Reviewer’s report

Title: The role of multipotent cancer associated fibroblasts in hepatocarcinogenesis

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Reviewer: Pascal Pineau

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In their manuscript Sukowati and coworkers described the analysis of fibroblasts isolated either from 9 resected hepatocellular carcinomas (HCC) or the corresponding peritumoral livers. The authors conducted a comprehensive characterization of the cancer-associated fibroblasts (CAF) including purely morphologic features and molecular phenotypic traits (gene expression). The authors carried out, in addition, co-culture analyses with two HCC cell lines (HuH7, JHH6) and xenograft assays.

The present work has some merits as it tries to go beyond the simple gene expression to explore more functional aspect through in vivo analyses. Furthermore, the issue of CAF in HCC is important as it has been already associated with modulation of prognosis. However, some lack of clarity regarding data presentation and some weird technical options make the message difficult to grasp.

Major compulsory revisions

Primary cell isolation: What was the duration of cell isolation process? How many cells were obtained (range in the 9 patients)? The first picture of Figure 1B showing polygonal cell obviously more akin to hepatocytes than to spindle-shaped fibroblasts is not completely reassuring concerning what we are examining in the current manuscript. The presence of positive hepatocytes and/or epithelial markers in Table 1 reinforces this impression just as the expression of human Albumin in xenograft. Could the authors discuss these aspects?

Morphology and surface markers: The authors presented the outcome of the analysis of a single sample in figure 1A and some quantification about markers presence on cell surface. However, no global figure is provided with percentage of positivity and statistical analysis accounting for the 9 CAF and NTF. It should be done.

Table 1: Presenting data of PCR in a table is totally inappropriate. What is the reason for using qualitative PCR in this instance whereas later in the manuscript qRT-PCR was employed. The authors should explain clearly why the “qualitative option” was retained sometimes.

Co-Culture: How many different populations of fibroblasts were analyzed? Where are the difference between CAF and NTF (p values). The purpose here should be to demonstrate difference between CAF and NTF and not between any
induced cells and non-induced controls.
Xenograft: The formulation “two primary cells” is equivocal. Is it fibroblastic, hepaticytic or a mix? What was the reason for excluding NTF from these analyses.

Minor essential revisions
Differentiation: Adipocytic differentiation seems to be more efficient than the two others. Is this related to the origin of the tumors? 6 out 9 HCC were associated with metabolic disease. Is there some differences between these samples and those from the 3 HCV-infected patients? Is there some pathological data evaluating the degree of stromal presence in the tumors of the different patients? Please discuss these aspects.

Level of interest: An article of importance in its field

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