Reviewer's report

Title: The role of multipotent cancer associated fibroblasts in hepatocarcinogenesis

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Reviewer: Kati Rasanen

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The manuscript by Sukowati et al. titled “The role of multipotent cancer associated fibroblasts in hepatocarcinogenesis” reports characterization of cancer-associated and cirrhotic tissue-derived stromal cells using various stem cell markers and presents some data regarding their role in the HCC tumor-stroma interplay. Overall, this is manuscript is within the journal’s scope. Regarding the data, the experiments conducted are presented in a clear, chronological order. However, this paper follows very closely the one by Cesselli et al. (Role of tumor associated fibroblasts in human liver regeneration, cirrhosis and cancer, Int J Hepatology 2011), thus for the main part this manuscript lacks novelty.

The following Revisions, detailed below, should be addressed.

- Major Compulsory Revisions

1) Both the Results and Discussion sections should be expanded, as now they present a cursory overview. In particular in the Discussion the correlation of the data presented here to previous publications should be expanded as now for the most part this is lacking.

2) One key reference (Mazzocca et al. Tumor-secreted lysophosphatidic acid accelerates hepatocellular carcinoma progression by promoting differentiation of peritumoral fibroblasts in myofibroblasts, Hepatology 2011) is missing and should be included and discussed in the manuscript, as it describes similar data as presented here. For example, ACTA2 expression is indicated in the Table1. Was this higher in the CAFs compared to NTFs, correlating with the Mazzocca results?

3) Figure1Bb: CAF or NTF? In either case, this image is not informative and from the Results or Discussion it does not become clear what is the purpose of the experiment or the meaning of the data.

4) Figure 1B d-f, were images obtained from an aliquot of FACS samples? The current images are not informative, proper immunofluorescence experiment (cells stained on cover slips) should be performed in order to show the morphology and localization of the staining.

5) Figure 2a: results from CAFs or NTFs? Representative images of both should
be shown; in particular as only in the NTFs PPARG was induced.

6) Figure 2Bc and Results section (line 212-215): Statistics required to demonstrate the significance of the differential expression.

7) The paper by Mazzocca et al. (Hepatology 2010, Ref4 in this manuscript) describes the role of CTGF and TGFbeta1 in the tumor-stroma crosstalk in HCC. The findings and their correlation to the results presented here should be discussed as up-regulation of both of these genes as an effect of CAFs and NTFs is shown (Figure3).

8) The xenograft experiment is rather unclear; primary cells refer here to CAFs? Were any NTFs injected? Are these nodules benign or malignant? An H&E staining of the nodules should be performed, as in the Discussion it is suggested that there was a negativity of human HCC after injection. Also, a staining for CD90 of the nodules should be done, as the stem cell theory, as discussed by the authors, is based on its expression. Notably, the n is not very strong, 3 and 2 for nude and NOD-SCID, respectively, to draw any meaningful conclusions from the experiment.

9) A professional language editing is required; hence spelling and grammar mistakes are not detailed in the Minor Essential Revisions.

- Minor Essential Revisions

1) Results section, line 200: qualitative PCR. Quantitative PCR?

2) Figure 1A and 1Ba: results from CAFs or NTFs? Representative images of both should be shown for both FACS and phase contrast morphology and labelled accordingly in the Figure 1.

3) Figure 1B: in all images scale bar is 100 micro m, even though in the legend images a-c are 10x and d-f are 40x. Figure 2a scale bar also 100 micro m, yet magnification according to figure legend is 63x.

4) Figure 1Bc: These images are too small, are the same colonies photographed at Day1 and Day3? What was the concentration of the Matrigel used?

5) Figure legend 2, lines 473 and 474: macro- and macroscopic. Macro- and microscopic?

- Discretionary Revisions

1) The order of results could be changed, starting with the Table1 RT-PCR, followed by other results.

2) Quantitative RT-PCR was presumably performed, yet the data indicates only whether the genes were expressed or not. A comparison of the relative expression levels between CAFs and NTFs could be performed to expand the genotypic expression data.
Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Not suitable for publication unless extensively edited

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.