Reviewer's report

Title: Sphere-forming cell subpopulations with cancer stem cell properties in human hepatoma cell lines

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Reviewer: Nicholas Barker

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Cao et al:

This study evaluates the existence of cancer stem cells in human liver cancer cell-lines using sphere-forming and in-vivo transplantation assays. Cancer stem cells are undoubtedly a hot topic and formal demonstration of their existence in liver cancer would have major therapeutic implications. However, the findings of this study are preliminary, lack mechanistic insight and do not represent a significant advance on those already published.

Comments:

1) The authors report that different cell-lines harbor sub-populations with sphere-forming capabilities in-vitro. However, there is no attempt to compare the efficacies of non-tumorigenic versus highly tumorigenic (eg, HepG2 vs MHCC97H) cell-lines, or Hep+ versus Hep- in this assay. Such information is crucial for drawing generally applicable conclusions.

2) Related to the above point, it is also important to directly compare the sphere-forming and in-vivo tumorigenic efficacies of at least one other cell-line in addition to PLC/PRF/5 and relate this to CSC activity/frequency.

3) The observations on resistance to chemotherapeutic agents should be extended to include other common drugs with different mechanisms of action, eg, doxyrubicin (ABC transporter blocker). Is there preferential survival of candidate CSC markers in this assay (easily tested by IHC for CD133, CD13, CD44 etc)?

4) The correlation between increased tumorigenicity and enhanced expression of candidate cancer stem cell markers in figure 4 is highly tentative. As a minimum, the Western blots need to be accurately quantified. However, it would be far more informative to demonstrate by IHC analysis that populations of cells co-expressing markers such as CD133 and CD44 etc are enriched in the spheres. CD13 should also be included here (See Haraguchi et al., JCI 2010 120:3326).

5) The link with aberrant activation of Notch signaling, although preliminary are potentially interesting given the current interest in defining signaling pathways linked to maintenance of CSC function in-vivo. This could easily by functionally evaluated by blocking the Notch pathway in the sphere colonies with commercially-available gamma-secretase inhibitors.
Level of interest: An article of limited interest

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests