Roles for Ndel1 in keratin organization and desmosome function

Yong-Bae Kim, Daniel Hlavaty, Jeff Maycock, and Terry Lechler

Corresponding author(s): Terry Lechler, Duke University

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

RE: Manuscript #E21-02-0087
TITLE: Ndel1 promotes keratin assembly and desmosome stability

Dear Terry,

Two reviewers have now seen your manuscript. Both definitely think that this work will be of interest to the readership of Molecular Biology of the Cell, and they especially find "the central selling point", as Reviewer 2 refers to it, to be very interesting. However, Reviewer 1 in particular has a sufficient number of suggested additional experiments or concerns that your paper will require revision and re-review. Many of your change may simply require some change in emphasis in the writing, but others may require some additional experiments.

Both Reviewers express concerns about the central point reflected in the title of the manuscript regarding keratin assembly. While they agree that your work demonstrates that loss of Ndel1 has powerful effects, they both raise questions about your interpretations. Reviewer 1 points to the "chicken-and-egg" issues of keratin and desmosomes, and Reviewer 2 feels that more work could be done to address peripheral keratin disorganization.

Reviewer 1 also has many specific comments that you should address in your revision. Many relate to controls and quantification, as well as some discrepancies between images in the main text and the Supplemental data. Some of these issues may be addressable through clarifications in the writing and others through some additional data analysis, but it may be necessary to perform a few additional experiments if some of the controls requested are not easily available. I will leave it to you as to how to prioritize these suggested changes.

Many thanks for submitting your work to MBoC. I look forward to receiving your revisions.

Best regards,
Jeff Hardin
Monitoring Editor

Molecular Biology of the Cell

Dear Dr. Lechler,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you haveopted out of publishing the review history.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us at mboc@ascb.org.

Revised manuscripts are assigned to the original Monitoring Editor whenever possible. However, special circumstances may preclude this. Also, revised manuscripts are often sent out for re-review, usually to the original reviewers when possible. The Monitoring Editor may solicit additional reviews if it is deemed necessary to render a completely informed decision.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised manuscript, and figures, use this link: Link Not Available

Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

Kim et al, set out to address the potential role of Ndel1 in promoting keratin assembly at desmosomes. They demonstrate that Ndel1 can bind to keratin filaments in a highly conserved region. Furthermore, they demonstrate that Ndel1 is important for the localization of intermediate filaments (IF) to desmosomes. Membrane localized Ndel1 required Lis1 and this membrane association was important for desmosome stability. The importance of Nde1 for desmosome structure/function was demonstrated both in vitro and in vivo. Ndel1 cKO animals exhibited decreased membrane staining for desmosome components and smaller desmosomes. These data begin to fill a major gap in our understanding of how IF are organized at desmosomes and should be of broad interest to the MBoC readership.
However, while the authors do demonstrate that Ndel1 has a powerful effect on keratin organization in cells, they have not directly demonstrated that Ndel1 promotes keratin IF assembly at desmosomes. The authors suggest in the discussion that desmosome defects are likely secondary to the keratin disorganization in the absence of Ndel1, I'm not sure they can formally rule out that it is the other way around, and that through desmosome impairment there is an indirect effect on IF. In addition, EMs clearly show IF at desmosomes, albeit smaller ones, in Ndel1 deficient animals. This could be in part due to compensatory mechanisms that are not addressed here in an in vivo model. When all the data are considered, the authors need to be cautious about not over-reaching in their interpretation, including the title. The title also states that Ndel1 promotes desmosome stability. While the mobile fraction of DP is measured, other junction molecules are not analyzed in the same fashion. It would be more accurate to refer to desmosome function, based on the dispase assay.
While the larger question of exactly how Ndel1 is affecting IF (polymerization or otherwise) can wait for future reports, the current work nevertheless requires some additional work to make it acceptable for MBoC, including additional controls and quantification of data. Other questions or clarifications can be handled by textual changes.
Detailed figure-by-figure comments are given below:
Textual issues and statistics
1. Minor: In the introduction on page 3 the authors mention arrhythmogenic right ventricular cardiomyopathy which is now referred to as arrhythmogenic cardiomyopathy.
2. The authors mention that Ndel1 is important for epidermal barrier formation in the abstract, but this is not analyzed in the manuscript. The animals do not have a severe barrier defect as they reach adulthood exhibiting only a scruffy coat. This description should be more carefully stated in the manuscript. If desired, milder barrier defects could be determined in the animals using inside-out and outside-in barrier assays and if added could support the statements in the current version of the manuscript.
3. All the figures must include information about the number of independent experiments performed, indicate what the p values are and what statistical analyses were performed.
Figure 1:
a. Figure 1D: Is it known that mutation of the conserved leucine does not disrupt the structure of the keratin construct; that is, are we sure it is directly inhibiting the interaction with Ndel1?
b. Figure 1 E: Do the authors have confirmation that the K1 and K10 formed filaments in their ultracentrifugation assay? In all of these assays it would be helpful to show negative stained EM images of filaments plus/minus increasing amounts of Nde1 in order to directly visualize filament status.
c. In the assay, an increasing amount of soluble K1 and K10 were seen in the supernatant with increasing amounts of the Ndel1 and this does not happen with the mutant keratins. Does this raise the possibility that Ndel1 binding sequesters monomers or small oligomers, inhibiting polymerization instead of promoting assembly?
Figure 2:
a. Authors may want to check to make sure Figure 2B is not out of focus, and replace, if it is.
b. With respect to the observed perinuclear accumulation of keratin filaments in the Ndel null cells (2A, B) what do Z-stacks reveal? Is the size/shape of Ndel1 altered and are they taller than the WT cells?
c. It would be beneficial to share higher magnification insets of the areas adjacent to the membrane (2A,B).
d. It appears that there is less keratin at the periphery in the cells cultured in low calcium as well as high calcium (2C, D). If Ndel1 is important for promoting keratin assembly at the desmosome, why is the difference still apparent in low calcium? Does Ndel1 play a role in keratin filament assembly throughout the cell and not just at cell-cell contact sites?
e. The expression of the C-terminus of Ndel1 (2E, F) can cause the collapse of the keratin filaments in a dominant negative fashion. This supports the idea that Ndel1 might in fact inhibit polymerization since WT Ndel is present in the cells and is unable to overcome the expression of this construct. What does WT Ndel1 look like in Ndel-CT expressing cells? Does this occur in all

of the Ndel CT expressing cells, now many cells were observed with this phenotype?
f. In figure 2G, H there is less keratin in a filamentous network in the Ndel1 null cell overall, not just changes at the periphery. There also appears to be more DP in the cytoplasm of the Ndel1 null cells. Does Ndel1 play a role in DP recruitment to the desmosomes? In spite of the result in 3F, this difference seems interesting, and one wonders whether DP could be involved in the mechanism by which Nde1 influences IF polymerization status in the normal junctional context (as organization at the mitochondria seems very unclear to me)? Quantification as was done in 2C, D would be important here as expression of the K14-GFP can vary from cell to cell independent of the expression of Ndel1.

Figure 3:
a. The figure 3 legend is missing a description of F, G, H and I.
b. 3C should include labeling for consistency.
c. mito-PAGFP is not defined in the text.
d. The authors make the argument that slow recovery time is consistent with the mitochondrial keratin being in a stable filamentous form. However, the structures in the mito-Ndel1 in 3D do not appear to be normal. Why not just do some conventional EM to get a direct indication of how these structures are organized? Whether they consist of 10nm filaments or something else?? Graphs should be included for recovery experiments.
e. 3F, G: control panels for DP null and Lis1 null with mito-PAGFP should be included.
f. 3H, I: A panel showing the localization of Ndel in DP null cells should be added. Is Ndel at the membrane in the absence of DP? What does the keratin organization look like in Lis null cells? Is it collapsed in the same manner as seen in Ndel1 null cells?
Figure 4:
a. 4A-H should be quantified.
b. In 4N what is the phenotype of the Ndel1/Nde1 null cells in terms of desmosome formation and keratin organization? Clearly there is a larger effect on epithelial sheet fragmentation. Is Nde1 promoting keratin organization at the membrane in the Ndel1 null cells? Immunofluorescence for keratin should be provided for Nde1 null and Ndel1/Nde1 double null cells. The organization of desmosome components at the cell membrane appears much less dramatic in Supplemental Figure 4C than that shown in Figure 4A-F. Quantification should be provided for the immunofluorescence for both of these experiments.
Figure 5:
a. There is a dramatic decrease in Dsg1 in 5J, yet there is no barrier loss phenotype in the animals. A recent paper by the Waschke and colleagues demonstrate a severe barrier phenotype upon Dsg1 loss. What are the levels of the desmosome and adherens junction proteins in Ndel1 null epidermis when analyzed by western blot?
b. IN 5P the quantification of DP and Dsg1 do not appear to match the changes in cortical staining intensity in the representative images shown G-J.
c. 5E, F. Are the microblisters between the basal layer and the basement membrane? Could there be an effect of Ndel1 deletion on hemidesmosomes? The areas of microblister should be clearly indicated in 5D.
Supplemental Figure 2:
a. Controls for the DP GFP expression and drug treatments in the absence of mito-Ndel1 should be included here.
Supplemental Figure 4:
a. The effect of Ndel1/Nde1 loss on the desmosome protein localization appears milder here than in the main text Figure 4. Controls that need to be included are Ndel1 null and Nde1 null cells to compare to the double null cells. Keratin staining should be included, or K14GFP, to look at keratin organization in single and double null cells.
b. 4C, D should include quantification.
Supplemental Figure 5:
a. Include quantification of desmosome and adherens junction proteins in whole cell lysates.

Reviewer #2 (Remarks to the Author):

The manuscript by Kim et al examines the role of Ndel1 in driving the assembly of keratin filaments at sites of desmosomal cell-cell contacts. The work identifies novel mechanisms of keratin organization in keratinocytes and how keratin filaments locally assemble at sites of desmosomal contacts. This study is important because, in contrast to actin and microtubule networks, we know strikingly little about control mechanisms for local keratin polymerization. Overall, the manuscript is well written and the results reveal important new mechanisms for keratin regulation. Some weaknesses should be considered, as outlined below:

1) Peripheral keratin organization is not well characterized/displayed in the manuscript for control vs. Nde1/Nedl1 null and in the double knock outs. In Fig. 2 A,B there does not seem to be a large difference in keratin filaments near cell-cell borders. Perhaps this difference would be more pronounced in the double knock out cells but keratin organization is not shown in Supp. Fig. 4. The role for Ndel1 in organizing keratin filaments at desmosomes is really a key aspect of the work, and the paper would be improved by showing additional keratin localization data at the cell periphery of control vs single and double knock out cells. The changes in cell-cell adhesion strength are presumably due to changes in keratin filament attachment, so additional emphasis should be placed on documenting how keratin attachments to desmosomes are altered.

2) The manuscript would be improved by the addition of a model for how Ndel1, Lis1, and DP coordinate activities to locally organize keratin filaments at the desmosome.

Minor comments:

Additional references and reviews on desmosome mutations in cardiac disease (pg. 3) and K10/ K1 distribution in the epidermis (pg. 6) would be helpful.

Fig. 2A in the figure panels as well as legend, please specify if these cells are in low calcium or 4h calcium.

Legend for Supp, Fig. 2a is switched. WT should be blue and Keratin null should be red. The graph itself is correct.

Fig. 3 F,G,H,I is not mentioned in the legend.

No Electron microscopy protocol is mentioned in the Materials & Methods section. The manuscript should state how many desmosomes were counted in Fig. 5O graph?

The authors should clarify why mito-Nde1 is used instead of mito-Ndel1 in Supp. Fig. 2A?

RE: Manuscript #E21-02-0087R
TITLE: "Roles for Ndel1 in keratin organization and desmosome function"

Dear Terry,

Many thanks for submitting your revised manuscript. After looking through your responses to the Reviewers' comments, I am please to say that your work is now ready for publication in Molecular Biology of the Cell.

Many thanks for submitting to MBoC.

Best regards,
Jeff Hardin
Monitoring Editor

Dear Dr. Lechler:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

Within approximately four weeks you will receive a PDF page proof of your article.

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