Author’s response to reviews

Title: Effects of concentration of amyloid β (Aβ) on viability of cultured retinal pigment epithelial cells

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Author’s response to reviews:

Stephan Hoffmann, MD, Ph.D
BMC Ophthalmology

Re: Ms. Ref. No.: BOPH-D-18-00556

October 23, 2018

Dear Dr. Hoffmann,

Thank you for your letter and comments and suggestions on our manuscript (BOPH-D-18-00556). We have revised our manuscript according to the comments of the reviewers.

RESPONSES and CHANGES (Changes are indicated in RED in the REVISED Manuscript (Word file):

Answers to Reviewer 1

“A good rationale for the choice of aB1-40 instead of 1-42 in the reported experiments has not been established. It is accepted that the 1-42 is more associated with disease, although 1-40 is more commonly distributed. Furthermore, the structures of the 2 forms are very different. As such, it is expected that the 1-42 form will be used in these experiments. Alternatively, the 2
different forms (1-40 and 1-42) need to be compared. These would have provided internal controls.”

Answer: We agree that the 1-42 form is an important form to investigate for understanding the pathogenesis of amyloid β-related diseases. But in diseased eyes, especially AMD, we believe that the 1-40 form is also very important. For example, Liu et al. demonstrated that NF-κB/RelA activation was enhanced in RPE cells after the stimulation of Aβ1-40 (Exp Eye Res. 2014 Oct;127:49-58). In addition, Sun et al. showed that intravitreally injected Aβ1-40 mice develop AMD-like pathologic changes (Cell Death Dis. 2017 Oct 12;8(10): e3115.). These findings suggested that Aβ1-40 plays important roles in the pathogenesis of AMD. As we mentioned, we believe that high concentrations of Aβ are important for the pathogenesis of AMD. Compared to other forms of Aβ, the concentration of Aβ1-40 is the highest among the different forms of Aβ (Invest Ophthalmol Vis Sci. 2010 Mar;51(3):1304-10.). That is why we chose Aβ 1-40 for this study. We have added comments and references about why the Aβ 1-40 was used in this study in the Discussion section. As controls for this experiment, we have added equivalent mass of DMSO that is used as a solvent for Aβ1-40. So, we add some comments about the controls in the Methods section.

“There are inhibitors of beta/gamma selectase available. Adopting one of these would also have established whether the observed changes were blocked by introducing such inhibitors.”

Answer: We agree blocking experiments are important to determine how Aβ functions in RPE cells. We did not present data in this manuscript but we have already investigated that knockdown of RAGE, a receptor of Aβ inhibitor, by siRNA attenuated the increase/decrease of viable RPE cell number induced by Aβ addition. These results showed not only that Aβ cause the increase/decrease of viable RPE cell number but also that Aβ-RAGE pathway is related to this observed change.

I have added a new figure as Figure 7C and added some comments on this in the manuscript.

“The authors have attempted to explain the increased proliferation of ARPE-19 cells to 5um AB as due to increased PEDF expression by blocking a nuclear inhibitor of PEDF. This explanation needs further support. This is especially as there doesnt seem to be a difference in PEDF mRNA levels at AB concentrations of 5 and 25 uM.”

Answer: We have added more details in the Discussion section. In brief, even in the 25 µM group, PEDF is expressed originally and the inhibition of PEDF attenuated the cell protective effect of PEDF resulting in a decrease the number of living cells. Thus, although PEDF has a cell protective effect with both 5 µM and 25 µM groups, the strong apoptosis signal in the 25 µM Group decreased the number of living cells. This effect was enhanced by PEDF inhibition, and the PEDF inhibition caused a further decrease in the number of living cells in the 25 µM Group.
“The rel VEGF mRNA at the different AB concentrations as shown in Fig 4A and 7A seem to be discordant.”

Answer: This is because of the difference in the controls. In Figure 4A, we showed the original data in which the VEGF mRNA original copy number was divided by the Actin original copy number. But in Figure 7A, we defined the control group as 1 and showed the order of relative comparisons in the experimental group. To try to clear these differences, we have add more details in the Figure legend to make these differences more easily.

“The references need updating e.g. to include the recent review by Lynn et al. / Neural Regeneration Research. 2017;12(4):538-548.”

Answer: We have updated the references including the recent review by Lynn et al. / Neural Regeneration Research. 2017;12(4):538-548.”

Reviewer #2’s comments,  

This manuscript demonstrates that the effect of Aβ on the viability of RPE is dose-dependent in which number of living ARPE-19 cells was increased by 5 μM but decreased by 25 μM of Aβ. The pro-apoptotic effect of Aβ on RPE is linked to its effect on PEDF and VEGF and that modulation of RAGE could be an important player in this process. The manuscript is well-written and sounds, however there are minor concerns: - The study is solely based on in vitro approach in which RPE culture is the main target. Although RPE dysfunction is crucial in the pathogenesis of AMD, choroidal endothelial cells may also play an important role. It would be interesting to dissect the potential role of Ab in the cross talk between RPE and choroidal endothelial cells particularly with the increased VEGF expression by Ab. - There was no real functional assessment of RPE, instead the study focused on molecular changes. It would be interesting of the investigators considered studying, RPE barrier or phagocytic function.

Answer: We agree that the choroidal endothelial cells may also play important roles, and the cross talk between RPE and choroidal endothelial cells may also be important. We have added some comments in the Discussion section on this and added reference that showed the role of endothelial cells. We have also added some comments about the real functional assessment of RPE caused by Aβ in the Discussion section.

We thank the reviewers for the helpful comments and hope that we have now produced a more balanced and better account of our work.

Sincerely,

Nahoko Ogata, MD, PhD