Reviewer's report

Title: All-trans retinoic acid stimulates the secretion of TGF-β2 via the phospholipase C but not the adenylyl cyclase signaling pathway in retinal pigment epithelium cells

Version: 0 Date: 05 Sep 2018

Reviewer: Khrishen Cunnusamy

Reviewer's report:

Review of "Effect of U73122 and SQ22536 on all-trans retinoic acid in stimulating the secretion of TGF-β2 in human retinal pigment epithelium cells: a randomized controlled trial" manuscript.

This is an elegant and simple study which aims to investigate the difference between inhibitions of the phospholipase C versus inhibition of the adenylyl cyclase signaling pathways on the production of TGFβ2. In that respect, the study uses two compounds which differentially act on the respective pathways that may potentially regulate the production of TGFβ2. SQ22536 acts as an adenylyl cyclase inhibitor, while U73122 operates as a phospholipase C inhibitor, and a no treatment group complements the study. The use of RPE cells further reinforces the adequacy of this investigation. Based on the results of the dose response experiment, TGFβ2 expression appears to be regulated by phospholipase C and not by adenylyl cyclase expression. However, important questions such as ultrastructural changes induced by the modulating effect of TGFβ2 expression still need to be addressed. Accordingly, the authors of this manuscript should attempt to respond to the following points:

1. The author of the article should consider the use of an additional agent that inhibits exclusively the phospholipase C pathway to further validate the claim from the original findings with U73122.

2. The same should be attempted with SQ22536 as it pertains to the inhibition of adenylyl cyclase to see if similar results are obtained.

3. The author should further demonstrate that those agents are effectively inhibiting both of those pathways are indeed working as suggested.

4. In addition to inhibiting those pathways, the authors should also investigate the effects of inducing those pathways further on the amount of TGFβ2 produced.
5. Validate the results with TGFB2 ELISA kits which can are readily available from Ebioscience or R&D biolabs. Since the TGFB2 is secreted, it should be readily quantified by the ELISAs.

6. Last but not least, the main problem is that the study does not conclusively show that modulating the expression of TGFB2 has a beneficial effect on the incidence of myopia - either ultra structurally or functionally. This study is done in vivo but is not be functionally validated i.e. does modulation of TGFβ2 signaling affect the incidence of myopia.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable
Declaration of competing interests

Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests.

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal.