Author's response to reviews

Title: MicroRNA-144 suppresses cholangiocarcinoma cell proliferation and invasion through targeting platelet activating factor acetylhydrolase isoform 1b

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The BMC Cancer Editorial Team:

Thank you for your letter and for the editorial request' comments concerning our manuscript entitled “MicroRNA-144 suppresses cholangiocarcinoma cell proliferation and invasion through targeting platelet activating factor acetylhydrolase isoform 1b” (MS: 1910821527127704). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have revised editorial request carefully and have made correction which we hope meet with approval.

Reviewer's report

Reviewer # 1: Kinya Okamoto

Major Compulsory Revisions:

1. Authors should explain their deliberate CCA cell line selection in some experiments. Why have HCCC-9810 and CCLP1 been enrolled into the almost all experiment, but not HuCCT1 and RBE? I presume that the cell line selection depended on the expression levels of LIS1. If at all, the authors should declare the selecting criterion. The deliberate cell line selection is also found in miR-144 ectopic over expression and selective inhibition and in vivo tumor model.

Thanks a lot for the Kinya Okamoto’s suggestion and reminding. We chose HCCC-9810 and CCLP1 because they had lower miR-144 expression among the cell lines detected (Figure 1B). As the low expression of miR-144, we could not effetely inhibition of miR-144 by transient transfection, let alone in vivo tumor model.

2. The estimation of miR-144 putative target genes is one of the important parts of this manuscript. The authors should explain the software predicting method in detail and indicate other candidate target genes and their calculated parameters.
We have amended it according to reviewer’s comments in our revised manuscript.

3. The authors should show more information about LIS1 in detail. For example, unabbreviated name, other referring name; platelet activating factor acetyldihydrolase isoform 1b (PAFAH1B1) and the known functions from the latest studies.

Thanks a lot for the Kinya Okamoto’s suggestion and reminding. We have added latest studies of LIS1 in discussion of our revised manuscript.

Minor Essential Revisions

1. The manuscript sentences are written clearly and easy to understand. However, please check the manuscript carefully. There are many lacks of space and word duplications. In addition, I recommend the authors taking proofread by a native English speaker to refine the manuscript more.

I have revised our language editing by Elsevier.

2. In Materials and Methods, the authors should show the abbreviation of human embryonic kidney 293T at the first referred sentence.

We are so sorry about our mistakes. We have already modified it in revised manuscript.

3. Please unite the company reference style as the submission instruction of the journal.

Thank you for your kind attention. We have already amended it in revised manuscript.

4. The authors use the word “infection” as the plasmid transfection by the viral vector. I think the word “transfection” may be better than infection.

Thank you for your good suggestion. We have already amended it in revised manuscript.

5. In figure 1: Please indicate which expression profiles are CCA tissue samples or adjacent normal bile ducts clearly.

We have added some explanation in revised figure legends.

6. In figure 3A: please correct has-miR-144 to hsa-miR-144. I think these misspellings are due to the auto word correcting function of the word processing application.

Thank you for careful examination, we have revised it.

7. In figure legends, some special characters are seen between “p” and “<”. Have the authors used some Chinese fonts for space? Please replace them with English fonts.

We have replaced them with English fonts.

Reviewer # 2: Arthit Chairoungdua

Major compulsory revision

1. How did the overexpression of miR-144 and/or reduction of LIS1 expression
resulted in suppression of Akt phosphorylation and MMP2 expression? How is it involved in suppression CCA cell proliferation, invasion and motility?

Activation of the Akt signaling pathway was a prototypic survival pathway that played a central role in diverse cellular functions, including proliferation and metastasis. So we detected the expression of P-AKT (ser473) and found it downregulation.

2. It would be interesting to examine the effect of miR-144 inhibition of CCA cell phenotypes.

We are so sorry about it. As the low expression of miR-144, we could not effectly inhibition of miR-144 by transient transfection because the efficiency of transient transfection was not stable. But we would try to resolve it in our further study.

3. As the author mention in discussion, in contrast with this study, “ectopic expression of LIS1 inhibited hepatocellular carcinoma cell proliferation”. The author should discuss this discrepancy in the discussion.

We have added this section in our revised manuscript.

4. Figure 3E, what is the level of miR-144 after transfection with anti-miR-144?

Because of the low expression of miR-144 in CCA cell lines, we just inhibition of miR-144 40%.

5. What kind of mice are used in this study between nude mice and SCID mice?

In result part, the author described that nude mice were used. However, the author mention that SCID mice were used in figure 5.

We are sorry for our incorrect expression which results in the reviewer’s misunderstand. The animal we used was nude mice and we have amended it in revised manuscript.

Minor essential revision

1. Spelling and grammar should be carefully checked such as “cholangiocarcinoma not choriocarcinoma”.

We are sorry for our incorrect spelling. We have amended it in revised manuscript.

2. Phosphorylation site on AKT should be indicated in Figure 2 and 4.

We have amended it in revised Figure.

3. The author should indicate the statistic that used in each Figure.

We have did gray analysis and there was more space insert the statistic data.

4. Is LIS1-UTR-MUT (Figure 3B) double mutations?

Yes.

5. How many mouse was used in Figure 5?

Eight mice were used in each group and we just showed typical results.

6. The exogenous miR-144 expression decreased cell viability only after 72 h not 48 h (the author mention in the result) after infection.

We are sorry for our incorrect expression which results in the reviewer’s
misunderstand. We detected cell viability at the time point of 0, 24, 48 and 72 hours, but the difference was in 48 hour and 72 hour.

7. For cell proliferation assay, what kind of method was used between CCK-8 cell counting kit (in materials and methods) and MTT assay (in result and figure legends).

We used CCK-8 cell counting kit. We have amended it in revised manuscript.

Sincerely yours,
Shengquan Zou, PhD, MD