EML4—ALK V3 oncogenic fusion proteins promote microtubule stabilization and accelerated migration through NEK9 and NEK7

Laura O'Regan, Giancarlo Barone, Rozita Adib, Chang Gok Woo, Hui Jeong Jeong, Emily L. Richardson, Mark W. Richards, Patricia A. J. Muller, Spencer J. Collis, Dean A. Fennell, Jene Choi, Richard Bayliss and Andrew M. Fry
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Original submission

First decision letter

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MS TITLE: The EML4-ALK V3 oncogenic fusion protein promotes microtubule stabilisation and accelerated migration through NEK9 and NEK7 kinases

AUTHORS: Laura O'Regan, Giancarlo Barone, Rozita Adib, Chang Gok Woo, Emily L Richardson, Mark W Richards, Patricia A J Muller, Spencer J Collis, Dean Fennell, Jene Choi, Richard Bayliss, and Andrew M Fry
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 reviewer gave favourable reports 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.

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 reviewer 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

Different EML4-ALK oncogenic fusion variants exist in NSCLC, which are associated with different patient outcomes. However, the role of the different variants is unclear. Here the authors demonstrate that variants associated with increased metastatic potential, V3 and V5 increase microtubule stabilisation and lead to the formation of cytoplasmic protrusions and increased cell migration. Similar phenotypes are observed upon overexpression of activated Nek9, suggesting a common mechanism. Indeed, both EML-ALK and activated Nek9 mediated protrusions and cell migration require Nek7. This seems to be relevant for tumours since cancer cell lines overexpressing the EML4-ALK v3/v5 show similar phenotypes. Interestingly Nek9 overexpression in NSCLC is mostly associated with v3/v5 and is a predictor of poor prognosis.

While the mechanism by which microtubule stabilisation leads to morphological alterations is still unclear, the authors convincingly show a role of Nek9 and Nek7 kinases in this process. Furthermore, they also showed that this signalling cascade also occurs downstream of the EML4-ALK oncogenic fusion, suggesting a role in cancer.

Comments for the author

Overall, the experiments performed are appropriate and conclusions are clear. This work will certainly be of interest to the cell biology community and therefore I recommend publication at Journal of Cell Science. There are however few minor issues the authors should address/clarify prior to publication:

Figure 1I: in the images provided microtubules inside protrusions appear “reticulocyte-like”. This is somewhat surprising as I would expect them to be organised in a more parallel manner. In fact, this is what the authors see in Figures 3G and 5B where bundles of parallel microtubules seem to be inside protrusions. Is this special organisation due to deltaRCC1 Nek9 mutant overexpression?

Figure 2B: there is a mistake in graphic legend of 2B.

Figure 2D: Images seem to be of low resolution and are difficult to see.

Figure 2H: can the authors chose a better description of y axis? Cell migration, which is used throughout this manuscript, is not very specific and it is unclear what the authors are measuring.

Figure 3: is there a specific reason the authors overexpressed Nek7 in HeLa cells as opposed to U2OS cells used throughout the manuscript?

Figure 3G: missing scale bar. Images do not look the same size.

Figure 6D: did the authors test also if Nek9 interacts or not with V2?

First revision

Author response to reviewers' comments

We thank the referee for noting the interest of this study to the cell biology community and recommending it as suitable for publication in the Journal of Cell Science. We have added points of clarification as requested.

There are however few minor issues the authors should address/clarify prior to publication:
Figure 1I: in the images provided microtubules inside protrusions appear “reticulocyte-like”. This is somewhat surprising as I would expect them to be organised in a more parallel manner.

In fact, this is what the authors see in Figures 3G and 5B where bundles of parallel microtubules seem to be inside protrusions. Is this special organisation due to deltaRCC1 Nek9 mutant overexpression?

The organization of microtubules inside the protrusions in this image is unusual and we have not yet been able to determine whether this organization is significant or an artefact of the fixation used for phalloidin staining or the post processing performed on these images. These images are intended purely to demonstrate the presence of both actin and α-tubulin in protrusions and not to infer any specific details of the organization of these networks at this juncture.

Figure 2B: there is a mistake in graphic legend of 2B.

We have corrected the error in the legend of figure 2B.

Figure 2D: Images seem to be of low resolution and are difficult to see.

The panels presented in this figure have been replaced with higher magnification images to more clearly illustrate the difference in track length and persistence of movement between control and NEK9-ΔRCC1 expressing cells.

Figure 2H: can the authors chose a better description of y axis? Cell migration, which is used throughout this manuscript, is not very specific and it is unclear what the authors are measuring.

We have changed the description of the y axis to cell migration index, which is the convention used in the literature for such experiments. For clarity, we have also added a description of how this index is calculated into the Materials and Methods section of the manuscript.

Figure 3: is there a specific reason the authors overexpressed Nek7 in HeLa cells as opposed to U2OS cells used throughout the manuscript?

HeLa cells were used to express the Nek7 proteins as this was the cell line in which we were able to generate inducible expression of these proteins. Whilst consistency in cell lines would have been ideal, we feel that the fact that activation of this pathway promotes similar phenotypes in a range of cell lines adds weight to our conclusion that this pathway is important in promoting cell migration.

Figure 3G: missing scale bar. Images do not look the same size.

We have added scale bars to both the upper and lower panels in this figure as the referee is correct that these are shown at slightly different magnification.

Figure 6D: did the authors test also if Nek9 interacts or not with V2?

We have tested the interaction of NEK9 with EML4-ALK v2 but have been unable to produce conclusive data on this point, perhaps because the large size of this protein makes it difficult to work with in such experiments. However, given their relative levels of incidence, we feel that V1 and V3 are the most clinically relevant proteins and so have not pursued V2 further.

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.