Reviewer's report

Title: IL-6 signaling between ductal carcinoma in situ cells and carcinoma-associated fibroblasts mediates tumor cell migration

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Reviewer: Kyung Sung

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Osuala et al. present an intriguing study regarding the role of IL-6 signaling between ductal carcinoma in situ (DCIS) cells and carcinoma-associated fibroblasts (CAF) in stimulating tumor cell migration. Further validation and characterization of this signaling could bring a practical impact on the diagnosis and treatment of patients with DCIS, as there are currently no reliable markers as well as drugs targeting more aggressive type DCIS. However, the manuscript will require major revision prior to be published in the BMC Cancer for the following reasons.

1) The authors need to better describe the 3D co-culture system they used. Even though the authors cited a reference (#31) showing the MAME model that was used in the study, it could benefit from a little more details. For example, how did the authors conduct the co-culture? Were the cells embedded in the matrix or cultured on top of the matrix? The authors also described that cancer cells migrate toward fibroblasts; however with the minimal information given in the current manuscript, it is hard to interpret the data. A simple schematic illustration depicting the co-culture conditions would be very helpful.

2) On page 8, the authors mention that the “MAME model is unique as it allows for co-culture of multiple cell types in the context of a three dimensional microenvironment.” This statement is not very persuasive because the authors did not add any comparison with other existing in vitro models developed to enable 3D co-culture. One could possibly think that even a well-established trans-well platform would allow 3D co-cultures of cancer cells and stromal fibroblasts. Even though the development of a platform is not the focus of this paper, it would be better if the authors elaborate the advantages of the system a little bit more.

3) On page 9, the authors present that IL-6 induced MCF10.DCIS growth is reversible, which was confirmed by adding complete media after the cells were treated with an IL-6 neutralizing antibody. It is not very clear how the experiment was performed. The authors mentioned that the complete medium was added for 48 hrs, but they did not mention when the medium was added. Was the medium added after 8 days of blocking antibody treatment?

4) On page 10 (Ln 204), it is not clear what the phrase “under MAME culture conditions” indicates. Did the authors collect mRNA and conditioned media from
the 3D mono-cultures of CAFs?

5) Figs. 3D and 3E need to be quantified. The authors only described the overall size and volume of the multicellular structures without supporting data. Presenting representative images is not persuasive enough especially for this type of data.

6) What is the density of fibroblasts in co-culture? What is the ratio of cancer cells and fibroblasts? Co-culture images presented in this manuscript present very few fibroblasts (only one or two around the DCIS clusters). This reviewer believes that the density of fibroblasts in co-culture is also an important factor regulating the growth and invasion of cancer cells. Are there only very few fibroblasts because the loading density of fibroblasts is much lower than that of cancer cells?

7) In the 1st paragraph in page 12, the authors mentioned that they observed many single DCIS cells and few CAFs (Fig. 4A). If the authors used the MAME model presented in reference #31, the cells were co-cultured in different layers (i.e., fibroblasts in the bottom gel and cancer cells on the top gel). Based on the information provided in the manuscript, it is not clear how the CAFs are near cancer cells on day 1 unless the CAFs have migrated toward the cancer cells within 24 hours.

8) The evidence that IL-6 controls CAF leading the tumor through the material is not clear and not completely supported because the authors only presented representative images. This is very interesting data, but it needs more supporting data or discussion. Figs. 4B and 4D show one fibroblast (in each figure) near the leading edge of DCIS clusters. How many times did the authors observe this? Maybe high resolution images of the junction between cancer cells and fibroblasts would be helpful to show the cells actually formed a hetero-cellular contact.

9) The gradient of IL6 at the leading edge of DCIS clusters near CAFs (Fig. S6) is very interesting. It would be more convincing if the authors present IL6 images of control DCIS clusters (i.e., with no fibroblasts contact) side by side. Does this gradient only happen when the cancer cells and fibroblast come into contact? Or would DCIS cells showing signs of invasion without the contact with fibroblasts show a similar gradient of IL6 at the leading edge?

10) Are WS-12Ti cells normal fibroblasts or cancer-associated fibroblasts? They are mentioned in the Materials and Methods but not explained.

11) On page 16, Ln 322-324, the authors mentioned that the DCIS cells migrated toward CAFs and upon attachment to CAFs, DCIS cells remained attached and migrated through the matrix following the lead of the CAFs. It is not clear how this was confirmed. CAFs are more motile than DCIS cells, so it could also be that CAFs migrated to the DCIS cells and, upon attachment to CAFS, DCIS cells followed the CAFs. The authors need to include more discussion about this issue.
12) The authors did not clearly state the limitations of the study.

13) The title of this paper is a bit misleading. The majority of the presented data is associated with the growth of DCIS cells, not the migration of the DCIS cells.

**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.