

Biphasic regulation of glutamine consumption by WNT during osteoblast differentiation

Leyao Shen, Deepika Sharma, Yilin Yu, Fanxin Long and Courtney M. Karner
DOI: 10.1242/jcs.251645

Editor: John Heath

Review timeline

Original submission:	14 July 2020
Editorial decision:	20 October 2020
First revision received:	27 October 2020
Accepted:	19 November 2020

Original submission

First decision letter

MS ID#: JOCES/2020/251645

MS TITLE: Biphasic regulation of glutamine consumption by WNT during osteoblast differentiation

AUTHORS: Leyao Shen, Deepika Sharma, Yilin Yu, Fanxin Long, and Courtney Karner

ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript. I do apologise for the delay in reviewing this paper. We have one review after approaching 26 referees including some who agreed to review and then did not produce a report. As a result, I have looked at the paper myself in the light of the one report we have.

To see the reviewer's report 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 reviewer gave favourable reports but raised some points that will require amendments to your manuscript. I hope that you will be able to carry these out, because I would like to be able to accept your paper.

We are aware that you may be experiencing disruption to the normal running of your lab that makes experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

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*

WNT induces Slc7a7 and Slc1a5 expression to increase glutamine uptake. Inhibiting either transporter reduces WNT induced glutamine uptake and prevents osteoblast differentiation establishing Slc7a7 and Slc1a5 as critical mediators of WNT induced osteoblast differentiation.

Comments for the author

Biphasic regulation of glutamine consumption by WNT during osteoblast differentiation, by L. Shen, et al., is an interesting paper demonstrating that Slc7a7 and Slc1a5 are critical mediators of WNT induced osteoblast differentiation. The methods are mostly well described and the work shown demonstrates the conclusions well, although there are a few points needing clarification. The figures in general are very nicely done and clear.

Specific comments

1) Please do a careful review for grammar. Example in the Abstract, Osteoblasts are the principal (not principle) bone forming cells.

2) If possible, please shorten the introduction to two pages.

3) I think the alkaline phosphatase shown in Fig 1 is enzyme activity, not "staining". I don't see this in Methods; might be very brief but indicating the substrate and how the product is visualized is recommended.

Similarly, stating that von Kossa is silver nitrate labeling of mineral and giving a reference is recommended.

4) More detail on the antibody targets is recommended, and if available data that the antibody reaction is eliminated by antigen or some such as references to the manufacturer or another paper is recommended particularly since the Western results are given in "letterbox" form. The basis of product visualization might be given in a few words (enhanced chemiluminescence perhaps) rather than stating a kit name.

First revisionAuthor response to reviewers' comments

Reviewer 1:

Advance Summary and Potential Significance to Field: WNT induces Slc7a7 and Slc1a5 expression to increase glutamine uptake. Inhibiting either transporter reduces WNT induced glutamine uptake and prevents osteoblast differentiation establishing Slc7a7 and Slc1a5 as critical mediators of WNT induced osteoblast differentiation.

Reviewer 1 Comments for the Author: Biphasic regulation of glutamine consumption by WNT during osteoblast differentiation, by L. Shen, et al., is an interesting paper demonstrating that Slc7a7 and Slc1a5 are critical mediators of WNT induced osteoblast differentiation. The methods are mostly well described and the work shown demonstrates the conclusions well, although there are a few points needing clarification. The figures in general are very nicely done and clear.

Specific comments

1) Please do a careful review for grammar. Example in the Abstract, Osteoblasts are the principal (not principle) bone forming cells.

We thank the reviewer for the positive review of our manuscript. We have carefully reviewed the revised manuscript and corrected all grammatical errors we found including the one noted by the reviewer.

2) If possible, please shorten the introduction to two pages.

We have shortened the introduction to under two pages.

3) I think the alkaline phosphatase shown in Fig 1 is enzyme activity, not "staining". I don't see this in Methods; might be very brief but indicating the substrate and how the product is visualized is recommended. Similarly, stating that von Kossa is silver nitrate labeling of mineral and giving a reference is recommended.

We thank the reviewer for this comment about the functional assays in the manuscript. The author is correct, the staining in figure 1 is an enzymatic activity assay. We have modified the text in the methods as follows:

“Osteoblast differentiation was assayed by visualizing alkaline phosphatase activity using 5-bromo-4-chloro-3'-indolyphosphate/nitro blue tetrazolium (BCIP/NBT) (Katagiri et al., 1994) or von Kossa staining of deposited calcium phosphate (Rungby et al., 1993). Alkaline phosphatase assay was performed 24 hours after WNT stimulation. Von Kossa staining was performed 6 days after addition of osteogenic media.”

4) More detail on the antibody targets is recommended, and if available data that the antibody reaction is eliminated by antigen or some such as references to the manufacturer or another paper is recommended, particularly since the Western results are given in "letterbox" form. The basis of product visualization might be given in a few words (enhanced chemiluminescence perhaps) rather than stating a kit name.

We have included more detailed information including the dilution, manufacturer, antigen specificity and references for the antibodies used in this study. This information is located in supplementary table S2. We have also modified the methods as follows: “All blots were developed using enhanced chemiluminescence (Clarity Substrate Kit, Bio-Rad).”

Second decision letter

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

Reviewer 1

Advance summary and potential significance to field

This is a re-review, please see the original review for this section.

Comments for the author

The authors have responded appropriately with change in the manuscript addressing the issues raised. I have no further comments.