Author's response to reviews

Title: Hypoxia induces epithelial-mesenchymal transition via activation of SNAI1 by hypoxia-inducible factor -1a in hepatocellular carcinoma

Authors:

Lin Zhang (zhanglinxm@gmail.com)
Gang Huang (huanggcq@sohu.com)
Xiaowu Li (fengxiaobin200708@yahoo.com.cn)
Yujun Zhang (jiangyan-0124@163.com)
Yan Jiang (jiangyan-0124@163.com)
Junjie Shen (junjiesh@gmail.com)
Jia Liu (leinama@gmail.com)
Qingliang Wang (qingliangwang@yeah.net)
Jin Zhu (zhujinfly@yahoo.com)
Xiaobin Feng (fengxiaobin200708@yahoo.com.cn)
Jiahong Dong (dongjh301@163.com)
Cheng Qian (cqian3184@yahoo.com.cn)

Version: 2 Date: 24 January 2013

Author's response to reviews: see over
Author's response to reviews

Title: Hypoxia induces epithelial-mesenchymal transition via activation of SNAI1 by hypoxia-inducible factor -1a in hepatocellular carcinoma

Authors:
Lin Zhang (zhanglinxm@gmail.com)
Gang Huang (huanggcq@sohu.com)
Xiaowu Li (fengxiaobin200708@yahoo.com.cn)
Yujun Zhang (jiangyan-0124@163.com)
Yan Jiang (jiangyan-0124@163.com)
Junjie Shen (junjiesh@gmail.com)
Jia Liu (dadaliujia@gmail.com)
Qingliang Wang (qingliangwang@yahoo.net)
Jin Zhu (zhujinfly@yahoo.com)
Xiaobin Feng (fengxiaobin200708@yahoo.com.cn)
Jiahong Dong (dongjh301@163.com)
Cheng Qian (cqian3184@yahoo.com.cn)

Version: 2 Date: 23 January 2013
Author's response to reviews: see over
Reviewer's report

Title: Hypoxia induces epithelial-mesenchymal transition via activation of SNAI1 by hypoxia-inducible factor -1a in hepatocellular carcinoma

Version: 1 Date: 23 October 2012

Reviewer: Wenlin Huang

Reviewer's report:

This manuscript aimed to investigate the molecular mechanism by which hypoxia promotes HCC invasion and metastasis through inducing EMT. The authors demonstrated that hypoxia-stabilized HIF1# promoted EMT through increasing SNAI1 transcription in HCC cells. I think the experiments were well defined and innovative. The results provided a potential therapeutic target for HCC treatment. However, there are some problems that should be solved before consideration of publication.

Please find below my specific comments.

Major Compulsory Revisions

1. For the representative images of CoCl2 treatment groups in figure 3C, the cell number of migration experiment was similar to that of the invasion experiment. However, the mean values of these two groups showed great difference. The authors should check the raw data.

   We have checked the raw data and this data is correct. The representative images for experiments migration and invasion were not well presented. We have chosen appropriated images with high quality to replace old ones in Figure 3C.

2. The resolution of figure 3C and 4C was too low to be seen in detail. The authors should provide new figures with appropriate clarity.

   We have chosen appropriated images with high quality to replace old ones in Figure 3C and Figure 4C.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests
Reviewer's report

Title: Hypoxia induces epithelial-mesenchymal transition via activation of SNAI1 by hypoxia-inducible factor -1a in hepatocellular carcinoma

Version: 1 Date: 20 December 2012
Reviewer: Cecilia Sahlgren

Reviewer's report:

Although the question posed by the authors is interesting and important, the work does not raise our knowledge on the association between hypoxia and aggressive metastatic tumor growth nor does it enhance the molecular insight of the link between HIF and Snail, an inducer of epithelial mesenchymal transition (EMT). The link between hypoxia and EMT is already demonstrated for a number of cancer types, as is the direct regulation of Snail by HIF. The novelty is restricted to the extension of this already quite established paradigm to hepatocellular carcinoma and hence is of highest interest for those focused on this particular disease. As the authors include data pointing to the clinical relevance of the findings the work merits publication given that the comments below are addressed. This will require a major revision as the data as it stands is merely correlative and not conclusive.

Major:

The authors do not conclusively show that Snail is responsible for the hypoxia/HIF induced EMT. Experiments with down regulation of Snail should be included.

It is well demonstrated that Snail is an inducer of EMT and it plays an important role in induction of EMT in HCC cells. Thus, we did not carry out experiments with down regulation of Snail. Instead, we focus on experiments how HIF-1a regulates Snail. We have discussed this issue in the revised manuscript.

The authors demonstrate that Co2Cl treatment and HIF induces expression of luciferase form a snail promoter construct containing HRE sequences. The direct association of HIF on the Snail promoter should be demonstrated by ChiP assays.

Our data showed that CoCl2 treatment induced Snail expression and knock down of HIF-1a expression reduced the CoCl2-induced Snail expression. In order to know whether HIF-1a directly regulate Snail expression, we performed reporter assays with different forms of Snail promoter with wild-type and mutated HRE or without HRE. Our data indicate that HRE at -541 site plays an important role in transcription of Snail by HIF-1a. These functional studies are sufficient to support direct regulation of Snail by HIF-1a. Thus, we don’t think it is necessary to do ChiP assays.

The statistical data in the first result section is not correctly expressed.

According to your suggestions, we have modified this information in the revised manuscript.

The experiments with Co2Cl treatment should be repeated with hypoxia.
It is well accepted treatment with CoCl2 can represent hypoxia condition. In this study, we performed experiments of EMT marker expressions and migration and invasion assays in conditions of hypoxia and CoCl2 treatment. Similar results were obtained in both conditions (See Figure 2 and 3). Therefore, we performed the rest experiments only in condition of CoCl2 treatment.

The authors should include references to work establishing both direct and indirect links between hypoxia and Snail expression.

We have included references to work establishing both direct and indirect links between hypoxia and Snail expression and we have discussed this issue in the revised manuscript.

The reoxygenation mechanisms should be studied in more detail. What happens on the protein and cellular level? Is it a EMT-MET transition?

We have studied reoxygenation mechanisms carefully. Our results showed that reoxygenation decrease the hypoxia-induced expression of EMT markers, HIF-1α and Snail. In addition, reoxygenation decline the hypoxia-induced increase of migration and invasion (See Figure 2 and 3). Therefore, we speculated reoxygenation could induce a MET (See Figure 5C).

Minor

The text should be proof read and corrected

We have corrected all errors.

The E-cadherin blots should be repeated and quantified

We have replaced E-cadherin blots with high quality of images.

A control/normoxia image with more confluent cells in Figure 2A should be included.

We have included a new control/normoxia image with more confluent cells in Figure 2A