Reviewer’s report

Title: CD133+ Liver Cancer Stem Cells Resist Interferon-gamma-induced Autophagy

Version: 2 Date: 22 Oct 2015

Reviewer: Alessandro Lugli

Reviewer's report:

Unfortunately, there are still some major points that need to be addressed:

Major Compulsory Revisions

Revision 1 PCR data: additional quantitative evidence was requested to substantiate the claim of differing CD133 expression between the cell lines used. Additional PCR data has been provided but is given as relative expression levels only (Figures 2A, 4A). In both figures, the base level is cell line QGY7701 which contains only 0.06% positive cells as per FACS analysis. Please provide the absolute expression levels ("quantitative data") of CD133 between the cell lines used as requested. In the form the data is provided it is impossible to see the "significant differences" in expression for BEL7402 and QGY7701 (4A). Further, as per the PCR data in Figure 2A and 4A, PLC8024 cells show a 3 fold higher relative CD133mRNA level than Huh7 which contradicts the FACS data. Please comment on this unexpected result.

Revision 1 Our reply: Thanks to the reviewer's suggestion and the absolute expression levels ("quantitative data"), delta Ct, of CD133 mRNA in different cell lines were provided in the revised figure 2A and 4A in the revised manuscript. (please see the 1st paragraph in page9, 2st paragraph in page23 and 1st paragraph in page24 and Figure2A and 4A). As FACS data detect the percentage of CD133 positive cells (only the relative number) from protein level and QPCR results test the total relative CD133 mRNA expression level in the whole population which was determined both by the number of CD133 positive cells and the CD133 mRNA expression strength in single cell, we thought that it might be the difference expression strength of CD133 mRNA in single Huh7 and PLC8014 cell as well as the different detection methods between protein and mRNA level that led to the results in Figure 2A.
Revision 2: The authors have added deltaCt data. Maybe I have missed it but I did not find the absolute expression levels as claimed above. Differences between ct values of two samples may be caused simply by the fact that two samples contain a different amount of tissue material. Information on how the ct-values were normalized is missing, the data is thus still incomplete. Please provide the complete information regarding the PCR analysis or the absolute expression levels. The legend to Figures 2 and 4 also have not been adapted accordingly ("relative CD133 mRNA expression levels").

Revision 1 Figure 1: High quality images of serial sections were requested. The authors claim to provide serial sections, yet the isotype control in Figure 1 is clearly from a different area. Further, the serial sections show that the AFP+ and CD133+ cells are actually not identical. This contradicts the claim made by the authors that the CD133+ cells are actually AFP+ "liver cancer stem cells". The requested FACS data (to prove that there is a CD133/AFP-positive population in the transplanted tumors) was not provided.

Revision 1 Our reply: Thanks to the reviewer's concern. We are sure that the isotype control was come from the same area as CD133 and the only difference might be that we captured the photo at a little different angle. We agreed with the reviewer's suggestion that if we wanted to prove that the remaining cells were "AFP+ liver cancer stem cells", we should prove that AFP+ and CD133+ cells were identical cells with double staining in IHC or FACS. But, AFP was one of the differentiated special marker for HCC, which were expressed on only about 40% to 55% HCC tumor cells. And CD133 was one of the important liver tumor stem cells marker, which was normally expressed in undifferentiated HCC cell (the definition of stem cell) and which was only expressed on about a thousandth to several hundredths liver cancer cells. So the possibility of a liver cancer cell expressed both AFP and CD133 is very very small. We performed the AFP Powered by Editorial Manager® and ProduXion Manager® from Aries Systems Corporation and CD133 IHC staining in the manuscript meant to prove that these tumor cells were liver tumor cells and in which CD133 positive liver stem cell existed. We agreed with the reviewer that word "AFP positive HCC CSCs" was mistaken and which had been rewritten in the revised manuscript.(please see the 2st paragraph in page10).

Revision 2: Serial sections and images of identical areas including controls were requested. This has not been met. An image "from a different angle" is not an adequate visual control for the reader. Please provide strict serial sections and images from the same area including controls as requested.
The title of the current manuscript is "CD133+ Liver Cancer Stem Cells Resist Interferon-gamma-induced Autophagy". Results paragraph one claims that "we detected AFP-positive cells (Fig. 1). To further test whether HCC CSCs existed in this spot or not, we stained tissues with the HCC CSC marker CD133. Results showed that a subset of tumor cells were CD133+ (Fig. 1), indicating that CSCs could live for long periods of time in tested animals." This strongly suggests to the reader that CD133+ AFP+ cells were detected in the tumor transplants. However, the evidence provided by the authors does not support this conclusion. Also, the requested FACS data (to prove that there is a CD133/AFP-positive population in the transplanted tumors or to show the correlation or non-correlation between these markers) was not provided. If AFP and CD133 are mutually exclusive as claimed in the comment above, AFP expression in the cancer cell population should decrease with IFN-treatment (which upregulates CD133). Please provide further data supporting the conclusions made in the paper.

**Are the methods appropriate and well described?**

If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**

If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**

If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**

If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

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Please indicate the quality of language in the manuscript:

Needs some language corrections before being published
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