Reviewer’s report

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

Version: 0 Date: 28 Aug 2015

Reviewer: Khalid Matalka

Reviewer's report:

Li et al. described experiments regarding IFN-γ treatment on CD133+ liver cancer stem cells. The work was on 4 human cell lines; 2 cell lines expressing a high percentage of CD133 and other cell lines expressing low percentage. The authors concluded that CD133+ resist Interferon-gamma-induced autophagy. The major problems with study are A) results are not presented sequentially to represent a better flow of the ideas and there are insufficient presentation of the figures, B) the discussion part is also not sufficient and does not address the important results presented.

Specific Comments:

1. Why the authors used a 20000 IU rhIFN-γ every day for four weeks? They should cite a reference for such protocol or give a reason.

2. The number of replicates and repetition for each in vitro experiment should be mentioned in the figure legends.

3. The expression CD133 on the four cell lines should be presented at the beginning of the results section.

4. The first thing the authors mentioned in their results that they want to show that cancer cells can live s.c. long enough (8 weeks) in nude mice. They chose PLC8024 cell line that expresses a high percentage of CD133. Why did they not choose or present also a low-CD133 expressed cell line? Why did they pick 8 weeks? In addition, they indicated that CSC may escape immune cells attack in nude mice. The latter sentence is not an appropriate indication since this is seen in an immunocompromised animals and no data shows what happens next (will the tumor re-grow?)

5. Fig1C and D should be combined with Figure 2!
6. The second part of the results the authors stated that "CD133+ HCC cells resist IFN-γ-induced growth arrest". I would suggest changing the title especially the word "arrest" to "delay" since growth was seen also in low CD133+ cells.

7. The subtitle "IFN-γ treatment enriches the CD133+ cell population in vitro and in vivo" does not correspond to the results seen. IFN-γ treatment in vivo enriches CD133+ regardless of the CD133 expression, but this is not seen in vitro. This point needs to be discussed in detail in the discussion section.

8. "Results demonstrated that the percentage of CD133+ cells in BEL7402 and QGY7701 cells slightly increased after IFN-γ treatment, in which the percentage of CD133+ cells in BEL 7402 was doubled and the percentage of CD133+ in QGY 7701 was increased by seven times after IFN-γ treatment." The terms "slightly increased" and "increased by seven times" do not match. Were these changes statistically significant? The sentence above should be re-written.

9. It is not clear how Fig 5A and 5B "demonstrated that IFN-γ treatment increased autophagy in BEL7402 and QGY7701 cells, but not in the Huh7 and PLC8024 cells". The text needs a much better presentation of the data shown. The presentation is subjective. It should be presented in an objective or semi-quantitative manner.

10. Fig. 5C does not show the expression of ATG5 without and with IFN-γ treatment (i.e. no controls were seen).

11. The discussion part is not sufficient; it does not discuss the results for instance: why IFN-γ treatment in vitro and in vivo resulted in different behavior on low versus high-expressed CD133+? What are the proposed mechanisms? Why IFN-γ treatment induced a different behavior on low expressed CD133 cells with ATG5 silencing?

12. An important review that describes the relation between autophagy and apoptosis and the crosstalk between the process should assist the authors in their discussion.

13. Fig 4A the y-axis INF-γ should be corrected to IFN-γ

14. The article needs to proof-read for English language usage.

Are the methods appropriate and well described?

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

Yes
Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

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.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organisation that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?
If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below

I declare that I have no competing interests’

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal