Author's response to reviews

Title: Effect of P21WAF1/CIP1 on retinal pigment epithelial cells and experimental proliferative vitreoretinopathy

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Author's response to reviews: see over
Dear Editor,

Thank you editor and reviewers for constructive comments and suggestions to this manuscript (MS: 3903436101358187). Some corrections or revisions were made according to your and reviewer’s comments. We’ll try our best to answer or explain all questions you and reviewers have presented. Below are our explanations on comments reviewer(s) presented.

Sincerely,
Ying Wang

Reviewer(s)' Comments to Author:
Reviewer: 1
Comments to the Author
The experiment was well designed and the English was written well. There was no statistical error.
There were some flaws in the paper.
1. In abstract, line 7, PRE cells should be RPE cells.

Reply: we are sorry for our incorrect writing. I have checked the grammars carefully and changed PRE cells to RPE cells.

2. The groups described were confused.
Reply: I have changed the name of groups in the figure 3 and figure 4 to make sure the groups are consistent.

3. Although the function of p21 was clear, which plays an important role in the regulation of cell cycle progression. An increase in p21 expression and other CDKIs results in an activation of suppression molecules of CDKs and cyclinE, allowing accumulation of hypophosphorylated Rb and cell cycle arrested in G1 phase, thereby inhibits cell proliferation. However, the signaling pathway was not clear. Maybe, it should be discussed in the discussion.

Reply: Thanks for your suggestions. I have added the reference and discussed according to the reviews’ suggestion.

Reviewer: 2
Comments to the Author
It is an interesting study; the authors show that P21 may play a role in the parthenogenesis of PVR. However, in order to get the manuscript to be published in the BMC ophthalmology, following issues has to be addressed:

Major points:
1. There is no evidence of the connection between P21 and PVR in the paper; at least the authors should show some result of P21 expression in human PVR membranes by
IHC.

Reply: Thanks for your constructive suggestions. Previous study reported that p21 showed low expression level in PRE-19 cells delivered from human RPE cells which are important in the parthenogenesis of PVR. I have rewrite and added references in this part. Moreover, our previous study also showed that the mRNA and protein expression of P21 in retina were decreased in experiment PVR model. We can offer the figures of WB and PCR results if necessary.

2. The major part of the research is the inhibition of experimental PVR in rabbit by P21 vector injection, but this part is too weak to support their conclusion. First, what kinds of RPE cells were used for the induction of PVR, human RPE or rabbit RPE? If it is human RPE cell, how did you take care of the immune response? Second, the authors did not give enough description of methods of the vitreous injecting; third, the authors should make a fig to show the outcome of experimental PVR after the vector injection and also include statistical analysis according the PVR classifications; Forth, we want to know which cell is the target cell by the P21 vector intra-vitreous injection, RPE, glia cell or others. Fifth, authors should indicate if there is any difference of the expressions of the major fibrosis factors (TGF-β and CTGF) after p21 vector application in the rabbit PVR model.

Reply: First, we used the human RPE cells to induction PVR. Immune response of human RPE cells leading to the inflammation is an important cause of PVR.

Second, considering the reviewer’s suggestion, I have added the methods of vitreous injection.

Third, as reviewer suggested that I have added a fig and table to show the outcome of PVR and statistical analysis.

Forth, the main target cell is RPE. Our result in vitro also suggested that P 21 inhibit the proliferation and migration of RPE cells.

Fifth, it is really true as reviewer suggested that the fibrosis factors also play the key words in the progression of PVR. It’s a pity that we didn’t test the expression of the fibrosis factor in this study because of the experimental design. The fibrosis factors may be the further target in the future study.

Minor:
1. In the introduction, the parthenogenesis of PVR should be briefly reviewed and then indicated what is the significant of your study.

Reply: Thanks for your constructive suggestions. I have introduced the parthenogenesis of PVR briefly.
2. How the density of the western blot was measured?

*Reply:* Protein bands were quantified by ImageJ software. I have added the method into the manuscript.

3. Why the migration was lasted for 24 hours, regularly it only takes 4-6 hours.

*Reply:* Thank you for pointing out the mistake to me. We are very sorry for our negligence of it. The cells were starved for 24 hours and migrated for 6 hours. I have changed the mistake in manuscript.

4. The writing of the manuscript needs to be re-written, check the grammars carefully and try to use Standard English.

*Reply:* I am sorry for our English writing. I have asked a native English speaking colleague to help me copyedit the paper according to the reviewers’ suggestion.

We appreciate for editors and reviewers’ work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions.