Reviewer's report

Title: Sphere-forming culture enriches liver cancer stem cells and reveals Stearoyl-CoA Desaturase 1 as a potential therapeutic target

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Reviewer: Manlio Vinciguerra

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This manuscript analyzes the role of sphere-forming culture in enriching subpopulations of CSC within HCC. The authors used two HCC cell lines (Huh-7 and Hep3B) and fresh primary tumor cells cultured in serum-free and ultra-low attachment conditions, to allow formation of HCC spheres. Subsequently, the authors used also accepted and established n vitro and in vivo approaches to evaluate CSC characteristics. The major findings were that both cell lines and primary tumor cells formed spheres. These spheres possessed the capacity for self-renewal, proliferation, drug resistance, and contained different subpopulations of CSCs. Furthermore, thanks to transcriptomic analyses the authors identify a PPAR-SCD1 signaling axis that might play an important role in the maintenance of the CSC properties of HCC sphere cells by promoting nuclear accumulation of β-Catenin. The authors thus suggest that targeting the SCD1-pathway might be relevant therapeutically for HCC treatment.

The study is of good quality, well conducted and the results in general supports the conclusions drawn. However, I have two major comments that I ask the authors to address:

1. 5-FU is not a good drug to treat HCC, it is mainly used as coadjuvant therapy. The chemoresistance of the sphere should be tested using Sorafenib and Doxorubicin, separately.

2. Recent reports show that epigenetically modified aggressive HCC cell lines with CSC properties have an enhanced content of lipid droplets, acetyl-coA, and activation of LXR (Lo Re O et al. Epigenetics, 2018; Lo Re O et al, Hepatology 2018). These features are very consistent with the activation of PPARalpha/SCD-1 pathway observed by the authors. I recommend a) to cite these papers and, more importantly, b) to discuss how these changes in lipid metabolism reconcile with a metabolic rewiring required for the CSC phenotype, self renewal, independence of growth factors/serum etc.

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.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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I am able to assess the statistics

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