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Molecular mapping of transmembrane mechanotransduction through the β1 integrin-CD98hc-TRPV4 axis

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Original submission

First decision letter

MS ID#: JOCES/2020/248823

MS TITLE: Molecular mapping of transmembrane mechanotransduction through the Î²1 integrin-CD98hc-TRPV4 axis

AUTHORS: Ratnakar Potla, Kobayashi Mariko, Hao Wu, Hong Chen, Akiko Mammoto, Benjamin D. Matthews, and Donald E. Ingber
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 are enthusiastic about the ultimate suitability of the paper for publication in the JCS, but raised some critical 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 currently be unable to access the lab to undertake experimental revisions. 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

The paper "Molecular mapping of transmembrane mechanotransduction through the β1 integrin-CD98hc-TRPV4 axis" by Potla et al. utilized co-immunoprecipitations, proximity ligation assays, mutational analysis molecular dynamics simulation, and a pair of functional assays: mechanical and chemical driven calcium flux and strain-driven cell alignment to implicate ternary complex formation between the β1 integrin-CD98hc-TRPV4 as a principal mechanism for force induced channel activation through a focal adhesion. Through this work, the authors identified that the juxtamembrane cytoplasmic region of CD98hs engages the β1 integrin tail, whereas the Ankyrin-rich repeats of TRPV4 engage CD98hs cytoplasmic regions.

Comments for the author

An appreciable amount of this paper's significance is in identifying the protein:protein interactions critical to the proposed ternary complex. Lee and colleagues (Y Lee, Nature Structural and Molecular Biology, 2019), recently found little interaction between the transmembrane domain of CD98hs and TM4 of Lat2 as it approached the inner leaflet. As such, it might be expected that ISM mutations identified in this work (179 and 181) would be non-participatory by locality in LAT1 binding. To better support the conclusion that both the domain interaction assignment and proper folding of the mutant CD98hs protein is present, the authors are encouraged to investigate whether both FLAG-CD98hc and FLAG-CD98hc(ISM) can be immuneprecipitated with Lat2, as expected, while only FLAG-CD98hc is capable of precipitating B1 integrin as shown here.

The HH domain of CD98hs participates in other engagements, such as the LAT1 interaction mentioned above. It is possible that the dominant negative effects of HH expression on cell alignment may be through disrupting CD98hs engagements other than B1-integrin. The inability HH(ISM) overexpression, compared to HH overexpression, to uncouple mechanoactivation and block cell alignment (both in Figure 6) would add significant support to the conclusion the results assigned to figure 6 are direct disruption of the proposed ternary complex, especially if successfully coupled with the proposed FLAG-CD98hc(ISM)/Lat1 co-IP above.

Other:

Figure 1F – the image doesn't agree with the 1G data, are the delta1 and delta5 images switched?

In results, page 7, the authors conclude "These results suggest that TRPV4 does not bind directly to the HH domain of CD98hc or β1 integrin tail, but rather to some other region of CD98hc that might be exposed via allosteric conformational changes upon its binding to β1 integrin" While integrin binding to CD98hc may drive conformational change to support TRPV4 engagement, the data presented here does not exclude the possibility that direct binding occurs between HH region and TRPV4 but such interaction is not sufficient for stable IP interaction. Please consider revising this conclusion.

The increase of Talin engagement with B1 in the presence of the CD98hs mutants (Figure 5) is of regulatory interest. Paper discussion might be improved by its inclusion.

Reviewer 2

Advance summary and potential significance to field

This work shows that β1 integrin, CD98hc, and TRPV4 proteins form a ternary complex in focal adhesions capable of sensing mechanical force.It is a significant contribution to our understanding

of mechanotransduction. This manuscript is significantly improved compared to the one I reviewed previously, and I recommend it for publications with some minor revisions.

Comments for the author

1. Section on Ankyrin rich domains….
The authors refer to a transmembrane mutant called TRPV4(N) that does not have transmembrane elements. Also, there is no discussion of results related to this mutant. It should be removed.
2. I suggest a summary figure (cartoon) to aid in understanding the Discussion section.
3. Perhaps the authors should include work that compares Piezo1 and TRPV4 since it seems to buttress their arguments for tethered versus through lipid forces (Servin-Vences, M.R., et al., Direct measurement of TRPV4 and PIEZO1 activity reveals multiple mechanotransduction pathways in chondrocytes. Elife 2017. 6).

First revision

Author response to reviewers' comments

RESPONSE TO REVIEWERS

MS# JOCES/2020/248823 (Potla et al.):

Reviewer 1:

1. An appreciable amount of this paper's significance is in identifying the protein:protein interactions critical to the proposed ternary complex. Lee and colleagues (Y Lee, Nature Structural and Molecular Biology, 2019), recently found little interaction between the transmembrane domain of CD98hs and TM4 of Lat2 as it approached the inner leaflet. As such, it might be expected that ISM mutations identified in this work (179 and 181) would be non-participatory by locality in LAT1 binding. To better support the conclusion that both the domain interaction assignment and proper folding of the mutant CD98hs protein is present, the authors are encouraged to investigate whether both FLAG-CD98hc and FLAG-CD98hc(ISM) can be immuneprecipitated with Lat2, as expected, while only FLAG-CD98hc is capable of precipitating B1 integrin, as shown here.

The paper referenced above by Lee et al. reports the cryo EM structure of CD98hc interacting with LAT1; however, human CD98hc associates with 5 other SLC7 light chains (LAT2, y+LAT1, y+LAT2, xCT and asc-1) in addition to LAT1. They report four interfaces between the heavy chain and the light chain: TM1′-TM4, linker-Cβ2-Cβ3-Cβ8-EL2, Aα8-EL4a and Aα1-Aα2-EL3. Except for TM1′-TM4, all of the other interfaces involve the extracellular domain of CD98hc. The mutant constructs used in our study lack the extracellular domain of CD98hc beyond amino acid 210. We therefore believe that a Co-IP using our mutant construct will not address the Reviewer's question. Moreover, our work focuses on the highly flexible cytosolic tail and point mutations at specified amino acids involved in binding β1-Integrin or TRPV4 and we thereby believe that understanding the consequences for Lat1 binding is beyond the scope of this manuscript. While we acknowledge that Reviewer's suggestion will provide evidence for membrane localization and proper folding of a full length mutant CD98hc, we believe that the proximity ligation data from basal slices of the cell provide similar evidence on membrane localization and thereby proper folding of the mutant protein.

2. The HH domain of CD98hs participates in other engagements, such as the LAT1 interaction mentioned above. It is possible that the dominant negative effects of HH expression on cell alignment may be through disrupting CD98hs engagements other than B1-integrin. The inability of HH(ISM) overexpression, compared to HH overexpression, to uncouple mechanoactivation and block cell alignment (both in Figure 6) would add significant support to the conclusion the results assigned to figure 6 are direct disruption of the proposed ternary complex, especially if successfully coupled with the proposed FLAG-CD98hc(ISM)/Lat1 co-IP above.

We carried out additional experiments using a Flexcell culture system to apply mechanical strain in order to address the Reviewer's question regarding specificity of the β1 integrin – Cd98hc interaction to the proposed mechanical coupling. These studies revealed that uncoupling only occurred in HUVE cells overexpressing CD98hc(WT), and not in cells overexpressing CD98hc(ISM), which continued to align perpendicular to the direction of uniaxial stretch. We include these data that respond to this concern in new Figures 6 E & F, and the Results section has been updated accordingly.

3. Figure 1F – the image doesn't agree with the 1G data, are the delta1 and delta5 images switched?

Thank you for pointing this out. The images labels have been corrected.

4. In results, page 7, the authors conclude "These results suggest that TRPV4 does not bind directly to the HH domain of CD98hc or β1 integrin tail, but rather to some other region of CD98hc that might be exposed via allosteric conformational changes upon its binding to β1 integrin" While integrin binding to CD98hc may drive conformational change to support TRPV4 engagement, the data presented here does not exclude the possibility that direct binding occurs between HH region and TRPV4 but such interaction is not sufficient for stable IP interaction. Please consider revising this conclusion

We have revised the statement to include both possibilities.

5. The increase of Talin engagement with B1 in the presence of the CD98hs mutants (Figure 5) is of regulatory interest. Paper discussion might be improved by its inclusion.

Discussion has been updated to include regulation of Talin engagement of β1 integrin by Kindlin and potential competition between CD98hc and Kindlin for the distal NxxY motif.

Reviewer 2:

1. Section on Ankyrin rich domains:The authors refer to a transmembrane mutant called TRPV4(N) that does not have transmembrane elements. Also, there is no discussion of results related to this mutant. It should be removed.

Mention of TRPV4(N) has been removed from the text and Figures S3B,C.

2. I suggest a summary figure (cartoon) to aid in understanding the Discussion section.

A cartoon has been added in new Figure 7.

3. Perhaps the authors should include work that compares Piezo1 and TRPV4 since it seems to buttress their arguments for tethered versus through lipid forces (Servin-Vences, M.R., et al., Direct measurement of TRPV4 and PIEZO1 activity reveals multiple mechanotransduction pathways in chondrocytes. Elife, 2017. 6

Discussion has been updated with the suggested reference to convey the differential roles of TRPV4 and PIEZO1 in mechanotransduction.

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.