Reviewer’s report

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

Version: 0 Date: 28 Aug 2015

Reviewer: Alessandro Lugli

Reviewer's report:

The manuscript by Li and colleagues investigates the response of CD133+ HCC cells to IFNgamma using functional assays, flow cytometry and mouse experiments. The authors suggest that CD133+ cell lines show an increased resistance to the anti-proliferative effect of IFNgamma in vitro. Further, the authors propose that the percentage of CD133+ cells increases after treatment of cell lines with IFNgamma. These effects are assigned to an increased resistance of CD133+ cells to autophagy.

The paper adds knowledge on the functional relevance of CD133 expression in cancer cell lines and mouse models. There is room for improvement, in particular regarding the experimental evidence provided.

Major Compulsory Revisions:

Introduction, P4/L9; Results P9/L48: The authors mention that in an HCC nude mouse model cells were "observed … that neither grew nor died" leading them to study this cell population further. In the results P9 these cells are described as "live cancer cells" without further information on how this conclusion is reached. Please provide details on the experiments performed and of the experimental evidence for the homeostatic condition (non-apoptotic, non-proliferative) of these cells in your paper.

Results P9/FIG1: The quality of the provided images need to be improved and does not allow to identify the stained population and to substantiate the claims made in the results section. In fact,
images from different tumor are provided using single stains. Please provide evidence that the indicated cell populations (CD133, AFP, H & E stains) are identical using either double stains or strict serial sections of the same tissue in high resolution.

The cell lines are reported to have either high or low percentages of CD133+cells (Results P9). Huh7 /PLC8024 show less than 0.15% positive cells, Huh7 shows a spectrum of positivity particularly in cells with high SSC values and only PLC8024 shows two distinct populations in the FACS analysis. Please provide further information on how "high or low percentages of CD133+cells" were defined. Further, please provide additional quantitative evidence to substantiate the claim of differing CD133 expression between the cell lines used (e.g. qPCR data). Please also provide FACS blots showing double stains for AFP / CD133 to better delineate the different populations and to allow correlations to be made with the IHC images provided in Figure I.

P10/L6-10: The authors claim that the fact that cancer cells are surrounded by immune cells suggest that these cells resist immune attack. There is no evidence provided to support this conclusion. Co-localization cannot be used to infer immune attack or immune resistance without additional data (e.g. killing assays).

P11/L22 The authors claim that CD133 expression is substantially increased after IFNgamma treatment. However, the calims are based on FACS data of cell lines that show almost complete absence of CD133 (Huh7 /PLC8024). The seven-fold increase is actually from 0.06 to 0.42 %. First, this could be a gating or staining issue; second this could be due to cell death of the CD133 negative population. Please provide additional quantitative evidence to substantiate the claim of an increase in CD133 expression between the cell lines used (e.g. qPCR data).

Please provide a more detailed explanation of the autophagy experiments and results. As provided, the interpretation is incomplete.
Minor Essential Revisions:

Methods: P5/L53 The authors indicate that positively stained cells were counted under a microscope. Please describe your scoring characteristics, cutoffs for marker positivity and how these were reached.

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.

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

Quality of written English

Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

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