LDL uptake-dependent phosphatidylethanolamine translocation to the cell surface promotes fusion of osteoclast-like cells

Victor J. F. Kitano, Yoko Ohyama, Chiyomi Hayashida, Junta Ito, Mari Okayasu, Takuya Sato, Toru Ogasawara, Maki Tsujita, Akemi Kakino, Jun Shimada, Tatsuya Sawamura and Yoshiyuki Hakeda
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Original submission

First decision letter

MS ID#: JOCES/2020/243840

MS TITLE: LDL uptake-dependent translocation of phosphatidylethanolamine to the cell surface promotes osteoclast cell-cell fusion

AUTHORS: Victor J. F. Kitano, Yoko Ohyama, Chiyomi Hayashida, Junta Ito, Mari Okayasu, Takuya Sato, Toru Ogasawara, Maki Tsujita, Akemi Kakino, Jun Shimada, Tatsuya Sawamura, and Yoshiyuki Hakeda
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise a number of criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

This is a well written study that examines mechanisms mediating osteoclast fusion and the potential role of low-density lipoprotein and phosphatidylethanolamine (PE) expression on the cell surface. A range of knockout animals and assays are used to address the hypothesis. The paper is well written and would be of interest to the journal's audience.
The experiments are described appropriately, and the results are presented to a good standard.
The conclusions drawn suggest that PE translocation to the outer leaflet of the plasma membrane is needed for osteoclast fusion. The authors also suggest that this translocation may be dependent on LDL uptake via LDLR mediated by ABCG1.
The level of discussion is good and relates to previous literature.

Comments for the author

The following should be considered to improve the impact of the science.
While the data suggests that the removal of key regulators impairs fusion, this conclusion would be strengthened by the addition of studies examining what effect restoration of PE levels in the KO models has on fusion capacity.
The addition of a figure outlining the sequence of cellular events that mediate fusion would add clarity.

Typo pg 6 stained instead of strained.

Reviewer 2

Advance summary and potential significance to field

LDL uptake-dependent translocation of phosphatidylethanolamine to the cell surface promotes osteoclast cell-cell fusion, by Victor Kitano et al., is a detailed study of the role of LDL and phosphatidylethanolamine on cell fusion in monocytes with M-CSF and sRANKL in vitro. This uses very good transgenic models and extensive supporting assays in vitro.

This is an important area of study relating to the relation of bone loss to hyperlipidemia. The data, while uniquely valuable, all relate to cell fusion of giant cells in vitro rather than osteoclast differentiation. Regarding this it is suggested to use a more descriptive title and to describe giant cell fusion more specifically, rather than calling cells osteoclasts that may or may not reflect bone resorbing activity.

Comments for the author

Comments
The work shown is exclusively related to cell fusion and related topics, without data on bone resorption or effects on expression of osteoclast-related proteins. This weakness might be remedied by resorption assays and assays for key osteoclast-activity related proteins. It is suggested, alternatively, to modify the title to indicate that this is a study of in vitro monocyte fusion in M-CSF and sRANKL, rather than specific osteoclast differentiation. If this is done carefully, the difficult work on actual osteoclast protein expression and bone resorption might not be needed while capturing the valuable data on cell fusion.

Regarding the main data on low-density lipoprotein receptor and oxidized LDL receptor-1 knockout mice studies are well performed and results are clear. Supporting data on phosphatidylethanolamine are strong and well described. Studies on the ATP binding cassette G1 transporter are similarly well planned and results are clear.

It is thus recommended to describe more specifically monocytic cells in RANKL and CSF in vitro rather than calling these osteoclasts. The reviewer realizes that the spread giant cells labeled with

TRAP activity are often called osteoclasts, but they are not. Osteoclasts in vivo are globular cells typically with about five nuclei, attached to bone; TRAP is a secreted enzyme at the apical surface.

The manuscript is in general well written and easy to understand, although the introduction is long and the authors might consider moving some of the review items there to the Discussion.

First revision

Author response to reviewers' comments

Response to reviewers’ comments

To Reviewer #1:

1. While the data suggests that the removal of key regulators impairs fusion, this conclusion would be strengthened by the addition of studies examining what effect restoration of PE levels in the KO models has on fusion capacity.

Reply: At present, we do not have ABCG1 expression plasmid. If we start to prepare the restoration experiment of knockout mice with the expression plasmid from now, we would not meet the revision deadline. Thus, instead of the knock-in experiments, we examine the effect of LXR agonist GW 3965 on OCL formation and the expression of ABCG1 mRNA, and we added the results in Fig. 8 of the revised version. As a result, LXR activation by the agonist GW 3965 restored the expression of ABCG1 mRNA and the OCL formation. In line with the additional experiments, we changed the figure legend of Fig. 8. Also, we added a citation on GW 3965 in the reference.

2. The addition of a figure outlining the sequence of cellular events that mediate fusion would add clarity.

Reply: In line with this suggestion, we added the figure outlining the sequence of cellular events from LDL uptake through LDLR to the cell-cell fusion of OCLs mediated by ABCG1 in supplementary Fig. S7.

3. Typo pg 6 stained instead of strained.

Reply: I am sorry for the typo. I corrected it into “stained”. As I found typos other than it, I also corrected them in the revised version; for example, we corrected mRMA into mRNA in supplementary Table S2, and we also changed 100 μm of scale bars into 250 μm in the legends of Fig. 7 and Fig. 8.

To Reviewer #2:

1. The work shown is exclusively related to cell fusion and related topics, without data on bone resorption or effects on expression of osteoclast-related proteins. This weakness might be remedied by resorption assays and assays for key osteoclast-activity related proteins. It is suggested, alternatively, to modify the title to indicate that this is a study of in vitro monocyte fusion in M-CSF and sRANKL, rather than specific osteoclast differentiation. If this is done carefully, the difficult work on actual osteoclast protein expression and bone resorption might not be needed while capturing the valuable data on cell fusion.

Reply: We appreciate the comment. As with the answer to Comment 2, I changed the title of this paper to "LDL uptake-dependent phosphatidylethanolamine translocation to the cell surface promotes fusion of osteoclast-like cells".

2. It is thus recommended to describe more specifically monocytic cells in RANKL and CSF in vitro rather than calling these osteoclasts. The reviewer realizes that the spread giant cells labeled with TRAP activity are often called osteoclasts, but they are not. Osteoclasts in vivo are globular cells typically with about five nuclei, attached to bone; TRAP is a secreted enzyme at the apical surface.

Reply: We partially agree with the reviewer's opinion. More than twenty years ago, our group developed a method for isolation of authentic osteoclasts from rabbit bones (Hakeda, Y. and Kumegawa, M. In: Principles of Bone Biology. Chapter 88. 1st Edition edited by J. P. Bilezikian., L. G. Raisz. And G. A. Rodan. Academic Press), and identified the osteoclast specific protease as cathepsin K (Tezuka et al., 1994. J Biol Chem. 269, 15006-15009). Moreover, using the authentic osteoclast, we reported some molecular mechanism for osteoclast functions; for example, the role of BMP-2 in osteoclast bone resorbing activity (Kaneko et al., 2000. Bone 27, 479-486). Indeed, the morphology of the authentic osteoclasts is different from the shape of osteoclast-like cells formed in in vitro culture in the presence of M-CSF and sRANKL, as mentioned by the reviewer. However, the expression profile of osteoclast differentiation-related molecules such as cathepsin K in the in the osteoclast-like cells is very similar with that of the authentic cells. However, the TRAP-positive multinucleated cells formed in in vitro culture with M-CSF and sRANKL are not authentic cells, as suggested by the review, but are only osteoclast-like cells. Thus, we carefully revised the terminology of the TRAP-positive multinucleated cells as osteoclast-like cells (OCLs), but not called "osteoclasts" throughout in the revised manuscript.

3. The manuscript is in general well written and easy to understand, although the introduction is long and the authors might consider moving some of the review items there to the Discussion.

Reply: According to this comment, we moved the citations about the correlation between bone and lipid metabolism in Introduction to "Discussion".

Minor Changes:

According to the author instruction, we changed the name of funder into "Japan Society for the Promotion of Science".

Second decision letter

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ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.