Reviewer's report

Title: Impact of Stem Cell Marker Expression on Recurrence of Hepatocellular Carcinoma Post Liver Transplantation

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Reviewer: Norman Sussman

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The paper by Zeng et al. addresses the biology of hepatocellular carcinoma in the context of TACE and OLT. This is an intriguing study, but it leaves the reviewer with a number of questions.

1. The aim of the study (page 2) is to determine whether TACE-treated tumors express CSC markers at a higher level, and whether this correlates with tumor recurrence. The implication is that TACE either induces CSCs, or fails to kill tumors that express CSCs. The remaining CSCs in turn, increase the risk of tumor recurrence after OLT. The more likely explanation (found in the discussion) is that TACE was done on patients with more rapidly growing tumors. Recognizing the limitations of a retrospective study, the authors must raise this point earlier in the paper, and must attempt to address the concern by giving us some pretransplant history including rate of tumor growth and/or rationale for TACE vs. no TACE. The study was done at a time when waiting times for transplant in Kansas was relatively short, so the decision to do TACE may have been related to concern about growth.

2. Can the authors explain why these are called stem cells since they don’t appear to be pleuripotent?

3. Methods (page 2) – the scoring system is not clear. They compared staining in viable tumors and embolized tissue. We frequently see very little or no viable tissue after TACE.
   a. What did they do in cases where little viable tissue was available for examination? Could they always find 5 hpfs?
   b. Did tumors that recurred after TACE have more viable cells than tumors that did not recur?
   c. Embolized tumors give the viewer limited fields to examine, whereas viable tumors leave one with a lot of flexibility. By examining only 5 hpfs, one might select high staining or low staining areas within a tumor unless staining is homogeneous. How did they choose areas to examine? Why not map staining of all viable tumor tissue in both viable and post-TACE tissue?
   d. Did the three observers view the same five fields, or were they free to choose any five fields?
   e. Since many post-TACE livers had multiple tumors, did they compare treated vs. untreated tumors? This might be a better internal control.
f. Can they compare synchronous tumors in a subanalysis.
g. How did they identify tumor cells in embolized tissue?
h. Was the scoring system (0-3) based on number of stained cells or density/cell or a combination? The methods states number and intensity, but the description includes only number. If the score is weighted towards the number of cells, why not report the actual number of cells in 5 hpfs (or in the entire tumor divided by number of hpfs) rather than a subjective scoring system?
i. What was the concordance among the investigators?
j. Please give complete information for the primary antibodies – were all antibodies from Dako?

4. Background
a. The rationale for downstaging is quite clear – it is a non-invasive observation that has proven useful. Size usually correlates with tumor behavior, and has made OLT reasonably successful.
b. They fail to acknowledge that tumor staining requires a resected liver – certainly not a practical solution to tumor recurrence.
c. They should reference the work of Francis Yao in tumor size and downstaging.

5. Why did they choose four tumor markers out of a possible six – why not CK19 and CD13?

6. The most puzzling thing about this paper is the suggestion that TACE causes expression of a stem cell marker that correlates with HCC recurrence. This is certainly not the universal experience. More discussion about this controversial finding is essential to making this paper meaningful and broadly applicable.

7. Table 1 needs some changes:
a. TNM classification – all patients were within Milan criteria, so T stage is the same as size – consider using tumor size instead of T stage.
b. Median size would be helpful.
c. Number of tumors rather than single/multiple.
d. Since they have only 40 patients, a table of all patient data should be considered (could be provided as a supplement if space is limited).

8. Figures
a. What is the significance of cytoplasmic staining with EpCAM – are CAMs normally localized to the cell membrane? Figure 1 shows staining limited to the cytoplasm in grade 2, but appears to show cytoplasmic and membrane staining in grade 3.

Level of interest: An article of importance in its field

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