Reviewer’s report

Title: Differential effects of arsenic trioxide on chemosensitization in human hepatic tumor and stellate cell lines

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Reviewer: Wei Jiang

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The present study aimed to investigate the mechanisms of ATO to treat hepatocellular carcinoma (HCC) and seek potential combinational options. The authors analyzed the effects of ATO in combination with 5-FU or sorafenib respectively on HepG2 and LX-2 cell lines and concluded that ATO in combination with 5-FU or sorafenib have enhanced cytotoxicity in HepG2 for the decrease in thymidylate synthase by ATO and the potent inhibition of MAPK activation by sorafenib.

Nowadays, cellular crosstalk in tumor microenvironments are hotly debated in many tumor types. In HCC secondary to liver fibrosis and cirrhosis, HSCs as the determinants of liver fibrosis progression and also as major cellular components of peritumor microenvironments are newly found to play an important role in tumor driving. The authors has put forward a valuable topic that the crosstalk between hepatic tumor cells and peritumor HSCs might be the critical modulator of chemoresistance, however the data presented in this study does not fully support this point of view. The specific comments are as below.

Major Compulsory Revisions

1) Although authors highlighted the tumor-stroma interactions as a potential mediator of chemoresistance in this study, they only analyzed the interaction between ATO and 5-FU or sorafenib respectively on HepG2 and LX-2 cell lines. To investigate effects of cellular talks in chemoresistance, the co-culture model of HepG2 and LX-2 cell lines using a transwell system should be firstly established and then the significance of HepG2-LX-2 cellular crosstalk in chemoresistance could be analyzed in vitro.

2) Although LX-2 was demonstrated to be more sensitive to ATO-induced cell death in comparison with HepG2 for the presence of a sub-G1 peak, it’s quite reasonable to deduce that ATO might also influence the TS expression in LX-2 since ATO dramatically decreased TS expression in HepG2 (showed in Fig.5). The authors have no reason to make an arbitrary conclusion that ATO-induced TS expression account for the enhanced apoptosis of HepG2 cell line after the treatment of 5-FU+ ATO, for the following points: TS expression in HepG2 after treatment of ATO+5-FU or single 5-FU was not analyzed; TS expression in LX-2 after treatment of single ATO, single 5-FU and ATO+5-FU was not analyzed; besides, the significant change of TS expression in HepG2 cell line by ATO treatment occurred at 25μM, which is much higher than the concentration of ATO
(5µM) in combination with 5-FU to induce cell death of HepG2 in Fig.4.

Minor Essential Revisions
1) In the second paragraph of background part, authors suggested that the missing assessment of ATO effect on the stromal compartment could explain the discrepant results of single ATO treatment in rat HCC models and a phase II clinical trial. Since the preclinical data come from an in vivo model, effects of ATO treatment must include all determinant factors such as crosstalk with stromal cells. Thus, I suppose this discrepancy might be caused by the heterogeneity of HCCs in mouse and human models.
2) In Fig.4A, the meaning of 

3) Authors have demonstrated that LX-2 was much more sensitive to ATO compared to HepG2 in Fig.1. However, in Fig.4B, in combination with 5-FU, the concentration of ATO to treat LX-2 was twice as that of HepG2, what’s your explanation for this. How do you define the concentration?
4) In Fig.4C, the protein levels of procaspase 9 in LX-2 cell lines treated with ATO alone or ATO in combination with 5-FU should also be presented to better explain the chemoresistance mechanisms of LX-2 to 5-FU.
5) In Fig.7F, authors should present the pMARK and MARK expression in LX-2 when treated with ATO or ATO+sorafenib, and also GAPDH expression as the internal control.

Discretionary Revisions
1) Actually, to treat HepG2 cell line, ATO performed as the chemosensitization of 5-FU for its ability to decrease the thymidylate synthase, however, sorafenib performed as the chemosensitization of ATO for its potent inhibition of MAPK activation.

Level of interest: An article of limited interest

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.