Human cells lacking CDC14A and CDC14B show differences in ciliogenesis but not in mitotic progression

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First decision letter

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MS TITLE: Human cells lacking CDC14A and CDC14B show differences in ciliogenesis but not in mitotic progression

AUTHORS: Elmar Schiebel, Patrick Partscht, and Borhan Uddin
ARTICLE TYPE: Short Report

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.

We are aware that you may be experiencing disruption to the normal running of your lab that makes experimental revisions challenging.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. Instead, please use yellow shading or different colour font to denote changes in your manuscript.

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

DC14 was originally described in budding yeast as a key cell cycle phosphatase essential for normal mitotic exit. Since the gene is conserved in higher eukaryotes, with two isoforms hCDC14A and hCDC14B in human cells, it was generally assumed that the human CDC14 proteins would have an equally critical role in the regulation of the human cell cycle. However, studies of hCDC14A and hCDC14B so far failed to demonstrate any key cell cycle roles for these proteins. Definite conclusions, though, have so far been hampered by technical concerns, e.g. potentially incomplete RNAi depletion efficiency. This carefully conducted study is therefore very important for the cell cycle field because it unambiguously shows that hCDC14A/B are not involved in the regulation of mammalian cell division but may have more specialised roles in DNA repair and regulation of ciliogenesis.

Comments for the author

For this study, Partscht and colleagues used human immortalised non-transformed retinal epithelial cells (RPE1). Partscht et al. demonstrate that these cells express hCDC14A and hCDC14B, but not the pseudogene hCDC14C. Having established that hCDC14C does not play a role in these cells, they then use RRPE1 cells with genomic deletions in hCDC14A, hCDC14B or both for further analysis of cell cycle progression. By all criteria (appearance in live cell imaging, duration of different phases of the cell cycle proliferation over several days) the loss of hCDC14A or B or both did not seem to make any difference.
Because the author's own data and studies in zebrafish had suggested a role for CDC14 proteins in the control of ciliogenesis, Partscht and colleagues then focus on the characterisation of ciliogenesis in the absence of the human CDC14 proteins. In the absence of hCDC14A cilia length seemed to be increased whereas cell lacking hCDC14B seemed to initiate ciliogenesis more readily suggesting that the human CDC14 protein may be important regulators of ciliogenesis although the molecular biology underpinning this functionality is currently not clear.
The study is on the whole well conducted. However, to make this report fully convincing it would be good to have more characterisation of the cell lines being used included in the main figures, minimally a Western blot demonstrating that in the hCDC14A, hCDC14B and hCDC14A/B KO cell lines the respective hCDC14 proteins are indeed lacking.

Minor comments:
The authors state: "In addition, depletion of CDC14A and CDC14B in zebrafish suggests a role in ciliogenesis." A reference should be added to this statement.

Reviewer 2

Advance summary and potential significance to field

This short and informative paper uses knock out approaches to address the function of the CDC14 family phosphates in the most widely studied model for cell cycle studies in human cell biology, RPE1 cells. The study of phosphatases is a major focus for the field at present. Insight into phosphatase function has lagged far behind the cataloguing of the impact, function and regulation of the kinases, however, with the ability to score clear phenotypes and the application of proteomics, studies in cell cycle are now shedding an entirely new light on phosphatase biology. This paper marks an important part of this emerging jigsaw puzzle as it categorically shows an absence of expression of CDC14C in the widely used RPE1 cell line and that CDC14A and CDC14B have no impact upon cell cycle progression, but do influence the frequency and length of primary cilia. As such these are important advances and merit publication in Journal of Cell Science.

Comments for the author

I have no suggestions for modifications to the manuscript beyond my surprise that the authors did not discuss the contributions of Cdk10-Cyclin M to the regulation of the re-absorption of the primary cilium. As the budding yeast Cdc14 is intimately connected to the regulation of Cdk1

substrates, some similar coupling between this other Cdk family member and these phosphatases may merit further investigation. Perhaps the impact of the loss of Cdk10-Cyclin M is exacerbated by CDC14AB knock outs?

First revision

Author response to reviewers' comments

Thank you very much indeed for the valuable suggestions which we all addressed as outlined below.

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We previously have shown that the genomic *hCDC14A* KO construct in RPE1 cells leads to expression of a phosphatase dead version of the hCdc14A protein (Chen et al. PNAS, 2016). The *hCDC14B* KO cell line was constructed by insertion of a loxP flanked neomycin resistant gene. The neomycin gene was subsequently removed by Cre recombinase. This leaves the loxP insertion in the central exon 4 of the human *CDC14B* gene. In addition, the insertion construct contains stop codons in all three open reading frames disrupting expression of *hCDC14B* (Uddin et al. BMC Genomics 2015). The *hCDC14B* KO cell line was confirmed by Southern blot analysis, RT-PCR and sequencing of the genomic *hCDC14B* locus. Thus, by all scientific standards the *hCDC14B* locus is disrupted and therefore the encoded gene is not expressed. Because of the lack of hCDC14B antibodies that detect the endogenous hCDC14B protein (a general problem in the field (see below - we tried to raise anti-Cdc14B antibodies multiple times but were unable to detect with these antibodies the endogenous hCDC14B) we are unable to confirm this result by immunoblotting (although all other data indicate the lack of hCDC14B). The lack of suitable

hCDC14B antibodies is also the reason why in all papers on the functional analysis of *hCDC14B* based on siRNA depletion hCDC14B protein levels were not analyzed (please see a recently published paper in Nat Comm (Dietachmayt et al, 2020 as one of many examples).
We apologies that we have not described these cell lines in greater detail in our original submission. We now describe on page 7/8 in Materials and Methods how these cell lines were constructed and tested: "RPE1 *hCDC14B*, *hCDC14A* single KO and *hCDC14A/B* KO cell lines were generated by zinc finger nuclease-based approaches and confirmed by Southern blotting, RT-PCR and sequencing previously (Chen et al., 2017; Chen et al., 2016; Uddin et al., 2015). In brief, exon 9 of *hCDC14A* was targeted to generate a deletion of 77 amino acids (203-279 aa) around the catalytic cysteine. *hCDC14B* KO cells were generated by Cre-recombinase mediated removal of the selection marker leading to incorporation of pre-mature stop codons in all three frames immediately followed by a single loxP site (34 bp) at exon 4 upstream of the catalytic site. *hCDC14A* knockout was carried out on top of Cre-infected *CDC14B* KO cells to obtain double knockout cells."

Minor comments:
The authors state: "In addition, depletion of CDC14A and CDC14B in zebrafish suggests a role in ciliogenesis." A reference should be added to this statement.

The reference was added accordingly: "In addition, depletion of CDC14A and CDC14B in zebrafish suggests a role in ciliogenesis (Clement et al., 2011; Clement et al., 2012).".

Reviewer 2 Advance Summary and Potential Significance to Field...

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Thank you very much for pointing this out. We have included this interesting point in our discussion (see on page 7): "Furthermore, the CDK10/cyclin M protein kinase, whose deficiency is linked to STAR syndrome, was reported to negatively regulate ciliogenesis (Guen et al., 2018; Guen et al., 2016). It is therefore conceivable that hCDC14A or hCDC14B also counteract members of the CDK kinase family in some process of ciliogenesis in interphase."

Second decision letter

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