppressed the MMP2/MMP9 signalling pathway and then suppressed tumour progression by upregulating TIMP2 via targeting miR-873-5p. Of interest, in vivo results demonstrated that tumour formation was

inhibited in xenograft mice injected with HAND2-AS1-bearing UTMBs. The study has merit since it explores novel hepatocarcinogenesis-related mechanisms, potentially providing an additional source for the treatment of HCC. The study is of current interest since HCC is one of the most letal and frequent cancer worlwide. However, to make the study of clinical impact, the authors should discuss and try to link the study findings with the efficacy of available systemic therapies for the treatment of HCC. In particular, in the current scenario of increasing number of systemic therapies, we urgently need to identify prognostic markers to identify patients who better respond to tyrosine kinase inhibitors (TKI) such as sorafenib, lenvatinib, regorafenib, cabozantinib, and immune checkpoint inhibitors (ICI) such as anti-PD1, PDL1, CTLA-4 agents. In fact, it has been recently demonstrated that combination treatment strategy based on TKI plus ICI significantly enhance treatment response as well-described in a comprehensive review addressing the improved efficacy of TKI-ICI combination treatments (Expert Rev Anticancer Ther. 2023 Mar;23(3):279-291. doi: 10.1080/14737140.2023.2181162). Importantly, there are also other systemic treatments that have demonstrated both good efficacy and safety profiles in patients progressing under first-line TKI agent or intolerant to sorafenib as capecitabine, a safe and unexpensive therapy, as recently demonstrated in cohort real-life/clinical practice studies (Dig Liver Dis. 2015 Jun;47(6):518-22. doi: 10.1016/j.dld.2015.03.010; J Cancer Res Clin Oncol. 2018 Feb;144(2):403-414. doi: 10.1007/s00432-017-2556-6). The authors should suggest future studies investigating the prognostic role of lncRNAs to select the best systemic treatment in the era of precision medicine.

Response: Good comments. In the discussion section, we tried to link the research results with the systemic treatment of HCC. At present, our research is still at the

level of molecular biology, and has been verified in a small range in mice. Therefore, we will further explore the relationship between HAND2-AS1 and tyrosine kinase inhibitors (TKI) and immune checkpoint inhibitor (ICI) in subsequent studies, in order to provide new evidence for the use of UTMBs carrying HAND2-AS1 in the systemic treatment of HCC.

As follows:

Discussion

At present, there are more studies focusing on systemic treatment of HCC, for example, patients with better response to tyrosine kinase inhibitor (TKI), such as sorafenib, lenvatinib, regorafenib, cabozantinib and immune checkpoint inhibitor (ICI), including anti-PD1, anti-PDL1 and anti-CTLA-4 drugs. A recent comprehensive evaluation report showed that the efficacy of TKI-ICI combination therapy for HCC was more significant (Ref. 24). In addition, studies have shown that HCC patients who failed first-line treatment with sorafenib also had good tolerance and safety to metronomic capecitabine (Ref. 25&26). The results of this study suggested that HAND2-AS1 may be used as a prognostic marker of HCC. Therefore, our subsequent studies will further explore the relationship between HAND2-AS1 and TKI and ICI, in order to find the best systemic treatment for HCC.

Ref. 24: TKIs in combination with immunotherapy for hepatocellular carcinoma. Expert Rev Anticancer Ther. doi: 10.1080/14737140.2023.2181162.

Ref. 25: Metronomic capecitabine as second-line treatment in hepatocellular carcinoma after sorafenib failure. Dig Liver Dis. doi: 10.1016/j.dld.2015.03.010.

Ref. 26: Metronomic capecitabine as second-line treatment for hepatocellular carcinoma after sorafenib discontinuation. J Cancer Res Clin Oncol. doi: 10.1007/s00432-017-2556-6.

Additional comment：

(5) The labeling of *P* value in the figures does not meet the requirements of WJGO, please don't include any *, #, †, §, ‡, ¥, @….in your manuscript; Please use superscript numbers for illustration; and for statistical significance, please use superscript letters. Statistical significance is expressed as $^{a}P < 0.05$, $^{b}P < 0.01$ ($P > 0.05$ usually does not need to be denoted). If there are other series of P values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used, and a third series of P values is expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$.

Response: Thanks. We changed the *p*-value label in the figures to "a" and modified the corresponding figure legends part. In addition, we updated the figures in the manuscript.

We appreciate for Editors/Reviewers' warm work earnestly, and hope that the correction will meet with approval.

Once again, thank you very much for your comments and suggestions.