Author’s response to reviews

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

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Oct 26, 2015

Dear Dr. Meng,

Re-"CD133+ Liver Cancer Stem Cells Resist Interferon-gamma-induced Autophagy” (BCAN-D-15-00066R2)

Thank you for reviewing the above-referenced manuscript submitted earlier to your office. We would like to take this opportunity to express our appreciation to you and Reviewers. In accordance with the Reviewers’ comments and suggestions, the manuscript has been revised accordingly. We feel that this revised manuscript has been strengthened by the Reviewers’
comments and suggestions. A point-by-point response to Reviewers’ comments and suggestions has been prepared and followed this cover letter.

I hope these changes and explanations satisfy the requirements of the Editorial Board. I thank you again for reviewing the manuscript and look forward to hearing your favorable reply soon.

Yours sincerely,
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A point-by-point response to Reviewers’ comments and suggestions

Reviewer #2:

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").

Our reply: Thanks to reviewer’s suggestion and the CD133 mRNA deltaCt was identified as the CD133 Ct minus 18S Ct in the same pure cell line and it did not contain any other tissue material, which was added in the Methods and Material in the revised manuscript. (Please see the 1st paragraph in page9) Thanks to the reviewer’s remind and legend for Fig2 and Fig4 were adapted accordingly in the revised manuscript. (Please see the 2st and 4st paragraph in page22)

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.

Our reply: According to the reviewer’s suggestion serial sections and images of identical areas including control were provided in the revised manuscript. (Please see the revised Figure1and the 1st paragraph in page22) We agreed with the concern of the reviewer that our mention in the text would give readers the mistaken impression that CD133+AFP+cells were detected in the tumor transplants. As it was not necessary to prove that those HCC CSCs were AFP positive cells and it was also not all the HCC tumor cells were AFP positive, we removed the part about AFP in the revised manuscript.(Please see it in the 2st paragraph in page5, the 1st paragraph in page10 and 1st paragraph in page22)