Reviewer's report

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

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Reviewer: Kari Nejak

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Description:
In this manuscript, Rangwala et al. demonstrate differential chemosensitization of a hepatocyte-derived tumor cell line (HepG2s) and a stellate cell line (LX2) to several chemotherapeutic agents. Overall, the study is well-designed and the conclusions are clear. The discussion is thorough and supported by the data. These findings highlight the importance of identifying synergistic combinations of chemotherapeutic compounds that will target the stromal as well as the epithelial compartment.

Minor Essential Revisions:

The authors should comment further on the significance of thymidylate synthase in ATO/5-FU treatment. The use of this enzyme as a marker for cytotoxicity needs some clarification – perhaps a line or two in the Background or Discussion as to its role in DNA synthesis/repair and importance in HCC.

It is unusual to evaluate the expression of a pro-caspase to demonstrate cell death, as it is an indirect measure of apoptosis activation. Can the authors simultaneously measure cleaved caspase-9 (or cleaved caspase-3) to complement the pro-caspase-9 data? Furthermore, caspase-9 is an initiator caspase activated during the process of intrinsic (mitochondrial) apoptosis; are the authors implying that the cell death seen in ATO/5-FU treatment occurs through activation of this pathway?

Why did the authors not examine the effect of ATO on TS in LX2 cells? It is also important to show by WB the levels of TS in both cell types treated with ATO.

Unsure why the dose of ATO was altered from 5µM in Figure 2 to 10 µM in Figure 4. Can the authors explain in the text?

Figure 4C is hard to see – the lines and data points are very light. Please make this graph darker.

Please add p-values in Figure 7E.

Discretionary Revisions

1) The authors should consider use of an in vitro co-culture model OR a
mixed-cell xenograft tumor model to demonstrate efficacy of ATO/5-FU and ATO/sorafenib in vivo (Ding XY et al., Oncogene 2011).

2) To determine potential toxicity of the proposed treatments on normal liver tissue, it would be useful to test ATO/5-FU and ATO/sorafenib on cultures of primary human hepatocytes and stellate cells.

3) The authors noted that in a phase II clinical trial, ATO had no effect against advanced HCC. Advanced HCC is often characterized as being moderately or poorly differentiated. However, the combination ATO/5-FU and ATO/sorafenib treatments described in this manuscript were used on HepG2 cells, which are a well-differentiated cell line. HepG2 cells are also a hepatoblastoma cell line, which is a tumor never associated with fibrosis or cirrhosis. In light of this, it would be interesting to test these treatments on a poorly-differentiated HCC cell line, such as SNU398, SNU475, SNU449, SNU387, FOCUS, Mahlavu, or SNU182 (Yuzugullu H et al., Mol Cancer 2009), or additional differentiated HCC cell lines (Hep3B, Huh7, or others).

Any one of these three experiments would significantly improve the scope and impact of the work.

Level of interest: An article whose findings are important to those with closely related research interests

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.