Response to Reviewers' comments

Dear editors,

We are very grateful for your feedback regarding our manuscript entitled “Impact of neurotrophic factors combination therapy on retinitis pigmentosa”.

We also thank the reviewer(s) for the sound comments that have helped us markedly improve our study.

Please find below a point by point response to all comments.

We hope that this new very will be suitable for publication in your renowned journal. Please do not hesitate to contact me if you need further information regarding this manuscript.

Best wishes,

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Reviewer Comments to Author:

Reviewer: 1

Comments to the Author
This is a well presented study on neurotrophic factors and their effect on retinitis pigmentosa.

Fig. 2:
Please present the negative control image for TrkB, TrkC and CNTFRα. For RD and
C57BL/6 mice. Not sure about the staining condition for TrkB, TrkC for rd mice PN-3w and PN4w.

**Response:**

Thank you for the instructive suggestion. All the negative control images for TrkB, TrkC and CNTFRα for rd and C57BL/6 mice have been attached in the file named “negative control images.docx” along with the manuscript. The samples for rd mice PN-3w and PN4w were incubated with rabbit anti-mouse TrkB (1:1000, Abcam, Cambridge, United Kingdom) and rabbit anti-mouse TrkC (1:1000, Abcam, Cambridge, United Kingdom) primary antibodies at 4°C overnight, washed, mounted and imaged at room temperature, incubated with Fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG polyclonal antibody (1:200, ZSGB-BIO, Beijing, China) for 45 min at 37°C.

Page-19: Line 38 to 53. Author described about Bcl-2 in the "Discussion" section. Please add references for every statement, which were known from other literatures and not from current study.

**Response:**

Thank you for the kind reminder. We have added the related references in the “manuscript-1”.

31 Adams JM, Cory S: The Bcl-2 protein family: arbiters of cell survival. Science 1998;281(5381):1322-1326.
32 Tsujimoto Y: Role of Bcl-2 family proteins in apoptosis: apoptosomes or mitochondria?. Genes Cells 1998;3(11):697-707.

Please elaborate Fig. 1 and Fig. 2 in the figure legend. Author only provided the headings.

**Response:**

Thank you for the valuable advice. We have added the figure legend as follows:

**Figure legends**
Figure 1. Structure of the rd and C57BL/6 mice’s retina at PN-0, 1, 2, 3, and 4 weeks (×400). PN, postnatal, IS/OS, inner and outer segments, ONZ, outer neuroblastic zone, INZ, inner neuroblastic zone, ONL, outer nuclear layer, OPL, outer plexiform layer, INL, inner nuclear layer, IPL, inner plexiform layer, GCL, ganglion cell layer.

Figure 2. Immunofluorescence localization of TrkB, TrkC, and CNTFRα expression in the retina of rd and C57BL/6 mice at PN-0, 1, 2, 3, and 4 weeks (×400). PN, postnatal, IS/OS, inner and outer segments, ONZ, outer neuroblastic zone, INZ, inner neuroblastic zone, ONL, outer nuclear layer, OPL, outer plexiform layer, INL, inner nuclear layer, IPL, inner plexiform layer, GCL, ganglion cell layer.

Reviewer: 2

Comments to the Author

The authors investigated the location of the neurotrophic receptors TrkB, TrkC, and CNTFRα in the retina of rd mice and explore the dynamic changes of Bax, Bcl-2, and LC3 expression and ultrastructure in the retina. The manuscript in general is well-written. Just a few minor points:

The literature reviews. Some important references should be added to the introduction (such as PMID: 29721974).

Response:

We thank the reviewer for this valuable comment. We have supplemented the relevant references.

The statistical methods used in this study are not clear. More details should be added
to the methods section.

**Response:**

We thank the reviewer for this valuable comment. In the Immunofluorescence and Transmission electron microscopy part of the study, the tissue localization, fluorescence color development characteristics of corresponding protein expression and ultrastructure in retinal tissues of mice of different age groups were mainly observed, which belonged to qualitative analysis. Morphological and ultrastructure characteristics were mainly analyzed and intergroup expression characteristics were described. No quantitative analysis.

The experimental results were expressed as mean ± standard deviation and statistically analyzed by SPSS18.0 software. normal distribution test and homogeneity test of variance were performed for each group of data. If the normal distribution and homogeneity of variance were satisfied, one-way ANOVA and pairwise LSD-T test were used. =0.05 was taken as the test level, and P < 0.05 was considered statistically significant. If variance was not homogeneous, Kruskal-Wallis H rank sum test and Tamhane’s T2 (M) test between groups were used, with =0.05 as the test level, and P < 0.05 as the difference was statistically significant.

**From the Associate Editor:**

1. Please make sure your manuscript contains the following information:

   Ethics/Review board approval with name, location and date of approval.

**Response:**

Thanks a lot for your reminder. The study protocol was approved by the Ethics Committees of the Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing Key Laboratory of Ophthalmology and Visual Sciences, Beijing, China at 2012.

2. How were the animals sacrificed?

**Response:**

Thanks a lot for your question. Cervical dislocation method: the left hand holds
the neck of the mouse, the right hand holds the tail of the mouse, both hands pull hard, do not hesitate, it is best to succeed, otherwise the mouse will be very painful, but pay attention not to pull off the head, this needs to control the degree of force, not only to say cervical vertebra break, but also can not pull off the head. And it is best not to show mice the execution of their own species.

3. Add a statement about adequate care of the animals. Did you follow guidelines (e.g. ARRIVE)?

Response:

Thanks for the kind reminding. The animals were treated according to the Statement for the Use of Animals in Ophthalmic and Vision Research made by the ARVO. All animal experiments are conducted in accordance with the animal experiment specifications.