Reviewer's report

Title: Molecular Signaling Pathways Mediating Osteoclastogenesis Induced by Prostate Cancer Cells

Version: 1 Date: 27 October 2013

Reviewer: Meenakshi Chellaiah

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Manuscript title: Molecular Signaling Pathways Mediating Osteoclastogenesis induced by Prostate Cancer Cells by Rafiei et al.

In this study the authors attempt to relate that soluble factors released from prostate carcinoma cells (PCa) significantly increased viability of bone marrow macrophages as well as osteoclastogenesis from bone marrow precursors primed with RANKL. They attempted to characterize the signaling pathways induced in OC precursors by soluble mediators present in the conditioned medium of PCa cells using inhibitor studies. Based on these studies, they concluded that their study reveals the molecular mechanisms underlying the direct osteoclastogenesis effect of PCa derived factors ERK1/2 as a unique target.

General comments

Idea is novel to find an alternative mechanism that may play a role in PCa mediated osteoclastogenesis. Most of the data is clearly presented; however, the study manifests as a large series of descriptive observations. It lacks mechanistic experiments. The novelty of the MS is moderate at best.

- Major Compulsory Revisions

1. Results section: page 9; 5th line. Supplemented with 10% serum free conditioned medium (CM) of PCa cells (PC3 and LNCaP)….How do you normalize the medium from these cells? Figure 3 shows 0-50% dilution effect of the media on osteoclast number. Media should be quantified for the protein content and diluted accordingly. It is not clear how the authors maintain the consistency in terms of protein amount when they use the CM in different experiments.

2. Why do you need to prime the cells with RANKL? It shows RANKL is a necessary factor for cell survival. Induction of NFATC1 expression occurred in RANKL primed cell and it is necessary for the acquisition of their sensitivity to PCa factors. It is not clear how soluble factors produced by PCa cells activate MEK/ERK signaling pathways. The cell lines (PC3 and LNCaP) should be genetically authenticated for this purpose to make sure the results are relevant to
prostate cancer.

3) Did you check the conditioned media in gel? Is there any difference in the expression of soluble factors in PC3 and LNCaP cells? PC3 cells are derived from bone metastasis and LNCaP from lymph node metastasis. There are differences in the signaling mechanisms in these cells because of their sensitivity to androgen. Do they have similar mechanism in the induction of osteoclastogenesis? Does this happen with DU145 cells which are derived from brain metastasis?

4) How did you determine the relative binding affinity of soluble OPG with RANK in order to come to a conclusion that osteoclast formation occurs independent of RANK in figure 4?

5) TGF beta has been shown to increase RANK mRNA levels in a time- and dose-dependent manner (Ref. J. Cell. Biochem. 2001; Yan et al., 83: 320-5). Quantification of TGF-beta in the CM is required. Will knockdown of TGF-beta or TGF-beta receptor reduces osteoclastogenesis?

6) It is possible inhibition of TGF-beta receptor could reduce the number of RANK on cell surface. TGF-beta receptor signaling which involves ERK1/2/NFATc1 may play a role in the regulation of RANK expression at mRNA and protein levels. It is important to determine the RANK level at mRNA and protein (cellular and surface) levels.

- Minor Essential Revisions

1. Page 2- 2nd line; MSCF. Do you mean M-CSF? Please check

2. Page 7: Resorption assay: How much dilution? Is this diluted 100X? Do you mean a solution containing 10mM NaCl in 0.2%TritonX-100. Please provide specific molarity of the NaCl?

- Discretionary Revisions

1) provide a schematic diagram of the pathway involved in cancer induced osteoclastogenesis

**Level of interest:** An article of limited interest

**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'