Responses to the peer reviewers' comments

Title: Knockdown of RACK1 by Regulating PKC-ε/ROS Effectively Slows the Progression of Early Diabetic Retinopathy

Dear Editors,

Thank you very much for your letter inviting us to submit a revised version of the above-mentioned manuscript. We have revised the paper according to the Reviewer's suggestions. Attached please find the revised version of the paper, with highlighting where changes have been made in the revised manuscript.

Below are our specific responses to the reviewers' comments.

Reviewer 1

1. It is not clear to me why they use the ARPE-19 cells line, neither the selection of the proteins studied, since their previous work their mentioned was done in endothelial cells.
RE: Thanks for your advice. Retinal pigment epithelium (RPE) is the main component of the outer blood retinal barrier (oBRB), and leakage caused by BRB injury is a sign of DR. However, to date, research on DR has mostly focused on the internal retinal barrier, while research on the damage mechanism of the oBRB in diabetes is limited. This study investigates the mechanism of outer blood-retinal barrier disruption in diabetic retinopathy by studying ARPE-19 cells.

2. Methods." while 400 μM cobalt chloride (CoCl2) (Merck, Germany) was added to the cell culture medium for 24 h before experimentation", please explain the use of CoCl2. How did you induce hypoxia in your model.
RE: Thanks for your advice. CoCl2 primarily induces cellular hypoxia by activating hypoxia-inducible factors (HIF), offering advantages such as prolonged action duration and stable hypoxic conditions.
3. Section 2.7. Western blotting analysis, the section is redundant.
RE: Thanks for your advice. We have removed the excess parts, please refer to the revised article for details.
4. Fig 2, it is rather difficult to observe which authors indicate. It might be usefula an immunohistochemistry for RPE65. On the other hand the image does not contain a bar indicating the size. Did you observed any change in retina thickness? Also it would be appropriate an immunohistochemistry for PKC-ε and/or RACK1 Fig 3. Results are from neural retina not from RPE.
RE: Thanks for your advice. Figure 2 mainly consists of observations on the structure and cell count of the retinal pigment epithelium. Additionally, we observed that the retinal nerve fiber layer in the DM group of rats became thinner from the 4th to the 10th week compared to the NC group.

However, since study focuses on the retinal pigment epithelium, it was not explicitly mentioned in the article. We acknowledge that performing immunohistochemistry experiments would be an excellent suggestion, but the sections we obtained were only stained with hematoxylin and eosin (HE). Further experiments may be necessary to address this. The results presented in Figure 3 indeed originate from neural retinal epithelium, as a part of the retinal tissue, it still holds certain value for our subsequent research.

5. Fig 5 and 6. Levels of protein increased are very low, about 15% these are statistically significant but please discuss about the biological meaning. Authors should discuss about the mechanisms by which high levels of PKC lead to increase in ROS production.
RE: Thanks for your advice. We have included some additional content in the revised manuscript based on the suggestions you provided. Thank you again for your advice.

Sincerely yours,
Jian Tan

Editor-in-Chief

Dear Editors,

Thank you very much for your letter inviting us to submit a revised version of the above-mentioned manuscript. We have revised the paper according to the Editor-in-Chief's suggestions. Attached please find the revised version of the paper, with highlighting where changes have been made in the revised manuscript.

Below are our specific responses to the Editor-in-Chief's comments.

1. The title is not supported by the experiments, since the authors did not see any slowing the progression of diabetic retinopathy. Even the authors have used cell culture to investigate the mechanisms, which was very superficial. (1) how cell culture work links to the DR is unclear; (2) (Cell culture work was also only one time-point, how the authors make the conclusion: "slowing the progression of DR" Therefore, the title should be changed, "Diabetes and high-glucose could increase.

   RE: Thanks for your advice. This is our revised title. " Diabetes and high-glucose could upregulate the expression of receptor for activated C kinase 1 in retina."

2. Result section, "However, at weeks 8 and 10, a notable decrease in the number of RPE cells was observed in the diabetic group. Furthermore, a significant decrease in the number of RPE cells was observed in the diabetic group compared to weeks 8 and 10 ($P < 0.01$)." which should be very important for the authors make the conclusion, but these quantitative results were not shown to exhibit the decreased cell results.

   RE: Thanks for your advice. We have made modifications to Figure 2B and replaced it with a chart of our REP cell count. In addition, we have made slight modifications to this sentence, which now reads as follows: "However, at weeks 8 and 10, a notable decrease in the number of RPE cells was observed in the diabetic group. Furthermore, a significant decrease in the number of RPE cells was observed in the diabetic group compared to the normal control group at week 8 and 10weeks 8 and 10 ($P < 0.01$)." Thank you for pointing out our shortcomings.

3. The several gel profiles (only one sample for each group, which was not much increased comparing to corresponding control), but the graphic results were significantly increased or decreased, please shown gel profiles of 2 or 3 in each group, to show the small variation in such small.

   RE: Thanks for your advice. Based on your suggestions, we have made modifications to the gel image by including the sample sizes for each group. We would like to express our gratitude once again for your valuable feedback.