

Differentiation of ciliated human midbrain-derived LUHMES neurons

Gilbert Lauter, Andrea Coschiera, Masahito Yoshihara, Debora Sugiama-Trapman, Sini Ezer, Shalini Sethurathinam, Shintaro Katayama, Juha Kere and Peter Swoboda
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Editor: Giampietro Schiavo

Review timeline

Original submission:	4 June 2020
Editorial decision:	24 July 2020
First revision received:	31 August 2020
Editorial decision:	28 September 2020
Second revision received:	30 September 2020
Accepted:	5 October 2020

Original submission

First decision letter

MS ID#: JOCES/2020/249789

MS TITLE: Differentiation of ciliated human midbrain-derived LUHMES neurons

AUTHORS: Gilbert Lauter, Andrea Coschiera, Masahito Yoshihara, Debora Sugiama-Trapman, Sini Ezer, Shalini Sethurathinam, Shintaro Katayama, Juha Kere, and Peter Swoboda
ARTICLE TYPE: Tools and Resources

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 raise a number of substantial criticisms that prevent me from accepting the paper at this stage. I believe, however, that a revised version might prove acceptable, if you can address their concerns. Indeed, primary cilia analysis would be enabled with an unprecedented level of detail using the experimental system described in your manuscript, making this work significant in the field of neural development.

If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

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 by Lauter et al. presents data on the thorough characterization of cilia and ciliogenesis in the LUHMES neuronal cell line. This study is going to be very useful for investigators studying cilia biology in neuronal development and disease. The experiments are performed well, the data are presented clearly with appropriate statistical analyses and the text is easy to follow. Overall, this paper is appropriate for the resource section of JCS and represents a valuable contribution to the burgeoning cilia field.

Comments for the author

None.

Reviewer 2

Advance summary and potential significance to field

This manuscript by Lauter et al presents the establishment of a human neuronal cell model as a tool to decipher the function of primary cilia in neural/neuronal development and function. Given the importance of these organelles in brain phenotypes, this is a very nice paper that offers a novel entry point to delineate the molecular mechanisms underlying developmental brain disorders, and it is definitely worth publishing in Journal of Cell Science. In general, the manuscript is well-written and the results shown are robust and largely consistent with the interpretation proposed by the authors. I only have a couple of remarks concerning a subset of experimental procedures and data description.

Comments for the author

One remark relates to ciliation during differentiation for which it would be highly valuable to show the presence of primary cilia during the initial steps of neurogenesis from their pluripotent stage. This could be addressed using markers such as SOX2, OCT3/4, PAX6 and TBR2/NEUROD combined with some quantification on ciliation frequency. Such data would complement the gene expression profiles. On a note, lines 127-129 in the first paragraph of the Results section state that “Immunofluorescence stainings revealed that ARL13B is expressed and specifically localized in LUHMES cells at the stage of cell proliferation (d0) as well as during differentiation into neurons (d1 to d6) (Figure 1A)”. This figure displays d0, d3 and d6. As per data presented in Fig. 3, which shows activatable HH signaling at d2 of differentiation, the authors conclude that primary cilia in LUHMES transmit HH signaling. This is somewhat overstated, since there are no data presented to show that signaling relies on ciliary formation.

Reviewer 3

Advance summary and potential significance to field

In the manuscript entitled “Differentiation of ciliated human midbrain-derived LUHMES neurons” Lauter et al. describe the presence of functional cilia on the undifferentiated and differentiated LUHMES neuronal cell line. They also provide a time course analysis of gene regulatory network during differentiation.

LUHMES cells were already described and characterised, thus the authors do not report a novel technique. Presenting data of quality, the study brings some advance on the molecular

characterisation of this cell line that might be of interest for those wishing to work with this model (68 entries on pubmed). However, it does not report sufficiently significant and novel elements that may convince to use this cell line as a model system for studying the involvement of cilia during neuronal differentiation.

Comments for the author

The fact that LHUMES cells present primary cilia has not been reported before. However, neuronal progenitors and neurons have been shown to bear primary cilia and these cilia respond to Shh. Thus, the finding on the LHUMES cells is not unexpected.

The gene-based differential analysis of undifferentiated and differentiated LUHMES cells complement already published study on LUHMES cells. It brings some advance on the characterisation of this cellular model that might be of interest for people using it. Ciliary genes are up-regulated as expected since comparing proliferative population (that mainly have cilia in G1 but short or no cilia during other phases of the cell cycle) with post-mitotic cells.

To have a significant impact, the study should present results on the functionality of the primary cilia. Do they affect migration, differentiation, proliferation or other processes known to depend on the primary cilia in vivo?

First revision

Author response to reviewers' comments

Stockholm, 31 August 2020

Re: JOCES/2020/249789 - response to first manuscript decision from 24 July 2020

Dear Giampietro Schiavo (Editor - J Cell Sci) / Dear anonymous reviewers,

Thank you very much for your work and for the constructive feedback and input on our manuscript (JOCES/2020/249789), which we had submitted to J Cell Sci back in June 2020, and of course also for the opportunity to re-submit a revised version of our manuscript. As you will see below, we have been able to address all the points raised by the reviewers. We have adjusted and updated our manuscript accordingly. There are textual changes as well as changes to figures and tables, which we hope will now make our work acceptable for publication in J Cell Sci. All the changes are clearly marked in the re-submitted manuscript files (in the text sections **IN RED**). Please find below point-by-point responses to the reviewers comments.

Reviewer 1

Advance Summary and Potential Significance to Field: The manuscript by Lauter et al. presents data on the thorough characterization of cilia and ciliogenesis in the LUHMES neuronal cell line. This study is going to be very useful for investigators studying cilia biology in neuronal development and disease. The experiments are performed well, the data are presented clearly with appropriate statistical analyses and the text is easy to follow. Overall, this paper is appropriate for the resource section of JCS and represents a valuable contribution to the burgeoning cilia field.

Reviewer 1 Comments for the Author: None.

Reviewer 2

Advance Summary and Potential Significance to Field: This manuscript by Lauter et al presents the establishment of a human neuronal cell model as a tool to decipher the function of primary cilia in neural/neuronal development and function. Given the importance of these organelles in brain

phenotypes, this is a very nice paper that offers a novel entry point to delineate the molecular mechanisms underlying developmental brain disorders, and it is definitely worth publishing in Journal of Cell Science. In general, the manuscript is well written and the results shown are robust and largely consistent with the interpretation proposed by the authors. I only have a couple of remarks concerning a subset of experimental procedures and data description.

Reviewer 2 Comments for the Author: One remark relates to ciliation during differentiation for which it would be highly valuable to show the presence of primary cilia during the initial steps of neurogenesis from their pluripotent stage. This could be addressed using markers such as SOX2, OCT3/4, PAX6 and TBR2/NEUROD combined with some quantification on ciliation frequency. Such data would complement the gene expression profiles.

We have carefully looked through the relevant literature on LUHMES cells and neurons (Lotharius et al 2005, Scholz et al 2011, Pierce et al 2018). For example, Scholz et al 2011 presents relevant mRNA expression data on OTX2, PAX6 and SOX2. Further, we have re-analyzed our own STRT RNA-seq transcriptomics time course data for how classical stem cell and neurogenic marker genes behave over time (d0 - d6). These markers are either not expressed or expressed at very, very low levels in our LUHMES neuronal cell model. We conclude that proliferating LUHMES cells (d0) are not pluripotent neural stem cells, but rather are “committed” cells in a (neuronal) progenitor-type cell state. By changing the culture medium this “commitment” can then - within one day - be turned into full post-mitotic neuronal differentiation. We have added to the manuscript the relevant text section (Materials and Methods, lines 468-475) and Supplementary Figure S6D, presenting these data and our conclusions.

We also have quantified ciliation frequency in our (unsynchronized) LUHMES neuronal cell culture model, both at the proliferation stage as well as during the first few days of neuronal differentiation. Ciliation percentages are around 20-25% during proliferation (d0) (roughly a quarter of cells is in G0 / G1 phase of the cell cycle, the major “ciliated” cell cycle stage). Ciliation percentages then quickly rise to well above 50% at d2 to d5 of neuronal differentiation. We have added to the manuscript the relevant text section (Results, lines 188-193) presenting these data.

On a note, lines 127-129 in the first paragraph of the Results section state that “Immunofluorescence stainings revealed that ARL13B is expressed and specifically localized in LUHMES cells at the stage of cell proliferation (d0) as well as during differentiation into neurons (d1 to d6) (Figure 1A)”. This figure displays d0, d3 and d6. As per data presented in Fig. 3, which shows activatable HH signaling at d2 of differentiation, the authors conclude that primary cilia in LUHMES transmit HH signaling. This is somewhat overstated, since there are no data presented to show that signaling relies on ciliary formation.

We agree with the reviewer that we have slightly overstated our results on cilia-dependent SHH signaling. We have in the meantime repeated and expanded on some of our SHH signaling work, and added these new results to both text and figure on the matter. Our manuscript now contains updated and re-phrased text sections on SHH signaling (Results, lines 150-163; Materials and Methods, lines 558-560) as well as an updated and expanded Figure 3.

Reviewer 3

Advance Summary and Potential Significance to Field: In the manuscript entitled “Differentiation of ciliated human midbrain-derived LUHMES neurons” Lauter et al. describe the presence of functional cilia on the undifferentiated and differentiated LUHMES neuronal cell line. They also provide a time course analysis of gene regulatory network during differentiation. LUHMES cells were already described and characterised, thus the authors do not report a novel technique.

We agree with the reviewer on this point. We present LUHMES as a novel * resource * for ciliary research in human neurons, a resource that is currently lacking; in particular, a cell culture-based resource with the time course precision and rapidity that the neuronal LUHMES cell model represents. Accordingly, we have submitted our manuscript for consideration in the J Cell Sci * Resources * section.

Presenting data of quality, the study brings some advance on the molecular characterisation of this cell line that might be of interest for those wishing to work with this model (68 entries on PubMed). However, it does not report sufficiently significant and novel elements that may convince to use this cell line as a model system for studying the involvement of cilia during neuronal differentiation.

Here we refer to our response to the last point that this reviewer has brought up.

Reviewer 3 Comments for the Author: The fact that LHUMES cells present primary cilia has not been reported before. However, neuronal progenitors and neurons have been shown to bear primary cilia and these cilia respond to Shh. Thus, the finding on the LHUMES cells is not unexpected.

Again, we agree with the reviewer on this point. We hope that the LUHMES neuronal cell model will be used as a “workhorse” for research on cilia and ciliary signaling in human neuronal progenitors and neurons, many of which are ciliated. In particular, a cell culture-based resource with the time course precision and rapidity that LUHMES represents is currently lacking.

The gene-based differential analysis of undifferentiated and differentiated LUHMES cells complement already published study on LUHMES cells. It brings some advance on the characterisation of this cellular model that might be of interest for people using it. Ciliary genes are up-regulated as expected since comparing proliferative population (that mainly have cilia in G1 but short or no cilia during other phases of the cell cycle) with post-mitotic cells.

Please see above for our response to reviewer 2, who also has brought up the issue of ciliation percentage and gene expression profiles over time (d0-d6).

To have a significant impact, the study should present results on the functionality of the primary cilia. Do they affect migration, differentiation, proliferation or other processes known to depend on the primary cilia in vivo?

To analyze and describe in detail the impact of primary cilia (signaling) on proliferation, differentiation or migration is - in our opinion - beyond the scope of this manuscript. Such type of work addressing these issues actually results in entire papers by themselves. Whereby, we cite some of this work carried out in the mouse and/or in mammalian cell culture: cilia and proliferation (A Pitaval et al, J Cell Biol 2010; MJ Ford et al, Dev Cell 2018); cilia and neuronal / axonal differentiation (J Ferent et al, Cell Rep 2019; J Guo et al, Dev Cell 2019); cilia and neuronal migration (J Stoufflet et al, BioRxiv Sept 2019; M Matsumoto et al, J Neurosci 2019).

We do agree with the reviewer on the general point. These issues are the reason why we have been setting up LUHMES, a ciliated human neuronal cell model. With LUHMES it is now possible to look at these issues in human neurons in the necessary detail and with great time course precision. As proof of principle for the usefulness of the LUHMES ciliated cell/neuron model we are happy to report that we have very recently generated evidence that LUHMES primary cilia strongly impact neuronal differentiation, specifically the timing and complexity of axon branching patterns. Obviously these aspects bear enormous importance for “follow- up” neuronal connectivity. We have added to the manuscript the relevant text sections (Results, lines 220-228; Discussion, lines 409-411) and Supplementary Figure S2, presenting these exciting new data and our conclusions.

Thank you very much for your considerations. We are looking forward to hearing back from you in due course. For all the authors I remain with kind regards.

Peter Swoboda, PhD, Associate Professor
Principal Investigator and Group Leader

Second decision letter

MS ID#: JOCES/2020/249789

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AUTHORS: Gilbert Lauter, Andrea Coschiera, Masahito Yoshihara, Debora Sugiaman-Trapman, Sini Ezer, Shalini Sethurathinam, Shintaro Katayama, Juha Kere, and Peter Swoboda

ARTICLE TYPE: Tools and Resources

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 one of them raised some final 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.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

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

In my view, all criticisms have been adequately addressed.

Comments for the author

None

Reviewer 2

Advance summary and potential significance to field

The authors have done their best to answer issues raised in the reviews. I was already positive about the first version, and the present manuscript is significantly improved and ready for publication, at least as far as I am concerned.

Comments for the author

The authors have done their best to answer issues raised in the reviews. I was already positive about the first version, and the present manuscript is significantly improved and ready for publication, at least as far as I am concerned.

Reviewer 3*Advance summary and potential significance to field*

My principle concern on the submitted manuscript by Lauter et al. was that the resource described by the authors is not a new resource. The added value of the description of the cilia in this system is not straight forward as the results are expected from in vivo studies.

However, the study confirms that this in vitro model indeed recapitulate part of the in vivo data. So in principle, it may be of interest for those working on in vitro models of neurogenesis and the enthusiasm of the two other reviewers suggests that it will. The presented data are of quality and present some advance on the molecular characterisation of the system. Thus, I am in favour of publication.

Comments for the author

I only have a few minor comments that should be addressed prior to publication:

-The two functions of the centriole as template for primary cilia and MTOC are not mutually exclusive as some microtubules are still organised at the level of centrosome even in ciliated cells (e.g. on subdistal appendages or in PCM).

-The cilium is required for the activation of the canonical SHH signalling cascade. However, organisms devoid of primary cilia in most tissue, such as Drosophila, present SHH signalling. So in principle SHH signalling may happen independently of cilia. Thus, in absence of experimental depletion of cilia, the conclusions of Figure 2 are overstated and should be re-phrased.

-Similarly, cilia are dynamic structure, growing and shrinking. So I am not sure that on fix cells the absence of Arl13b staining is a proof that the cells have never been ciliated. Thus, the conclusions of Figure S2 are also overstated and should be re-phrased.

Second revisionAuthor response to reviewers' comments

Stockholm, 29 September 2020

Re: JOCES/2020/249789 - response to second manuscript decision from 28 September 2020

Dear Giampietro Schiavo (Editor - J Cell Sci),

Thank you very much for your work and for the constructive feedback and input on our manuscript (JOCES/2020/249789), which we had submitted to J Cell Sci back in June 2020, and of course also for the opportunity to re-submit a re-revised version of our manuscript. As you will see below, we have been able to address all the points raised by the third reviewer. We have adjusted and updated our manuscript accordingly. There are textual changes, which we hope will now make our work acceptable for publication in J Cell Sci. All the new changes are clearly marked in the re-submitted manuscript files (in the text sections **IN BLUE**, while textual changes from the first round of revisions are still marked **IN RED**). Please find below point-by-point responses to the third reviewers comments.

Reviewer 1 Advance Summary and Potential Significance to Field:
In my view, all criticisms have been adequately addressed.

Reviewer 1 Comments for the Author:
None

Reviewer 2 Advance Summary and Potential Significance to Field:

The authors have done their best to answer issues raised in the reviews. I was already positive about the first version, and the present manuscript is significantly improved and ready for publication, at least as far as I am concerned.

Reviewer 2 Comments for the Author:

The authors have done their best to answer issues raised in the reviews. I was already positive about the first version, and the present manuscript is significantly improved and ready for publication, at least as far as I am concerned.

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My principle concern on the submitted manuscript by Lauter et al. was that the resource described by the authors is not a new resource. The added value of the description of the cilia in this system is not straight forward as the results are expected from in vivo studies. However, the study confirms that this in vitro model indeed recapitulate part of the in vivo data. So in principle, it may be of interest for those working on in vitro models of neurogenesis and the enthusiasm of the two other reviewers suggests that it will. The presented data are of quality and present some advance on the molecular characterisation of the system. Thus, I am in favour of publication.

Reviewer 3 Comments for the Author:

I only have a few minor comments that should be addressed prior to publication.

-The two functions of the centriole as template for primary cilia and MTOC are not mutually exclusive as some microtubules are still organised at the level of centrosome even in ciliated cells (e.g. on subdistal appendages or in PCM).

[We have adjusted the relevant text in the Introduction section \(lines 76-77\).](#)

-The cilium is required for the activation of the canonical SHH signalling cascade. However, organisms devoid of primary cilia in most tissue, such as Drosophila, present SHH signalling. So in principle SHH signalling may happen independently of cilia. Thus, in absence of experimental depletion of cilia, the conclusions of Figure 2 are overstated and should be re-phrased.

[We have adjusted relevant text in the Results section \(lines 150-165\), and in the legend to Figure 3 \(lines 1010- 1011\). We provide a new reference in support of this topic \(lines 760-762\).](#)

-Similarly, cilia are dynamic structure, growing and shrinking. So I am not sure that on fix cells the absence of Arl13b staining is a proof that the cells have never been ciliated. Thus, the conclusions of Figure S2 are also overstated and should be re-phrased.

[We have adjusted relevant text in both the Results \(lines 222-231\) and Discussion sections \(lines 413-414\), as well as in the legend to Figure S2 \(lines 51-56 in Supplementary Materials\).](#)

Thank you very much for your considerations. We are looking forward to hearing back from you in due course. For all the authors I remain with kind regards.

Peter Swoboda, PhD, Associate Professor
Principal Investigator and Group Leader

Third decision letter

MS ID#: JOCES/2020/249789

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AUTHORS: Gilbert Lauter, Andrea Coschiera, Masahito Yoshihara, Debora Sugiama-Trapman, Sini Ezer, Shalini Sethurathinam, Shintaro Katayama, Juha Kere, and Peter Swoboda
ARTICLE TYPE: Tools and Resources

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.