Author's response to reviews

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

Authors:

   Erin M McCoy (emccoy@uab.edu)
   Huixian Hong (huixian@uab.edu)
   Xu Feng (xufeng@uab.edu)

Version: 2 Date: 16 November 2012

Author's response to reviews: see over
Author's covering letter for initial submission

Title: IL-11 produced by breast cancer cells augments osteoclastogenesis by sustaining the pool of osteoclast progenitor cells

Authors:

Version: 1 Date: 14 November 2012

Comments: see over
November 14, 2012

Dr. Scott Kominsky
Associate Editor
BMC Cancer

Re: MS: 5132344307970277

Dear Dr Kominsky:

Thank you for the prompt handling of the review of our manuscript. Attached is the revised manuscript entitled “IL-11 produced by breast cancer cells augments osteoclastogenesis by sustaining the pool of osteoclast progenitor cells” (MS: 5132344307970277), which has been revised based on the concerns and comments from the reviewers. Also, we would like to thank the reviewers for their thorough and constructive review of our manuscript.

Our responses to the reviewer’s criticisms and comments are itemized below.

Reviewer 1
1. Is the question posed by the authors well defined?
The questions are clear but the focus is somewhat lost by the present order of the manuscript. I would switch the order and start with the observation that Breast cancer conditioned media are capable of supporting the development and/or survival of osteoclasts, then the IL-11 neutralizing antibody data, then that IL-11 does not stimulate osteoclastogenesis independently of RANKL or with low doses of RANKL, IL-11 does not affect survival, IL-11 promotes development/survival of osteoclast progenitor cells.

Based on the reviewer’s helpful suggestions, we have reorganized the manuscript to present the data in a more clear order with the breast cancer conditioned media first followed by the neutralizing antibody and recombinant IL-11. The IL-11 functional studies on osteoclast formation are presented after that. Please note that we highlighted subsection titles in the Results section to indicate the order change.

2. Are the methods appropriate and well described?
The authors use the appropriate methods, and they seem to be well-described. However, there are some details in the figures that need to be addressed.
1. While the differences are obvious, there needs to be some quantification of this data. Additionally, the resorption pits need to be highlighted or indicated more clearly to readers that may not be used to looking at these types of figures (I can clearly see the differences). Another option would be to show a higher magnification image.
2. What is the magnification on Figure 2? It would be helpful to show the scale on the figure. Need to show quantification.
3. You need quantification. Images of bone slices need to be clearer.

We have used a higher magnification (200x) from the Scanning Electron Microscope...
images to better show the resorption pits on the bone slices. Because only the control samples formed osteoclasts and resorbed bone, we did not quantify the resorption and show images that show no osteoclasts were formed in the plates on the experimental groups.

3. Are the data sound?
Yes, the data seem sound. They have large differences in their assays, and the experiments seem to be done thoroughly.

No response is required

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
Yes.

No response is required

5. Are the discussion and conclusions well balanced and adequately supported by the data?
The discussion does a very good job at describing the relevance of these studies. I thought it did a better job with the order than the rest of the paper. Once the order of the results is changed there will need to be some minor changes to the discussion (top of page 16) for the order.

Proper changes were made to the discussion to ensure it followed the new order of results.

6. Are limitations of the work clearly stated?
Acceptable.

No response is required

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
They do a fair job in acknowledging the previous work and discussing the controversies within the IL-11 field. I think they should mention either in the discussion or the introduction the other factors that MDA-MB-231 cells are known to express and that contribute to bone resorption. However, I thought they convincingly show that IL-11 is involved.

We agree with the reviewer and have added a sentence in the introduction on page 4 listing other factors secreted by MDA-MB-231. Furthermore, we added in the discussion on page 15 that it was necessary to look at IL-11 specific role through neutralizing antibody and use of recombinant IL-11 to ensure that IL-11 was indeed playing a specific role and not being confounded by the other factors in the conditioned media.

8. Do the title and abstract accurately convey what has been found?
The title accurately conveys the study, but it could be re-phrased in a more direct way to make more of an impact. Something along the lines of “Tumor-produced IL-11 increases the osteoclast progenitor pool leading to an increase in osteoclasts and bone resorption.”

The abstract still needs work. The relevance of the study should be more prominent. I would also argue that the authors have not delineated the precise mechanism underlying the role of IL-11 in breast cancer induced osteolysis. They have examined how IL-11 stimulates osteoclast differentiation and bone resorption, but more studies are needed to truly know the precise mechanisms here.

The title has been changed to “IL-11 produced by breast cancer cells augments osteoclastogenesis by sustaining the pool of osteoclast progenitor cells.” We agree with the reviewer that this re-phrasing helps make a bigger impact as well as addresses a concern with Reviewer 2 regarding the use of the word “increasing” in the former title.

The abstract has been largely rewritten based on the new order of the data and to better display the relevance of the study. Furthermore, we removed the phrase “delineate the precise mechanism” and replaced it with a more fitting wording as we address several different aspects of breast cancer secreted IL-11’s role in osteolysis.

9. Is the writing acceptable?
The manuscript is well-written and very interesting. They have strong data, and some minor changes in how the order the manuscript will help with the impact.

No response is required

Overall, I thought this was an interesting manuscript, and while I have several corrections that need to be addressed, the majority are minor.

Reviewer 2
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).

We originally used 10-20ng/ml based on previous reports (10ng/ml) and the ED50 of the IL-11 (0.04-0.4ng/ml). We have repeated the osteoclastogenesis assays with a high concentration of 200ng/ml IL-11. The new data are included as Figure 5C and Figure 6C.

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.

We are aware of these reports of IL-11 being able to act as a survival factor for some cells and to increase commitment of some stem cells as mentioned in the discussion section of the manuscript. This is the reason that we have stated that the increased cell number with IL-11 could be either from survival of osteoclast progenitor cells already in the bone marrow or IL-11-mediated development of osteoclast progenitors from cells at earlier stage of hematopoiesis, or both.

We are also aware that CD11b+ Gr-1lo c-Fms+ RANK+ cells are recognized as osteoclast progenitors. Since our current work has primary focused on addressing the mechanism by which IL-11 enhances osteoclastogenesis, we feel the functional data showing that the cells resulting from IL-11 treatment of the whole bone marrow cells being able to differentiate into functional osteoclasts are sufficient to address the key question of this work.

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.

We show significant differences in viable cells when comparing the IL-11 added to control bone marrow cultures (which are now Figures 1 and 2 not Figures 5 and 6). The number of cells that survive in the control bone marrow cultures are very low, making it difficult to collect the same number of survived cells to compare. However, because we already showed that density is important for proper osteoclastogenesis, we show that the ability of IL-11 to maintain a larger population of precursors has implications for increased osteolysis.

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).

This is a very good suggestion. However, given that these studies are beyond the scope of this work, we will address these interesting issues as future directions.

Once again, we would like to thank you and the reviewers for the thorough and constructive review, which has greatly helped improve the quality and accuracy of the manuscript.

Sincerely,

Xu Feng, PhD
Professor
Department of Pathology
University of Alabama at Birmingham
1670 University Blvd, VH G019E
Birmingham, AL 35294