RAC1 induces nuclear alterations through the LINC complex to enhance melanoma invasiveness

Paula Colón-Bolea, Rocío García-Gómez, Sue Shackleton, Piero Crespo, Xosé R. Bustelo, and Berta Casar

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Review Timeline:

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|----------------------------|------------|
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Editor-in-Chief: Matthew Welch

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.)
Dear Dr. Berta Casar,

Your manuscript E20-02-0127 entitled "RAC1 induces nuclear alterations through the LINC complex to enhance melanoma invasiveness" has been evaluated by two reviewers, both experts in the field. You will see that both reviewers had very positive comments about your work. They did however raise a few issues that need to be addressed. In particular, I agree that you should pay attention to the methods and statistics of Fig. 4. This would strengthen the points you are making and provide a clearer understanding of the data presented.

I will be happy to look at a revised version of your work that specifically addresses this point and all other concerns raised by the reviewers.

I thank you for submitting your work to MBoC and I look forward to receiving a revised version.

Yours sincerely,

Peter J.M. Van Haastert
Monitoring Editor
Molecular Biology of the Cell
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 cover letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL): Link Not Available

Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

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

Reviewer #1 (Remarks to the Author):

In this manuscript, Colón-Bolea and colleagues have utilized melanoma cell lines and gain of function assays to perform in vitro & in vivo (chick embryo) studies to elucidate novel pro-invasive functions of the Rho GTPase RAC1. They report a functional interaction in the cytosol between RAC1 and the LINC complex resulting in a microtubule-dependent nuclear deformation. This phenotype facilitates migration through confined spaces and could be key in the intravasation-extravasation stages of metastatic dissemination. While the experimental approach is in general clear and the RAC1 dependent pro-migratory phenotype sufficiently substantiated I feel that additional evidence should be incorporated in support of the main conclusions.

Major points:
1. One of the two most relevant findings reported in this manuscript is the implication of the microtubules and not the actin cytoskeleton. This has been sufficiently validated by the use of a constitutively active RAC1Q61L mutant harbouring a second mutation (F37A) that fails to alter the actin cytoskeleton and yet, still provokes nuclear deformation. Also, blocking tubulin polymerization by nocodazole greatly diminished nuclear abnormalities.

2. The data presented in Figure 4b regarding nuclear deformation is difficult to evaluate. It is unclear whether the assay followed the nuclear variations of the same group of nuclei during the 3 hr time
course. If not I do not see the need of performing a time course analysis. In any case, could these results be quantitated providing statistical significance?

Reviewer #2 (Remarks to the Author):

Recent studies show that tumor cells penetrate tissues through confining spaces such as tight extracellular matrices, which requires deformation and plasticity of the cell nucleus. In this manuscript, the authors proposed that the nuclear deformation is responsible for increase in the invasive activity of melanoma cells. Expression of constitutively active (CA-) Rho GTPases, especially Rac1, altered the nuclear shape. This change in nuclear morphology is suggested to be caused by assembly of microtubule (MT), since the nocodazole-treatment that prevents microtubule polymerization diminished the effect of CA-Rac1 on the nuclear shape change. To show whether MT assembly is downstream of Rac1 activation, the authors carried out experiments using two different mutants of CA-Rac1; one can activate the downstream effector PAK1 but the other cannot. Although both mutants prevented changes in nuclear deformation to some extent, the latter was less effective on nuclear deformation. In addition, expression of CA-PAK1 also induced marked nuclear shape alterations and this effect was abolished by the nocodazole-treatment, while the nuclear morphologies were different from those elicited by CA-Rac1. From these results, the authors suggest to be microtubules as mediators of nuclear shape regulation by the Rac1-PAK1 pathway. Then, they also showed that changes in nuclear morphology by Rac1 is mediated by LINC complex that may link cytoplasmic microtubules with intranuclear scaffolds. Furthermore, they clearly demonstrated that increase in temporal changes in the nuclear shape of living cells that expressed CA-Rac1 are suppressed by disturbance of LINC complex. Finally, invasive properties of cells expressing CA-Rac1 were suppressed by co-expression of a dominant negative form of KASH both in vitro and in vivo.

These findings are biologically important and interesting broadly for the cell biologists. Experimental results are clear and the writing is easy to understand. Therefore, I recommend the manuscript for publication. However, it may increase more impact to show a direct evidence between the nuclear shape change and increase in the invasive property. (Although this is not necessary,) is it possible that cell migrating rates are measured during moving through constrictions, for example, a dens collagen matrix? Or is it possible to measure changes in the stiffness or flexibility of nuclei of cells expressing CA-Rac1? In addition, the manuscript should be edited according to the instruction for authors (particularly references) and also English should be edited.

Minor concerns:
1) Antibodies and plasmid constructs used in the experiments should be represented in 'Materials and Methods'.
2) Page 8, the last sentence, (Fig. 3d) is wrong.
3) In Fig. 4, photos of cells appear to be a bit out of focus.
4) I think there are some misspellings. for example, depolimerization (page 8)---depolymerization
hypothesyzed (page 10)---hypothesized
intriguinly (page 12)---intriguingly
RESPONSES TO REVIEWERS

Reviewer #1

1. The authors use overexpression of a RANQ69L to inhibit importin-mediated nuclear import of transcription factors potentially activated by the RAC1Q61L. As a proof of concept they evaluate the transcriptional activity of a luciferase reporter under the control of a serum-response promoter (Figure 2b). The data presented only demonstrates a partial reduction of luciferase activity and therefore does not totally exclude the participation of a RAC1-dependent transcriptional response in the observed nuclear phenotype. Could the authors assess by qPCR the transcriptional status of the RAC1-dependent SRF/MRTF transcription program plus/minus RAC1Q61L and RANQ69L? As an example some valuable transcriptional changes have been recently evaluated in the context of active RAC1 (doi.org/10.1016/j.ccell.2019.05.015).

Agree
We explored effects of RAC1 activation on SRF/MRTF transcriptome program in A375p cells. However, RAC1Q61L and RANQ69L did not induce SRF/MRTF expression changes in BRAF<sup>V600E</sup> mutation harbor cells. Our data demonstrates that the ability of RAC1 to induce alterations in nuclear morphology is independent on its role in gene regulation (Figure 2a, 2b and Supplementary Figure 2).

2. The data presented in Figure 4b regarding nuclear deformation is difficult to evaluate. It is unclear whether the assay followed the nuclear variations of the same group of nuclei during the 3 hr time course. If not I do not see the need of performing a time course analysis. In any case, could these results be quantitated providing statistical significance?

Agree
The assay followed the nuclear variations of the same group of nuclei during the 3 hr time course. We analyzed the effects of the expression of KASH dominant-negative mutant on the dynamics of nuclear alteration parameters: nuclear area, perimeter and roundness, monitored by time-lapse microscopy in A375p cells during 3 hours. We have included a quantitation of nuclear alteration parameters showing changes relative to the control and performed statistical analysis (Figure 4, right panels).
Reviewer #2

Although this is not necessary, is it possible that cell migrating rates are measured during moving through constrictions, for example, a dense collagen matrix? Or is it possible to measure changes in the stiffness or flexibility of nuclei of cells expressing CA-Rac1?

Agree.
We now provide a live confocal imaging time lapse showing that RAC1QL induces invasion of A375p cells. We performed a transwell invasion assays of A375p cells expressing pEGFP RAC1Q61L for 24 hours. We analyzed changes in the flexibility of nuclei of cells expressing RAC1QL and we found that nuclear deformation correlated with greater invasive capacity through pore membrane (Supplementary Figure 3 and Video).

In addition, the manuscript should be edited according to the instruction for authors (particularly references) and also English should be edited.

Agree. The manuscript have been edited according to the instruction for authors, we have included references according MBoC EndNote style.

Minor concerns:
1) Antibodies and plasmid constructs used in the experiments should be represented in 'Materials and Methods'. Agree, we included cell lines, antibodies, and plasmid constructs in Materials and Methods.
2) Page 8, the last sentence, (Fig. 3d) is wrong. Corrected
3) In Fig. 4, photos of cells appear to be a bit out of focus. Corrected
4) I think there are some misspellings. for example, Corrected depolimerization (page 8)---depolymerization. Corrected hypothesized (page 10)---hypothesized. Corrected intriguingly (page 12)---intriguingly. Corrected
RE: Manuscript #E20-02-0127R
TITLE: “RAC1 induces nuclear alterations through the LINC complex to enhance melanoma invasiveness”

Dear Dr. Casar:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell.

With kind regards,

Peter Van Haastert
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Casar:

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.

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