Reviewer's report

Title: Interleukin-11, a cytokine produced by breast cancer cells, augments osteoclastogenesis by increasing the pool of osteoclast progenitor cells

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Reviewer: Osamu Takeuchi

Reviewer's report:

In this manuscript, Erin M et al. found that IL-11 was involved in inhibition of bone marrow cell death. Based on the data from in vitro cell survival assay, the authors conclude that IL-11 plays an important role in osteoclastogenesis by stimulating the development of osteoclast progenitor cells. The role of IL-11 in osteoclastogenesis is potentially interesting topic to the field of osteo-immunology. However, several data presented in this manuscript are unconvincing and lack of controls, which makes the conclusions doubtful. Extensive additional experimental work needs to be presented to fully support the conclusions.

Major compulsory revisions

1. The authors found that IL-11 is unable to stimulate osteoclastogenesis. However, Figure 1 and 2 only use very low concentrations (10-20ng/ml) of IL-11. Cells should be exposed to much higher concentrations of IL-11 (e.g. 100-1000ng/ml).

2. In figure 5, authors showed that IL-11 treatment suppresses bone marrow cell death. Based on this data, they concluded that IL-11 is able to promote the osteoclast progenitor cells. However, bone marrow cells are the mixture of various lineage cells. Furthermore, numerous previous reports have suggested that IL-11 has an anti-apoptotic function to diverse cells (Orazi A et al, Lab Invest, 1996; Frasca D et al, Int Immunol, 1996; Tafuri A et al, Exp Hematol, 1999; Scordi IA et al, Dermatology, 1999; Mahboubi K et al, Lab Invest, 2001; Kie JH et al, Stem Cells, 2002; Kiessling S et al, J Biol Chem, 2004; Naugler KM et al, Am J Physiol Gastrointest Liver Physiol, 2008; Idris NM et al, Regen Med, 2012). CD11b+ Gr-1lo c-Fms+ RANK+ cells are recognized as osteoclast progenitor. Thus, it is in particular important to investigate whether this cellular population is increased (or survived) by IL-11 treatment.

3. In Figure 5 and 6, authors showed the ability of osteoclastogenesis in bone marrow cells cultured in IL-11 and MDA-MB-231 conditioned medium. While the data is impressive, these experiments completely lack the proper controls to support the notion that IL-11 expands the osteoclast progenitors as alluded to above. Authors should collect the same numbers of “survived” cells from IL-11 added or control bone marrow cultures and stimulate these two groups by RANKL to compare the osteoclastogenesis.
4. It has been established that M-CSF cultured (2-3 days) bone marrow cells are used as osteoclast precursors in in vitro RANKL-induced osteoclastogenesis. Furthermore, previous report on M-CSF receptor (c-Fms) deficient mice found a lack of osteoclasts and severe osteopetrosis because of the lack of osteoclast progenitors. Thus, it is critically important to investigate the crosstalk between M-CSF and IL-11 signaling in the context of progenitor differentiation. The authors should examine the effect of IL-11 on M-CSF induced osteoclast precursor differentiation by using several parameters (e.g. cellular proliferation, mRNA levels of c-Fms and RANK).

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.