Reviewer's report

Title: The effects of the location of cancer stem cell marker CD133 on the prognosis of hepatocellular carcinoma patients

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Reviewer: WEI WEI

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Review

In this manuscript, Chen et al investigated the prognostic impact of CD133 expression level in hepatocellular carcinoma (HCC). Specifically, the authors have reported nuclear CD133 may independently predict favorable prognosis, which is unexpected yet novel and definitely worth further exploration. While the study is straightforward by itself, there are some concerns to be addressed before considering the acceptance of this manuscript.

1. The authors heavily cited the property of CD133 as a cancer stem cell (CSC) marker to build study rationale. However, according to the CSC theory, CSCs are believed to represent only a minority number of the tumor mass. This is directly contradictory to the data shown in Fig. 1, in which a ubiquitous staining pattern, regardless cytoplasmic or nuclear, was shown for CD133. It should be noted that it is the AC133 epitope (glycosylated N-terminal of CD133) but not the CD133 protein in general, accurately serves as marker of stemness (see Int J Biochem Cell Biol. 2005 Apr;37(4):715-9. Cancer Res. 2010 Jan 15;70(2):719-29. and many other studies). Apparently, the polyclonal CD133 antibody used in this study is not a suitable tool to interrogate stemness. Other biological roles associated with CD133 are thus suggested, which needs to be thoroughly discussed in the revised manuscript.

2. Considering this is the first time reporting the nuclear localization of CD133 in HCC, it is imperative for the authors to thoroughly validate the specificity of the CD133 antibody used in this study (preferably in a HCC cell line). Both immunoblot (specificity test) and immunofluorescence (or other type of immunostaining, to suggest staining pattern) are needed. Further, these tests should be also accompanied by a validated siRNA (or shRNA) to suggest the signal from the testing antibody can be abrogated by CD133 knock-down.
3. The authors also need to exclude the possibility (although less likely) that the nuclear staining is a kind of antibody-specific artifact. This can be done by repeating the staining with the same specimen showing nuclear CD133 with another CD133 antibody.

4. In fact, for a study exclusively focusing on IHC study, "no primary antibody" as negative control is not enough. Instead, normal rabbit IgG (in this case) is needed. For the six cases shown in Fig.1A, please supply the corresponding isotype control.

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.

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

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Acceptable
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