Reviewer's report

Title: Protein markers of cancer-associated fibroblasts and tumor-initiating cells reveal subpopulations in freshly isolated ovarian cancer ascites

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Review comments on the manuscript by Bjorklund M et al, titled “Protein markers of cancer-associated fibroblasts and tumor-initiating cells reveal subpopulations in freshly isolated ovarian cancer ascites”

The authors collected ovarian cancer ascites, separated cancer cells by gradient centrifugation, cultured the cancer cells and then extracted the total cell lysates for Western blotting analyses of various protein markers. They observed that the cancer cells could grow in vitro as monolayer (M-type), spheres (S-type) or both. They separately lysed M-type and S-type cancer cells and carried out Western blotting for several markers, including E-cadherin, vimentin, integrin β3, α-SMA, PDGFR and cancer stem cell markers (CD44, EpCAM, ABCG2, CD117, Oct-4A and Nanog). In addition, the authors carried immunoprecipitation for expression of cancer stem cell marker Oct-4A and associated SUMO-1. They thoroughly analyzed the statistical significance of protein band intensities in Western blotting.

Major observation: The authors showed that there are two populations, namely M-type (grown in monolayer) and S-type (grown in spheres). Among 22 ovarian cancer patients, 13 patients had M-type ovarian cancer cells, 4 had S-type cancer cells and 5 had both types. M- and S-type cancer cells differ in cellular adherence (monolayer vs. sphere) and thus in E-cadherin (S-type), vimentin (M-type) and CD44 (M-type). Other protein markers that the authors examined, including cancer stem cell markers, are essentially present in both types of cancer cells from ascites.

Major concerns:

1. The title is not appropriate. The authors only examined a few markers for cancer-associated fibroblast and tumor initiating cells by Western blotting. But they did not verify if these cells are really TIC by using in vitro and in vivo tumorigenicity assays.

2. Basically this manuscript describes expression of several protein markers in two types of ascitic ovarian cancer cells differing in cell adherence. It is unknown if these two populations are different in chemoresistance, metastasesis and tumorigenicity.

3. According to the concept of cancer stem cells and the description in the Introduction of this manuscript, CSC or TIC tends to form spheres. However, the
authors observed that S-type cancer cells, which are grown in spheres and similar to TIC/CSC, expressed less levels of all TIC markers than those on M-type cancer cells, which are grown in monolayer and similar to regular cancer cells. But the authors did not address the issues.

Minor concerns:
1. Labeling errors. There are two tables labeled as Table 1.
2. It would be more meaningful to compare the pathology, FIGO, Histology, Grade, etc of M-type cancer cells with S-type cancer cells.
3. As indicated, 30 ug of each protein sample was loaded to each lane for Western blotting. But the loading controls (GAPDH bands) are quite varied in Figures 1-3. It is also unclear how the protein concentration was determined.

Suggestions:
To clarify if CAF and TIC are really present in M- and/or S-type ovarian cancer cells, it is a good idea
(1) to immunostain the freshly isolated M- and S-type cells for CAF and TIC markers and observe the staining under microscopy;
(2) to determine the percent of TIC in M- and S-type cells;
(3) to test the tumorigenecity of M- and S-type cancer cells in vitro and in vivo. This is particularly important to support the authors’ hypothesis of CD44 high/Oct-4A high subgroup in M-type cancer cells.

Level of interest: An article of limited interest

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.