Reviewer's report

Title: Overexpression of Heat Shock Transcription Factor 1 enhances the resistance of melanoma cells to doxorubicin and paclitaxel.

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Reviewer: Yanzhong Hu

Reviewer's report:

General comments
Hsf1 has been found to be involved in regulating cell transformation, tumor development, metastasis and chemotherapy resistance by associating with different signaling pathways. However, the molecular mechanisms underlying Hsf1 in tumor chemotherapy resistance is still unclear. In this paper, the authors investigate the regulatory effects of Hsf1 on doxorubicin, paclitaxel, cisplatin, vinblastin and boretzom resistance in mouse and human melanoma cells by ectopically expressing wild-type Hsf1, dominant negative Hsf1 (Hsf1DN), constitutively active Hsf1 (cHsf1) and Hsf1 shRNA in these cells. The wild-type Hsf1 and Hsf1DN can induce tumor cell resistance to doxorubicin and paclitaxel but not to other chemotherapy drugs. By using the cellular accumulation of fluorescent dyes and side population assays to evaluate ABC transporters’ activity (Figures 2 and 3), the authors find that wild-type Hsf1 and Hsf1DN can induce mRNA expression of ABC family members(Figures 1,4,5 and 6), but cHsf1 and Hsf1 shRNA cannot induce such an expression pattern. According to these results, the authors conclude that “Mediated by Hsf1 enhanced resistance to doxorubicin and paclitaxel is not dependent on Hsps accumulation, but on an increased ability to drugs efflux by ABC transporters. Direct transcriptional activity of Hsf1 is not necessary for increased ABC gene expression which is probably executed by the hsf1 regulatory domain”. This conclusion is not novel because Dr. Dautry’s group has already reported a similar conclusion. (see reference 52). Author does did not dig further to investigate howHsf1DN regulates MDR1 mRNA expression. Author needs more experiments (e.g. knock-down of ABC transporter family members or their inhibitors) to show whether up-regulation of ABC transporters can elicit a response similar to that of Hsf1-mediated resistance to doxorubicin or paclitaxel. In abstract the result part needs reorganization properly. All the figures should be number at the right places. The lanes of all the immunoblots and RT-PCR should be numbered. The citation of Figures in text should be placed at correct places.

Specific comments
1. Figure 1 A, the expression of endogenous Hsf1 mRNA is very different between mouse and human melanoma cell lines in response of heat shock response. Do the two cell lines have similar response to doxorubicin resistance. Please explain.

2. Figure 2C. the efflux of fluorescent dyes are different in response to the
over-expression of Hsf1 in the three cell lines. What is the mechanism? Does Hsf1DN have similar effects on efflux of fluorescent dyes?

3. Figure 3, The place of Figure 3 should be corrected.

4. Figure 4, RT-PCR results show most of ABC transporter family members are upregulated in Hsf1-overexpressed cells. Quantification is necessary to determine the different expression level of the ABC transporters. The protein expression level should be tested also. The place of Figure 4 should be corrected.

5. Figure 5B and C, under control condition, the ectopic expression of Hsf1DN in WM793B and 1205Lu is shown to increase the expression of HspH1 and HspA1 compared to the neo-expressing cells. How do you explain this phenomenon? Correct numbering of lanes on all blots is necessary.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

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

**Declaration of competing interests:**

no