

Peer Review File

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Reviewer A

The paper titled “LncASAP1-IT1 promotes hepatocellular carcinoma progression through the regulation of the miR-1294/TGFBR1 pathway” is interesting. These results suggest that lncASAP1-IT1 promotes HCC development by targeting TGFBR1 through miR-1294. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) There are many lncRNA that regulate the cell proliferation, migration, invasion, and epithelial-mesenchymal transition progression of hepatocellular carcinoma. Why did the author choose lncASAP1-IT1 for research? Please describe the reason.

Answer: We have modified our text as advised (see Page 3, line 76-79)

2) What are the problems and challenges that need to be overcome in the clinical application of lncRNA? It is recommended to add relevant content.

Answer: We have modified our text as advised (see Page 11, line 333-336)

3) What is the impact of this study on the further treatment and prognosis of hepatocellular carcinoma? It is recommended to include relevant content in the discussion.

Answer: We have modified our text as advised (see Page 11, line 333-336).

4) There are many detection methods for cell proliferation, migration, and invasion. If multiple methods are used, the results may be more reliable. It is suggested to add test results of other methods.

Answer: Your suggestion is valid. We have carefully evaluated the funding and resources required to complete these additional studies and found that such an expanded study is not currently affordable. Meanwhile, we feel that the scope of work of the present paper can support its conclusions. Therefore, we suggest that the additional experiments be included in a follow-up paper.

5) Figure 5A are not clear enough. It is recommended to provide clearer figure again.

Answer: We followed the suggestions (Figure 5A).

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Comprehensive analysis of the relationship between competitive endogenous RNA (ceRNA) networks and tumor infiltrating-cells in hepatocellular carcinoma, J Gastrointest Oncol, PMID: 33457008”. It is recommended to quote the articles.

Answer: We have modified our text as advised (see Page 4, line 92).

7) Can lncASAP1-IT1 be used as a potential biomarker for patient risk stratification and local regional metastasis in hepatocellular carcinoma? It is recommended to add relevant content.

Answer: We have modified our text as advised (see Page 11, line 333-336).

Reviewer B

- 1) First, the title needs to indicate the experimental research methodology such as in vitro and in vivo.

Answer: We have modified our text as advised (see Page 1, line 4).

- 2) Second, the abstract needs some revisions. The background did not indicate the clinical needs for this research focus and what the knowledge gap is on the mechanisms of LncASAP1-IT1 in HCC. The methods part is not adequate. The authors need to briefly describe more search procedures and the questions to be answered by these procedures. Please also describe how the HCC patients and healthy controls were matched. The results need to quantify the findings by reporting statistics such as expression levels and accurate P values. The conclusion needs to have comments on the potential clinical implications of the findings.

Answer: We have modified our text as advised (see Page 2, line 28-29; see Page 3, line 35-36; 48-49).

- 3) Third, in the introduction of the main text, the authors need to have comments on the potential clinical significance of this research focus, briefly review known biomarkers involved in the clinic-pathological mechanisms in HCC and analyze why LncASAP1-IT1 pathways deserve to be studied.

Answer: We have modified our text as advised (see Page 4, line 89-92).

- 4) Fourth, in the methodology of the main text, please have an overview of the research procedures of this study, the questions to be answered by these procedures, and how the HCC patients and healthy controls were recruited. It is necessary to use a figure to describe these experimental procedures. In statistics, please ensure $P < 0.05$ is two-sided.

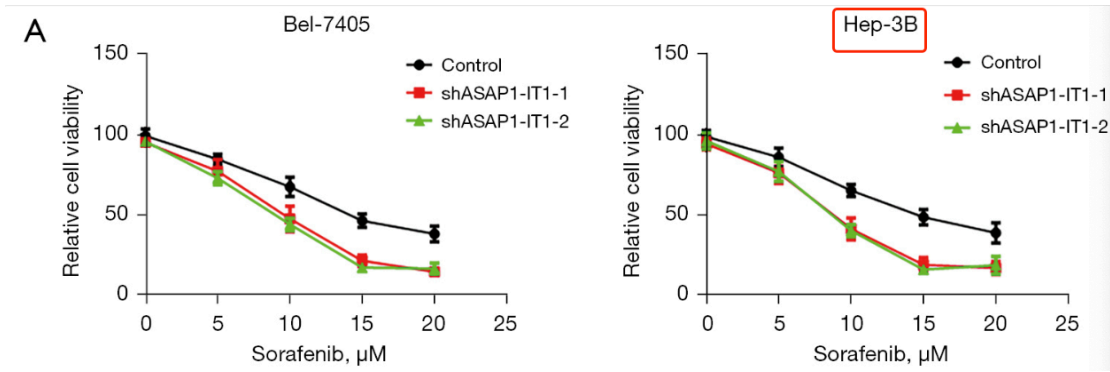
Answer: We have provided a graphical abstract and modified our text as advised (see Page 4, line 102-103; Page 7, line 218).

Reviewer C

1. Figure 3

The main text was different with the figure, please check and unify.

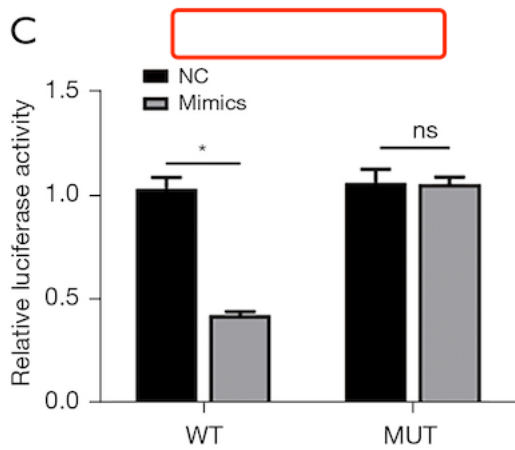
To better understand LncASAP1-IT1 function in chemoresistance, we generated sorafenib-resistant HCC lines (using Bel-7405-SR, Hep3B-SR) and evaluated drug resistance using the CCK-8 test. As seen in Figure 3A, we found that silencing



Answer: We have modified Figure 3A as advised.

2. Figure 4

Please check if a title is needed for 4C.



Answer: We have checked and there is no need a title.

3. Figure 5

The main text was different with the figure, please check and unify.

Figure 5D:

280 silencing miR-1294 reversed the decrease in TGFBR1 expression induced by
 281 lncASAP1-IT1 silencing (Figure 5D). In terms of cytotoxic behavior modulation, we

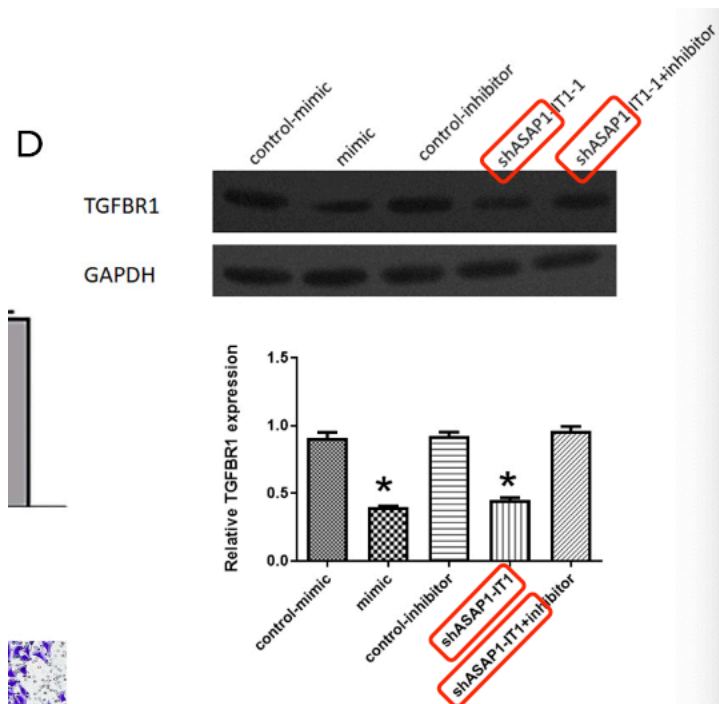
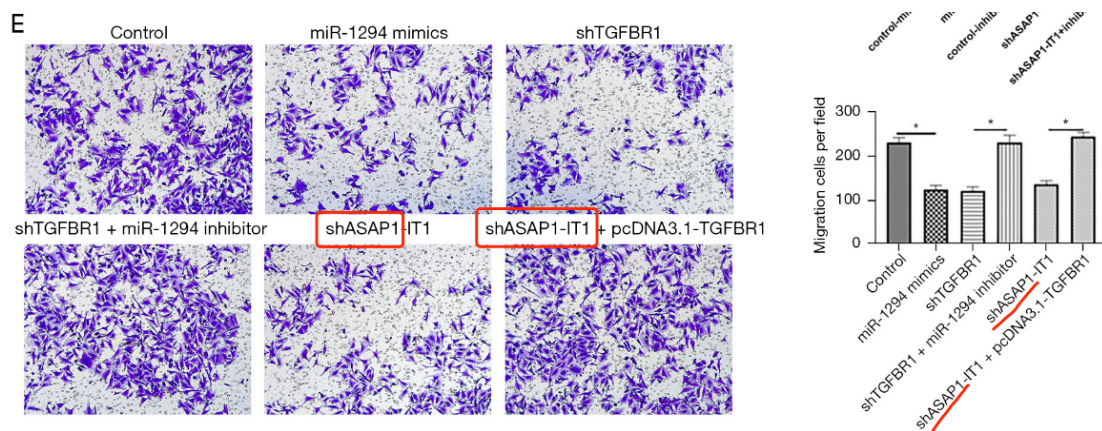


Figure 5E:

induced by the **lncASAP1-IT1** knockdown (Figure 5E).



Answer: We have modified our text as advised (see Page 10, line 273; 280)

4. References/Citations

Please double-check if more studies should be cited as you mentioned “studies”.

90 cancer, osteosarcoma, pancreatic ductal adenocarcinoma, and HCC (9-11). **Studies** have
 91 reported that ceRNA networks exhibit significant potential in the diagnosis and targeted
 92 therapy of cancer. However, the function of lncASAP1-IT1/miR-1294 in HCC remains
 93 incompletely defined (12).

Answer: We followed the suggestions (see Page 4, line 83).