Author’s response to reviews

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

Authors:
Xin-Yuan Guan (xyguan@hkucc.hku.hk)
Jian Li (lijian57@mail.sysu.edu.cn)
Jin-Na Chen (jinnachan2010@gmail.com)
Ting-Ting Zeng (zengtt@sysucc.org.cn)
Fan He (hefan2@mail2.sysu.edu.cn)
Shu-Peng Chen (chenshp@sysucc.org.cn)
Stephanie Ma (stephanie.ma@gmail.com)
Jiong Bi (bijiong2000@hotmail.com)
Xiao-Feng Zhu (zhuxfeng@mail.sysu.edu.cn)

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Oct 15, 2015

Dear Dr. Meng,

Re:"CD133+ Liver Cancer Stem Cells Resist Interferon-gamma-induced Autophagy" (BCAN-D-15-00066R1)

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,

Xin-Yuan Guan, PhD.
Professor
Director, Laboratory of Cancer Genetics,
Department of Clinical Oncology,
The University of Hong Kong,
Room L10-56, 10/F, Laboratory Block
21 Sassoon Road, Pokfulam, Hong Kong
Tel: (852) 28199782; Fax: (852) 28169126
E-mail: xyguan@hkucc.hku.hk

A point-by-point response to Reviewers’ comments and suggestions

Reviewer #1:

No discussion on why IFN-γ treatment induced a different behavior on low expressed CD133 cells with ATG5 silencing? For instance the last paragraph in the discussion section does not discuss anything and it needs to be rewritten (the English is awkward)!

Our reply: Thanks to the reviewer’ suggestion and this part had been rewritten in the revised manuscript. (please see the 2st paragraph in page15).
No relation between autophagy and apoptosis and the crosstalk between the two processes in relation to IFN-γ were addressed.

Our reply: Thanks to the reviewer’s suggestion and the potential mechanism of IFN-γ induced cell apoptosis and autophagy were added in the discussion part of the revised manuscript. (please see the 1st paragraph in page15 and reference 40 and 41 in page22).

Even though the authors in their letter mentioned that they added the number of replicates and repetition for each in vitro experiment should be mentioned in the figure legends but I do not see it!

Our reply: Thanks to the reviewer’s suggestion and the number of replicates and repetition for each in vitro experiment had been added in the Figure legend of revised manuscript. (please see the 2st paragraph in page23, the 1st, 2st and 3st paragraph in page24 and the 1st paragraph in page25 in Figure Legends).

Reviewer #2:

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.

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.
2. 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.

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 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).