Author's response to reviews

Title: Overexpression of SIRT1 promotes metastasis through epithelial-mesenchymal transition in hepatocellular carcinoma

Authors:

Chong Hao (haochong2006@126.com)
Zhi-peng Han (hanzhipeng0311@126.com)
Peng-Xi Zhu (zhupengxi5905@sina.cn)
Xue Yang (yangxue19900307@126.com)
Xu-Guang Zhang (xgzh1985@aliyun.com)
Wen-Ting Liu (liuwenting1015@163.com)
Qiu-Dong Zhao (zhaoqd@gmail.com)
Ting-Ting Fan (funttingting@126.com)
Meng-Chao Wu (wumengchao2012@126.com)
Li-Xin Wei (weilixin_smmu@163.com)
Li Zhang (zhanglicdma@189.cn)

Version: 2
Date: 25 October 2014

Author's response to reviews:

Point-by-Point Response to Reviewers' Comments.

Reviewer #1:

Major Compulsory Revisions

In this study, the authors showed a positive role of SIRT1 in the development of HCC in vitro and in vivo. They showed that the enhanced SIRT1 expression increased migration and invasion through EMT in HCC cell lines and further demonstrated that SIRT inhibition with nicotinamide diminished hepatic tumorigenesis in vivo.

Herein the authors clearly observed that SIRT1 play an important role in the development of HCC in vitro and in vivo. Results shown in this manuscript were solid to support their claims. However, we could not see originality except of HCC model because other studies already showed SIRT1 exerts as a promoter in prostate cancer and colon cancer model. The other studies even showed mechanism of SIRT1-mediated tumorigenesis through metalloproteinase-2 or some microRNAs in the studies. Although the authors nicely showed their results in vitro, in vivo and in clinical samples, which are phenotype of SIRT1 effect, we lose our interest in this study without the mechanism. We would like to urge a mechanism of SIRT1-mediated EMT for the cancer development in HCC model.

Reply: The reviewer raised an important point. Several studies showed SIRT1 exerts as a promoter in prostate cancer and colon cancer model. However, some other studies showed that SIRT1 could inhibited the development of cancer. So the role of SIRT1 in tumor development is still controversial. We have known
SIRT1 played an important role in the development of HCC. However, the effect of SIRT1 on HCC metastasis is still unknown. Therefore, the aim of this study is to investigate the role of SIRT1 in HCC metastasis and the potential mechanism.

Reviewer #2:
Chong Hao et al. found that SIRT1 expression was significantly overexpressed in the tumor tissues and HCC cell lines. A clinicopathological analysis showed that SIRT1 expression was significantly correlated with tumor size, tumor number, and TNM staging. Kaplan–Meier survival curves revealed that positive SIRT1 expression was associated with poor prognosis in patients with HCC. SIRT1 significantly promoted the ability of migration and invasion in HCC cells. In addition, experiments with a mouse model revealed that SIRT1 overexpression enhanced HCC tumor metastasis in vivo. Furthermore, they demonstrated that SIRT1 significantly enhanced the invasive and metastatic potential by inducing epithelial-mesenchymal transition in HCC cells. Though Chong Hao et al suggested that SIRT1 may play an important role in HCC progression and could be a potential molecular therapy target for HCC from their findings, many weakness limited the value of this research, even the publication of their findings.

Major Compulsory Revisions
1. The clinical data (one table and one figure) was not meaningful enough. Overall survival was too weak to reflect their conclusions. This research is about HCC metastasis, but no correlation was shown between sirt1 and metastasis. Additionally, disease free survival was more meaningful than overall survival to support their findings. I do not understand well why put these clinical data into this article as the first part. If they really want to show these weak clinical data, it is much better for them to write the clinical data in the second part to assist your in vivo and in vitro findings. Please check recent article correlation between P53 and SirT1 for disease free survival of HCC.

Reply: The reviewer raised an important point. We have modified the manuscript according to the suggestion.

2. Resveratrol data are quite different from previous ones. Most of the previous results showed that resveratrol repressed cancer cell proliferation and EMT invasion. But the authors showed increased invasive ability after treatment of resveratrol. Therefore, you should make the results of cell viability assay and apoptosis assay on their cell lines after reveratrol treatment to support their arguing findings.

Reply: This is an interesting question. In previous studies, most of the results showed that resveratrol repressed the development of tumors. However, the effect of resveratrol on HCC metastasis is poor documented. Our results showed that resveratrol could promote the metastasis of HCC cells by inducing EMT. According to suggestion, we also supplied the results of cell viability assay and apoptosis assay on HCC cells (supplementary figure 1). The results showed that in the concentration that we used could slightly inhibited the cell viability and enhanced apoptosis.
Minor Essential Revisions

1. No results were shown for Hep3B cells though you used Hep3B cells in this research as you mentioned in the text. I wonder the reasons. Will the authors get the same results from Hep3B cells as other cells?

Reply: As we have shown in figure 1, the expression of SIRT1 in Hep3B is same as SMMC7721 and HepG2. Furthermore, we have got the same results from Hep3B cells as other cells. So we choose SMMC7721 and HepG2 cells to carry out the study.

2. I think the authors wanted to insist on the suppression and stimulation effects of nicotinamide and resveratrol on the cell invasion relevant to sirt1. Then authors should verify their results through western blot and sirt1 activity assay using both of the experiments (1. shsirt1 to knock down sirt1; 2. Resveratrol and Nicotinamide treatment).

Reply: This is an important point. We have demonstrated in figure 2 that the efficiency of AdSIRT1 and shSIRT1 in regulation the expression of SIRT1. Several studies have proved that Resveratrol and Nicotinamide can effectively enhance and inhibit the function of SIRT1. Furthermore, Resveratrol and Nicotinamide treatment demonstrated the same phenomenon as AdSIRT1 and shSIRT1 in vivo and in vitro. So we can conclude that the suppression and stimulation effects of nicotinamide and resveratrol on the cell invasion relevant to sirt1.