Reviewer’s report

Title: The role of multipotent cancer associated fibroblasts in hepatocarcinogenesis

Version: 2 Date: 13 December 2014

Reviewer: Francesco Dituri

Reviewer’s report:

The authors report a morpho-functional characterization of stromal environment of HCC focusing on comparative analysis of human primary CAFs and NPCs phenotype as well as their contribution to tumor progression. Herein, Sukowati et al. highlight the plasticity of CAFs and NTFs showing their potential to differentiate into diverse mesenchymal stem cell derived lineages. The authors unveil an intriguing cross-tack between the mentioned stromal cell lines and HCC cancer cells. However some issues should be addressed to corroborate their conclusions.

Major Compulsory Revisions

1) The figure 1 is not much clear, according to title of related legend and description within the results. Indeed all the panels refer to CAFs, with no image showing both morphologic and phenotypic analysis of NTFs. To mirror the meaning of figure legend the authors should provide a comparative analysis including both CAFs and NTFs.

2) In the lines 190-191 the authors state: “CAF showed an higher percentage of antigens CD90 and CD44 (52 ± 27% and 59 ± 22%, respectively), as compared to NTFs (37 ± 28% and 74 ± 12%)”. This seems to be evident only about CD90. However, neither flow cytometry nor immunofluorescence data showing the expression of these markers in NTFs are reported.

3) In lines 231-232 the authors state “In the presence of CAF, an up-regulation of TGF#1, ACTA2, and FAP was observed in both HCC cell lines (p<0.05)”. However, watching the figure 3, only FAP is significantly up-regulated in both HCC cell lines, whereas TGFbeta1 is up-regulated only in HuH7, and ACTA2 mRNA does not appear significantly increased in both HCC cell lines. How the authors explain this discordance between text and related figure? Why FAP bar related to HuH7 is not shown In figure 3 panel B left?

4) In lines 237-240 the authors describe up-regulation of genes in NTFs upon co-culture with HCC cells, but statistic information referred to this effect is not supplied in the figure.

5) The authors should provide a more detailed description of the method they used to isolate and purify CAFs and NTFs from HCC specimens in the material and methods section.
Minor Essential Revisions

1) The panel C/a of figure 2 seems to be a western blot whereas it is described in the legend as a gel electrophoresis. Moreover the western blot procedure has not been mentioned in material and methods

2) The authors should point out that the figure B/a refers to alkaline phosphatase staining of CAFs

3) What is the meaning of cutting black bars in panel C/b, since y-axis is continuous?

Level of interest: An article of importance in its field

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

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