Reviewer's report

Title: Stimulation of ST3 expression in mouse fibroblasts by cytokines, collagen and co-culture with human breast cancer cell lines

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Reviewer: Ricardo D Coletta

Reviewer's report:

Matrix metalloproteinases (MMPs), a family of extracellular matrix-degrading enzymes, are considered to play important roles in cancer invasion and metastasis. The activity of MMPs is regulated at multiple levels, including the level of transcription, of activation of the precursors and of inhibition by endogenous inhibitors, in particular by TIMPs. In tumors, MMPs are expressed in both cancer and adjacent mesenchymal cells. To date, it is not clear whether MMP expression in the mesenchymal cells adjacent to tumor is induced by the tumors cells and whether this expression contribute to invasiveness of the tumor. This paper analyzes the effect of cytokines and extracellular matrix proteins on MMP-11 and MMP-14 expression by fibroblasts. The authors demonstrate MMP-11 stimulation by some factors and MMP-14 inhibition by others. Although the authors demonstrate increased/decreased expression of MMP mRNA, they do not demonstrate that this leads to increased MMP activity, as would be expected. Furthermore, the manuscript lacks critical controls and experiments to support the conclusions. Detailed criticisms follow:

1. The authors should fix the track changes before upload and submit the manuscript.
2. Title: Spell out ST3.
3. Pages 4 and 5: This content would fit better in the discussion section, with some modifications.
4. Page 6, Methods, Cell lines: 1) The primary cultures were established allowing the cells after homogenization to attach for a period between 6 and 12 hours prior to use. Was there any assess to determine the contamination with other cell types? 2) How was the invasive and metastatic potential of the breast cancer cell lines listed in the Table 1 determined? Are those based in Thompson et al. 1992? It is well know that cancer cells change their expression profile and behavior in the long time culture. 3) What are NCI-adr and MCF-7stv cell lines?
5. Page 6, Methods, Collagen Coats: 1) Inform the source of the collagens. 2) Were the cultures treated for 24 hours (last line of this section) or a ranged from 3 to 48 hours as shown in Figures 1 and 2?
6. Page 7, Methods, Con-A and Fibronectin Treatment: 1) Why was the fibronectin treatment different from the collagen treatment? 2) Inform source of reagents. 3) Were Treated cultures incubated for 24 hours before RNA isolation or incubated at different timepoints?
7. Page 7, Methods, Cytokine Treatments: 1) How was the specific concentration for each cytokine chosen? 2) What does TGF-b mean? Pan or specific b1, b2, or b3? 3) Was there serum in the treatment media? 4) How was the effect of residual serum avoided?
8. Page 10, Methods, RT-PCR: Were the RNA samples treated with RNase enzyme before RT reaction? How was DNA contamination controlled?
9. Page 12, Results: The authors start to abbreviate the primary fibroblast cultures as PMF (primary mouse fibroblasts). The English literature frequently uses MEF, as mouse embryonic fibroblasts. Furthermore, the use of the abbreviation is not consistent throughout the manuscript.
10. Results, Figures: 1) It is not clear in the text and in the legend figures if Figures 1-4 represent MEF or NIH3T3? 2) In Figures 1-4, it is very difficult to see the expression levels, since the lines frequently overlap. Furthermore, it is not clear if all genes are expressed in all timepoint or not. One way to improve that would be to convert the graphics into tables.
11. Page 12, Results: 1) Which are the basal levels of MMP-11 and MMP-14 expression in the
fibroblast cell lines, if there are? 2) Since all experiments were made in triplicate, why don’t show average ± SEM? Furthermore, why don’t apply statistical analysis? 3) Although the authors do a great job using competitive RT-PCR for amplification control, no controls for the treatment effects are included. For example, no timepoint 0 (no treatment) is included and used as reference to comparative effect.

12. Pages 12 and 13, Results, Induction Treatments: 1) The authors stated that MMP-14 expression was not affect by any cytokine. For example, comparing the timepoints 3 and 12 hours in Figure 1, an increase higher than 3-fold in observed for several cytokines. Minor variations? 2) Did other housekeep genes give the same results as 18S rRNA? 3) The authors state: “In our opinion, MT1-MMP expression should be considered unaffected by IL-1b, IL-2 and fibronectin treatments during this time schedule”. This conclusion can not be raised without additional controls and experiments. Furthermore, the results disagree to some extent with previously published data. In association with the expression studies, specific assays to measure amount of protein and/or activity are necessary.

13. Page 16, Discussion: If it is true that MMP-14 can be downregulated for some cytokines, how may this affect tumor progression?

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

None