Neural crest cells bulldoze through the microenvironment using Aquaporin-1 to stabilize filopodia

Rebecca McLennan, Mary C. McKinney, Jessica M. Teddy, Jason A. Morrison, Jennifer C. Kasemeier-Kulesa, Dennis A. Ridenour, Craig A. Manthe, Rasa Giniunaite, Martin Robinson, Ruth E. Baker, Philip K. Maini and Paul M. Kulesa
DOI: 10.1242/dev.185231

Editor: Stephen Wilson

Review timeline

Original submission:	2 October 2019
Editorial decision:	12 November 2019
First revision received:	26 November 2019
Accepted:	3 December 2019

Original submission

First decision letter

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MS TITLE: Neural crest cells bulldoze through the microenvironment using Aquaporin-1 to stabilize filopodia

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I apologise for the amount of time it took to be able to make a decision on your manuscript, especially given that I suggested we might be able to assess it quickly. I consulted one of our editorial board members who suggested we should send it out to review and I have now received two referees reports. The referees' comments are appended below, or you can access them online: please go to BenchPressand click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, the two referees are enthusiastic about your manuscript but one has some significant issues that she/he would like you to resolve before publication. Please attend to these issues in your revised manuscript and detail them in your point-by-point response. If you do not agree with any of their criticisms or suggestions explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

The authors show a novel role for the water transporter, Aquaporin-1 (AQ1) in the regulation of neural crest migration during early development. While its function in regulating the migratory ability of cells in other contexts, this developmental study allowed for a deeper elucidation of its mechanistic function. The authors show an expression pattern that is localised to the first row of the migrating neural crest cell cluster- corresponding to the leader cells. They further demonstrate an interaction with focal adhesions, integrin localisation, ECM degradation and Eph receptor

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expression. Together, the suggest a mechanism by which AQ1 promotes protrusion stability and hence help cells 'bulldoze' through their environment to promote cell migration.

Comments for the author

This manuscript has been thoroughly peer-reviewed elsewhere, and I am satisfied with the response to the previous reviewer comments. The results are well presented, analysed and the conclusions are drawn from a convincing series of results. I would recommend acceptance of this manuscript in Development.

Reviewer 2

Advance summary and potential significance to field

McLennan and colleagues present an analysis of aquaporin's role in neural crest migration. Through an analysis of AQP-1 expression, gain and loss of function experiments in vitro and in vivo, the authors establish roles for aquaporin in neural crest migration. The authors provide evidence that manipulating AQP-1 alters focal adhesions and MMP secretion, and that cells expressing AQP-1 have higher levels of ephB receptors.
The manuscript is comprehensive and data for the most part well-documented. The authors make a convincing case for a new regulator of nc cell migration, and findings are likely to be of general interest.

Comments for the author

It is not clear how many explants were used for cell migration analysis presented in figures 2C,D. As it is not at all clear that the measurements of each cell are independent of its neighbors (given the influences of nc cells on each other), data needs to be from multiple explants. This is particularly important given the variability between control and dmso control conditions. Similarly, the number of explants analyzed in Fig 6J is needed for straightness measurements. Why are speed measurements not shown? Why does AQP-1 FL have the opposite effect on straightness when BA2 is there (comparing Fig 2D to 6J)?

Sample sizes for filopodial analysis (fig 4) should be reported. Statistical tests should be ANOVA with posthoc comparison where there are more than two conditions, to account for multiple testing error, here and elsewhere.

I think the wrong N was used for analysis in Fig 5G - it should be the number of replicates (3), not the number of images taken.

First revision

Author response to reviewers' comments

Reviewer 2 Comments for the author
It is not clear how many explants were used for cell migration analysis presented in figures 2C,D. As it is not at all clear that the measurements of each cell are independent of its neighbors (given the influences of nc cells on each other), data needs to be from multiple explants. This is particularly important given the variability between control and dmso control conditions. Similarly, the number of explants analyzed in Fig 6J is needed for straightness measurements. Why are speed measurements not shown?

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>Response- We appreciate the reviewers comment and have added into the manuscript the number of neural tube explants that were used in the analysis. The number of neural tube explants used ranged from 4 to 8 cultures. We also double checked all of the in vitro neural tube explants analyzed and set our criteria more stringently to only include data sets where experimental and control cultures were timelapsed concurrently. This removed some of our initial data that was collected when our microscope capabilities did not allow multiposition acquisition. We did not think it was relevant to show the speed differences for this experiment as in this figure we are linking AQP-1 to guidance, not speed. However, we did go back and look at our data and in the presence of ba2 tissue, control cells became just as fast as the AQP-1 overexpressing neural crest cells. We think this may be from other unknown migratory factors coming from the BA2 which is speeding up the control cells as well as the AQP-1 cells and further investigation would be outside the scope of this paper.
Why does AQP-1 FL have the opposite effect on straightness when BA2 is there (comparing Fig 2D to 6J)?
>Response- Although on the graphs it does look like AQP-1 FL has opposite effects on straightness in the presence of BA2, the straightness values for AQP-1 FL cells are 0.32 and 0.27, with and without BA2 respectively. The dramatic change in the graphs is the straightness in control cells, which is 0.46 and 0.16, with and without BA2 respectively. Therefore, the presence of BA2 tissue substantially increases straightness for control cells but only mildly increases straightness for AQP-1 FL, suggesting that the typical chemoattractive response cells have towards BA2 is not as strong when AQP-1 is overexpressed.
Sample sizes for filopodial analysis (fig 4) should be reported. Statistical tests should be ANOVA with posthoc comparison where there are more than two conditions, to account for multiple testing error, here and elsewhere.
>Response- We appreciate the guidance from the reviewer and have added the number of cells and embryos to the materials and methods section for the filopodia analysis and performed an ANOVA with posthoc on the filopodia statistics. The ANOVA did not change which measurements were statistically different but did change the p values; the figures have been updated accordingly.
I think the wrong N was used for analysis in Fig 5G - it should be the number of replicates (3), not the number of images taken.
>Response- We have 3 replicates of each of the conditions in this experiment and then for the analysis, 5 random areas of each replicate were imaged and used. We have clarified this in the figure legend.

Second decision letter

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ARTICLE TYPE: Research Article

I have looked over your revisions and I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.

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