Author’s response to reviews

Title: Paracrine effects of CCN3 from non-cancerous hepatic cells increase signaling and progression of hepatocellular carcinoma

Authors:

Qingan Jia (qajia66@163.com)
Weimin Li (760230249@qq.com)
Xia Liao (373151023@qq.com)
Pengbo Ning (127858600@qq.com)
Yu Cao (18792629638@163.com)
Mei Zhang (xazhangmei@126.com)
Yang Bu (boyang1976@163.com)
Jun Lv (junlv0088@163.com)

Version: 3 Date: 27 Feb 2019

Author’s response to reviews:

Dear Prof. Cassady-Cain

Thank you very much for your letter and advice. We have revised the manuscript, and would like to re-submit it for your consideration. We have addressed the comments raised by the reviewers, and the amendments are highlighted in red in the revised manuscript. Point by point responses to the reviewers’ comments are listed below this letter.

We hope that the revised version of the manuscript is now acceptable for publication in your journal.

I look forward to hearing from you soon.

With best wishes,

Yours sincerely,
Qingan Jia

Department of Hepatobiliary Surgery, the First Affiliated Hospital of Xi’an Jiaotong University, 277 West Yanta Road, Xi’an 710061, China

E-mail: qajia66@163.com

We would like to express our sincere thanks to the reviewers for the constructive and positive comments.

Replies to Reviewer 1

1. In table1 the quantitative unit of AFP is μg/L, while in table2 the unit of quantity is missing. Please correct this minor problem.

   We had added the unit of μg/L into table2 in the revised version.

2. In figure legend 4, (Page22 line 20) the author described the E and F, while there is lack of relevant instructions in the result part.

   We had added the E, F, and G in the result part (page 11-page 12).

3. Cause figures in this manuscript is missing, I am not sure the legend describe well for figures.

   Thanks for the reviewers for their carefulness and patience. In order to ensure the accuracy of the content, we had reviewed the figures and the associated legends again, and the revised version of the manuscript should be accurate and acceptable.

4. On page 15 the description may be not accurate enough “we found that CCN3 expression was a useful but insufficient marker alone to predict prognosis of patients with HCC. Thus, exclusive targeting of CCN3 may have limited efficacy in treating HCC progression”. And I think the author may want to describe “ Although cirrhosis promoting tumors progression is very clearly, while cirrhosis was insufficient factor alone to predict prognosis of patients with HCC, cause of many cytokines such as CCN3 may influence the relationship between
cirrhosis and HCC progression. Maybe that is why anti-fibrosis is not an very effective way to anti-HCC.

We had reviewed the previous sentences, and which was inaccurate and vague indeed. In the revised version, we replaced the previous sentences with the suggested sentences from the reviewer. (Page15 line 18)

5. As described in the part 2 of Results, “Patients were then divided into four groups according to level of cirrhosis (severe, moderate, mild, and no cirrhosis)” . What is the criteria used in dividing the severity of cirrhosis. Please instruct it in the part of Method.

The level of cirrhosis refers to the general observation of pathological specimens and the standard of METAVIR scoring system. The severity of cirrhosis was classified into four degrees by histopathological evaluation of the liver tissue: No fibrosis, mild (portal fibrosis with few septa), moderate (numerous septa without cirrhosis), and severe (cirrhosis). And this content was added into the part of Method. (Page5 line 1-5)

6. Please describe the methods that activate Hepatic LO2 cells by HSC LX2 cells in detail.

Hepatic cells LO2 activated by HSC cells LX2

The Conditioned Media (CM) was collected from HSC LX2, and used for treating hepatic cells LO2 for 3 days. Then LO2 cells were activated by LX2 and referred to as activated LO2 (aLO2). When we silenced endogenous CCN3 in aLO2 using lentiviral transfection, the CM from HSC LX2 was continuously applied. Until the aLO2-CCN3-sh1 was successfully constructed, and CM was going to be collected, the supernatants were changed to low serum medium. And the content above was partly added into Method. (Page5 line 13-17)

Replies to Reviewer 2

1. Because this study follows a previous study already published on CNN3 expression in the tumor tissue (that was shown to be much higher than the surrounding "healthy" tissue, it would be interesting to show the relative level of expression of CNN3 in the tumor/ in the surrounding tissue/ in the tissue of a normal liver. This will allow a better appreciation of the relative amounts of protein in each tissue region. Because the effect observed on HSC could be the result of diffusion of CNN3 excreted by cancer cells (and not by healthy hepatocytes surrounding the tumor).
This is a very good suggestion. HSC could also be inhibited by the diffusion of CCN3 excreted by cancer cells. Based on our current findings, in the tumor microenvironment, the largest number is cancer cells, follow by fibroblasts, while in the microenvironment of surrounding hepatic tissue, the largest number is cancer cells, follow by fibroblasts. In the further studies, we will focus on the relationship between HCC and cirrhosis to identify a comprehensive treatment strategy.

2. The authors should describe the protocol for "activation" of hepatocytes by HSC in vitro. How long are the cells co-cultured? what is the ratio between HSCs and hepatocyte cell line...

Thanks, this is the same one as question 6 from reviewer 1. the Conditioned Media (CM) was collected form HSC LX2, and used for treating hepatic cells LO2 for 3 days. Then LO2 cells were activated by LX2 and referred to as activated LO2 (aLO2). When we silenced endogenous CCN3 in aLO2 using lentiviral transfection, the CM from HSC LX2 was were continuously applied. Until the aLO2-CCN3-sh1 was successful constructed. And the content above was partly added into Method. (Page5 line 13-17)