Reviewer’s report

Title: Cancer stem-like sphere cells induced from de-differentiated hepatocellular carcinoma-derived cell lines possess the resistance to anti-cancer drugs

Version: 3  Date: 9 June 2014

Reviewer: Sandra Muñoz-Galván

Reviewer’s report:

In this article, Hashimoto and colleagues provide a novel method for induction of cancer stem-like sphere cells from poorly differentiated hepatocellular carcinoma cell lines. These sphere cells express stemness and cancer stem cell markers and are resistant to a high variety of anti-cancer drugs. Consistently, they show increased expression of ABC transporters, G0/G1 cell cycle arrest and high expression of p21, and reduced levels of ROS together with high HIF1α mRNA levels.

Although the development of this new method is of relative interest, the authors must address some important issues before being suitable for publication.

a) Major compulsory revisions

- It is important to include the sphere-forming HLE cell line in the RT-qPCR, drug sensitivity, ABC transporter, cell cycle and ROS experiments, as well as the non-sphere-forming HuH-7 and Hep 3B cell lines. Only with these controls the authors will be able to conclude that their sphere induction method works specifically for de-differentiated HCC cell lines.

- There are a lot of “data not shown”. This is not acceptable and the authors must provide the cited results either in the main figures or as supplementary information.

- The reference gene used for RT-qPCR experiments should be always the same. If the authors have reasons for alternatively use GAPDH or PGK1 in different experiments, they must support these reasons and indicate which one was used in each case in the appropriate figure legend. If not, results have to be normalized only to one of them.

b) Minor essential revisions

- The Student’s t-test and Mann-Whitney U-test are used in the RT-qPCR experiments. Is there any reason to not to use the same test in all cases? The authors should clarify this issue.

- The percentages referred in the sixth paragraph of the Results section do not match with those in the referred Figure 8.

- The Results section would be highly improved if the authors included partial
conclusions in the different paragraphs and link them more fluently.

c) Discretionary revisions

- Panel labeling in Figure 1 should be in the same order as it is cited in the text to facilitate reading comprehension.

- Materials and Methods should be avoided in the Figure Legends.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests