Journal of Cell Science

Arih2 regulates hedgehog signaling through smoothened ubiquitylation and ER-associated degradation

Bo Lv, Xiao-Ou Zhang and Gregory J. Pazour
DOI: 10.1242/jcs.260299

Editor: Guangshuo Ou

Review timeline
Original submission: 1 June 2022
Editorial decision: 11 July 2022
First revision received: 14 July 2022
Accepted: 18 July 2022

Original submission

First decision letter

MS ID#: JOCES/2022/260299

MS TITLE: Arih2 Regulates Hedgehog Signaling Through Smoothened Ubiquitination and ER-Associated Degradation

AUTHORS: Bo Lv, Xiao-Ou Zhang, and Gregory J Pazour
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 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, depending on further comments from reviewers.

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 manuscript from Lv, et al “Arih2 Regulates Hedgehog Signaling Through Smoothened Ubiquitination and ER-Associated Degradation” is a refreshingly well written body of work with very nice, clear data that makes a simple but compelling argument for the role of Arih2Β in Smo processing and ciliary enrichment, resulting in basal activation of the SHH pathway. This work is a logical extension of their earlier work (Lv, et al 2021) in which Arih2 emerged from a screen of Ub

ligases with roles in cilia or ciliary proteins. The focus here turns specifically to the lesser studied variant, Arih2β, and a strong case is made for it (and not Arih2α) playing an important role in regulating the cellular levels of Smo, while acting at the ER. The data are strong, clear, and convincing.

Comments for the author

I find this work particularly interesting in that it highlights the potential for proteins active at the ER to be regulating aspects of ciliary biology as this is not something that is commonly considered but is likely quite important and where likely much more work is needed. The penultimate paragraph in the Discussion raises a number of points along these lines that if anything perhaps might be slightly expanded upon, simply to better highlight some of the potential impacts from this study. The authors focus here on the fact that simply increasing expression of Smo in cells has been found to be sufficient to increase its levels in cilia, presumably by overwhelming the export process. But as the authors point out, they see not only increased ciliary Smo in Arih2-/- cells but also increased basal SHH signaling (Fig. 1A) in Gli1 mRNA levels. This clearly points to a potential rate-limiting (or more accurately abundance-limiting) step in SHH pathway activation occurring at the ER. And begs the question how the SHH ligand and Ptch may propagate a signal that gets to the ER. Or are the authors suggesting that there are two separate limiting steps, one at the ER involving Arih2 and one at cilia that involves regulated import and activation? I find these fascinating questions to ponder and would be interested if the authors are interested in expanding a bit in the Discussion on such points. But I would not make it a requirement for publication as I believe the work stands on its own.

Reviewer 2

Advance summary and potential significance to field

This work describes a novel element of the hedgehog signaling pathway that emerged in an unbiased crispr screen performed by the authors. The authors show that Arih2β functions in the endoplasmic reticulum as a Ubiquitin transferase for smoothened and potentially other proteins. The discovery of Arih2β function adds a new layer of regulation to the hedgehog pathway, quality control in the ER, and also helps explain some Arih2β deficiency phenotypes observed in human patients.

Comments for the author

Its rare that I have no substantial comments for a paper. I found this paper to be easy to read and understand and also that it conveyed an important finding and provided sufficient supporting evidence to back up the author's conclusions. There are always additional experiments that could be performed (additional, more direct approaches to Arih2β ER localization and smo Arih2β interaction, and some in vivo evidence for Arih2β function in the hh pathway) but I think the paper as it stands presents an interesting finding that will have significant impact on the field.

First revision

Author response to reviewers' comments

Point By Point

Reviewer 1 Advance summary and potential significance to field

The manuscript from Lv, et al “Arih2 Regulates Hedgehog Signaling Through Smoothened Ubiquitination and ER-Associated Degradation” is a refreshingly well written body of work with very nice, clear data that makes a simple but compelling argument for the role of Arih2β in Smo processing and ciliary enrichment, resulting in basal activation of the SHH pathway. This work is a

logical extension of their earlier work (Lv, et al 2021) in which Arih2 emerged from a screen of Ub ligases with roles in cilia or ciliary proteins. The focus here turns specifically to the lesser studied variant, Arih2β, and a strong case is made for it (and not Arih2α) playing an important role in regulating the cellular levels of Smo, while acting at the ER. The data are strong, clear, and convincing.

Reviewer 1 Comments for the author

I find this work particularly interesting in that it highlights the potential for proteins active at the ER to be regulating aspects of ciliary biology as this is not something that is commonly considered but is likely quite important and where likely much more work is needed. The penultimate paragraph in the Discussion raises a number of points along these lines that if anything perhaps might be slightly expanded upon, simply to better highlight some of the potential impacts from this study. The authors focus here on the fact that simply increasing expression of Smo in cells has been found to be sufficient to increase its levels in cilia, presumably by overwhelming the export process. But as the authors point out, they see not only increased ciliary Smo in Arih2-/- cells but also increased basal SHH signaling (Fig. 1A) in Gli1 mRNA levels. This clearly points to a potential rate-limiting (or more accurately abundance-limiting) step in SHH pathway activation occurring at the ER. And begs the question how the SHH ligand and Ptch may propagate a signal that gets to the ER. Or are the authors suggesting that there are two separate limiting steps, one at the ER involving Arih2 and one at cilia that involves regulated import and activation? I find these fascinating questions to ponder and would be interested if the authors are interested in expanding a bit in the Discussion on such points. But I would not make it a requirement for publication as I believe the work stands on its own.

Our response:
We greatly appreciate your positive assessment of our work! In response to your request for more discussion about the mechanism driving increased basal expression of the pathway when Arih2 is absent. Our data suggests a role for Arih2 in regulating cellular levels of Smo rather than being directly involved in the signal transduction cascade. We added the following sentence to the discussion to clarify this point.
"It is likely that Arih2 is involved in the production of Smo and is not directly involved in the Hedgehog signal transduction cascade."

Reviewer 2 Advance summary and potential significance to field

This work describes a novel element of the hedgehog signaling pathway that emerged in an unbiased crispr screen performed by the authors. The authors show that Arih2β functions in the endoplasmic reticulum as a Ubiquitin transferase for smoothened and potentially other proteins. The discovery of Arih2β function adds a new layer of regulation to the hedgehog pathway, quality control in the ER, and also helps explain some Arih2β deficiency phenotypes observed in human patients.

Reviewer 2 Comments for the author

Its rare that I have no substantial comments for a paper. I found this paper to be easy to read and understand and also that it conveyed an important finding and provided sufficient supporting evidence to back up the author's conclusions. There are always additional experiments that could be performed (additional, more direct approaches to Arih2β ER localization and smo Arih2β interaction, and some in vivo evidence for Arih2β function in the hh pathway) but I think the paper as it stands presents an interesting finding that will have significant impact on the field.

Our response:
Thank you! We appreciate your efforts on behalf of our work.

Second decision letter

MS ID#: JOCES/2022/260299

MS TITLE: Arih2 Regulates Hedgehog Signaling Through Smoothened Ubiquitination and ER-Associated Degradation

AUTHORS: Bo Lv, Xiao-Ou Zhang, and Gregory J Pazour
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.