Reviewer's report

Title: Human breast cancer associated fibroblasts exhibit subtype specific gene expression profiles

Version: 2 Date: 30 March 2012

Reviewer: Kristian Pietras

Reviewer's report:

The report by Tchou et al describes a set of experiments involving transcriptional profiling of cancer-associated fibroblasts (CAFs) derived from different subtypes of breast cancer. Briefly, the authors find that CAFs from HER2+ breast cancers are transcriptionally distinguishable from CAFs of triple-negative or ER+ breast cancers. The authors speculate that CAF-derived genes involved in integrin signaling and/or cytoskeletal rearrangements are important for determining the invasive phenotype of the primary cancer. The study provides novel information on the properties of different subtypes of CAFs, but would benefit from placing the findings in the context of paracrine signaling between tumor cells and CAFs.

Major points

1. Tumor specimens of different size were processed differently in order to obtain the CAF cultures. What was the rationale behind using different strategies, especially since both procedures involved an initial mincing step and since this conceivably may yield distinct subpopulations of CAFs? The procedure used for each tumor specimen needs to be specified.

2. The in vitro culture step may introduce abnormalities, in particular since fibroblasts/CAFs are known to be highly plastic cells. The authors should provide validation of the gene expression differences in CAFs of different subtype using qPCR on freshly isolated materials.

3. The authors need to specify the stage of each tumor, and whether this parameter is different between tumor subtypes to rule out that the observed difference in gene expression is related to the invasive behavior of the tumor.

4. Flow cytometry using only one marker of each cell type is a blunt instrument to determine the purity of the isolated cell cultures. The authors should utilize qPCR to control for contaminating tumor cells that may have acquired mesenchymal-like properties through EMT.

5. The main finding of the paper that CAFs from different subtypes of breast cancer have a distinct transcriptional profile is very interesting. How much of the whole tumor classification of tumor subtypes can be explained by the variation in CAF gene expression? And can the authors distinguish the tumor subtype by assessing expression of the CAF signature on whole tumor material?

6. While it is recognized that this is mainly a genomics study, it is important even
within this field to provide functional insight. Based on the gene expression profile, the authors speculate about the importance of CAFs from the HER2+ subtype in assisting tumor cell invasion. Several questions are raised by this speculation, in particular since the CAFs are derived from the tumor center, presumably far from the invasion front of the tumor: Were the HER2+ tumors used in this study of higher stage and/or more locally invasive than TNBC and ER+ tumors? Are CAFs derived from HER2+ tumors superior at stimulating tumor cell invasiveness in co-culture assays?

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests