Reviewer’s report

Title: TRPV4 regulates migration and tube formation of human retinal capillary endothelial cells

Version: 0 Date: 12 Oct 2017

Reviewer: Haibo Wang

Reviewer's report:

Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Please overwrite this text when adding your comments to the authors.

This paper tested the effects of TRPV4-mediated Ca2+ influx in HRCEC migration and tube formation. Overall writing should be improved. I have listed my comments below regarding experimental designs and data presentation.

1. please insert page number.

2. Remove extra spaces between words and Comma (,) or period (.). Check throughout the text and fix the problems.

Abstract


4. Page 3, Lines 25-31: Please reframe these two sentences in the abstract: "And then we pretreated HRCECs with or without HC067047 and shRNA . By using fluorescence assay , migration and tube formation assay we verified TRPV4 function in HRCECs ."

5. Page 3, Lines 13-18: I recommend to move the sentence "It has been reported that TRPV4 is expressed in HRCECs and regulates Ca2+ entry" up to the front of the sentence "However, the function of TRPV4 in human retinal capillary endothelial cells (HRCECs) remains unknown.".

6. Page 3, Lines 46-49: please complete the sentence "TRPV4 is essential for HRCEC migration and tube formation , and maybe a potential therapeutic target". Did authors imply that TRPV4 may be a potential therapeutic target for retinal vascular diseases?
Introduction

7. Page 4, Line 45, change "the activation and migration of ECs is" into "the activation and migration of ECs are".

8. Page 5, Lines 1-4, remove the second "regulates".

9. Page 5, Line 9, change "involve" to "involved".

Materials and Methods

10. Please provide more information about "HRCECs", such as passages and where were they originally isolated, adults or fetus?

11. Please provide information (company, City, State or Country) for all the reagents used in this study.

12. Page 6, Lines 43-46, provide the concentrations of HC067047 and GSK101670A

13. For migration assay, are the transwells medium permeable? How did you control the FBS only staying in the lower chamber? What is the pore size of the transwell? Where were the migrated cells located?

14. There are many highlighted places (Yellow highlighting) throughout the text. Are there any specific reasons? Or just editing errors?

Results

15. Does the anti-TRPV antibody have good quality? To confirm TRPV protein expressed in HRCECs, did you have positive controls for TRPV?

16. Figure 2: how did the authors quantify intracellular Ca2+ concentration? By measuring fluorescence density? Fluorescence density can only be considered semi-quantification. Is there any other way to measure intracellular [Ca2+]?
17. Please provide the compared groups for the statistical significance, for example, *p<0.05 vs. Control.

18. Figure 4, "E treated with shRNA . F treated with shRNA and GSK", how did you treat cells with shRNA, by transfection or by viral vector delivery? Provide detail information in the Methods regarding shRNA to TRPV gene knockdown.

Discussion

Can the authors expand your discussion into angiogenic effects regulated by TRPV4 mediated Ca2+ influx?

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.

No

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:

Not suitable for publication unless extensively edited
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