Studying the contribution of aging to RGC degeneration relevant to glaucoma in a hPSC model

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Introduction

Glaucoma is a progressive neurodegenerative disease involving the degeneration of retinal ganglion cells (RGCs), which project visual information from the eye to the brain. Human pluripotent stem cells (hPSCs) have been established as a model of studying glaucoma that is more physiologically relevant to the disease presentation in humans compared to previously used rodent models. However, hPSCs have a "young" phenotype due to the reprogramming that allows them to assume a pluripotent state. While this is essential for the use of hPSC models, it does not create an accurate disease model for glaucoma, in which one of the largest risk factors is aging. Therefore, it is essential to create an aged hPSC model to study glaucoma in a more physiologically relevant human model.

In order to establish an aged phenotype in RGCs, we utilized a small molecule inhibitor of telomerase and stress inducer, BIBR1532 (BIBR). hPSCs were differentiated into 3D retinal organoids for the isolation of retinal ganglion cells (RGCs). RGCs were then incubated with BIBR for two weeks and aging-related phenotypes were determined in treated cells compared to untreated controls.

Methods/Experimental Design

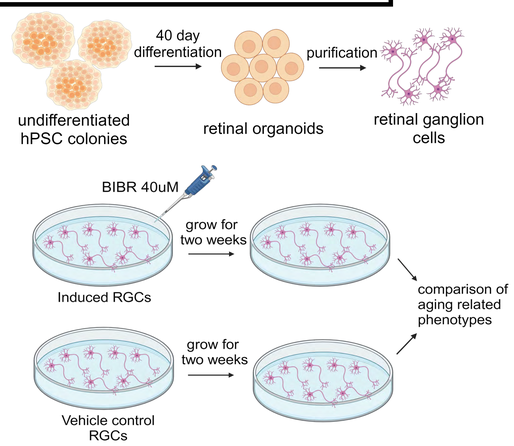


Figure 1. Schematic representation of retinal organoid differentiation, RGC purification, and induction of the BIBR small molecule to induce aging phenotypes in the experimental RGC group.

RGC Differentiation

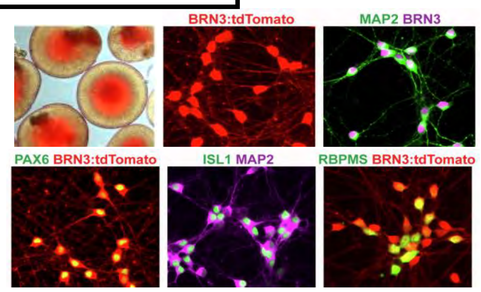


Figure 2. RGCs differentiated from hPSCs exhibited elaborate morphologies and expressed characteristic markers.
(Gomes, VanderWall et al. Stem Cell Rep.2022)

DNA Damage in BIBR-Treated RGCs

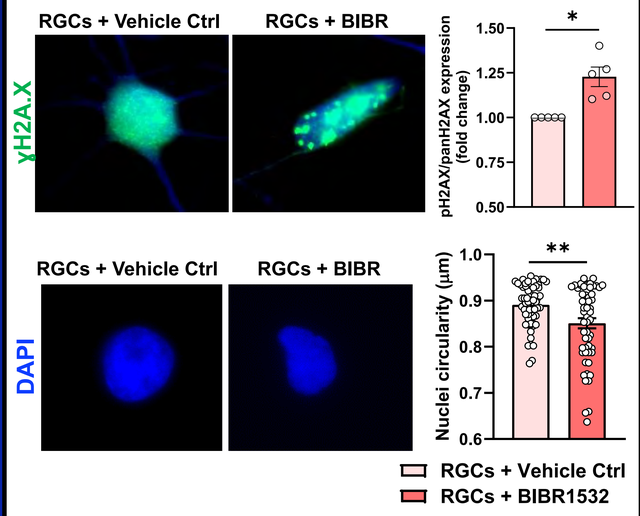


Figure 2. Increased γH2AX foci in the nucleus of BIBR-treated RGCs, as well as increased expression of γH2AX in BIBR-treated RGCs determined by ELISA. Decreased nuclei circularity suggest DNA damage, a feature of aging-related cells. n=3

Induction of Aging/Senescence Markers

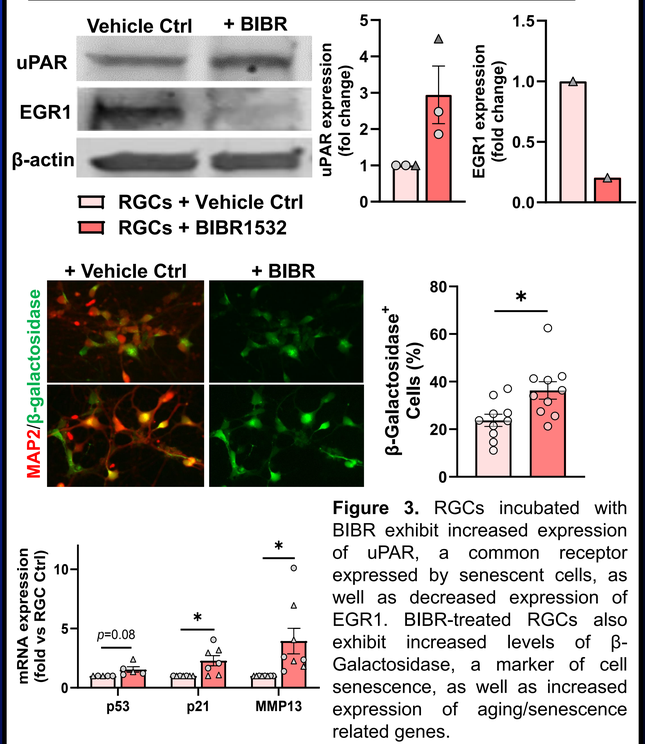


Figure 3. RGCs incubated with BIBR exhibit increased expression of uPAR, a common receptor expressed by senescent cells, as well as decreased expression of EGR1. BIBR-treated RGCs also exhibit increased levels of β-Galactosidase, a marker of cell senescence, as well as increased expression of aging/senescence related genes.

Morphological Changes in BIBR-Treated RGCs

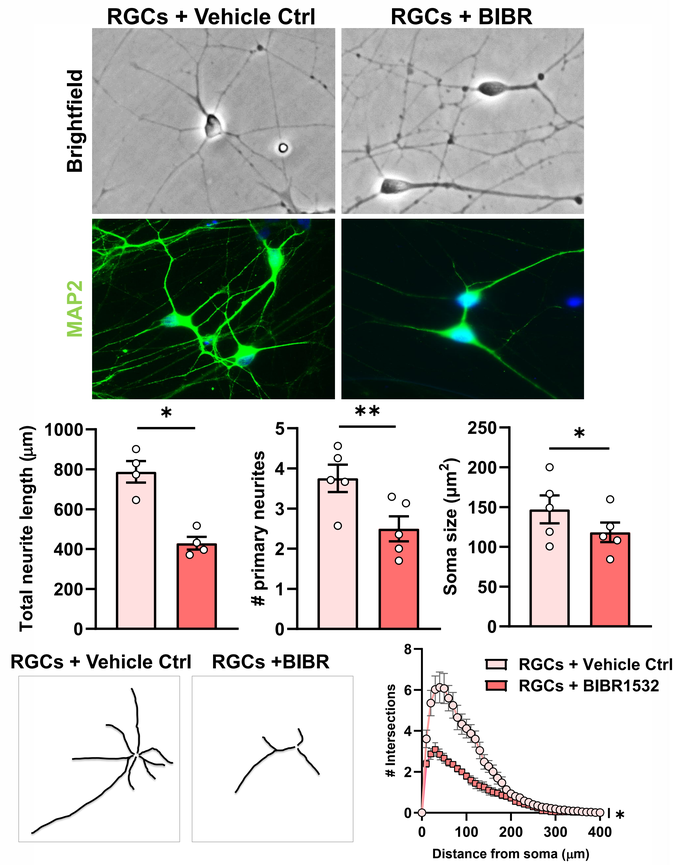


Figure 4. RGCs incubated with BIBR exhibited morphologies alterations including a reduction in the number of primary neurites, total neurite length and overall neurite complexity by Sholl analysis. n=4

Functional alterations in BIBR-treated RGCs

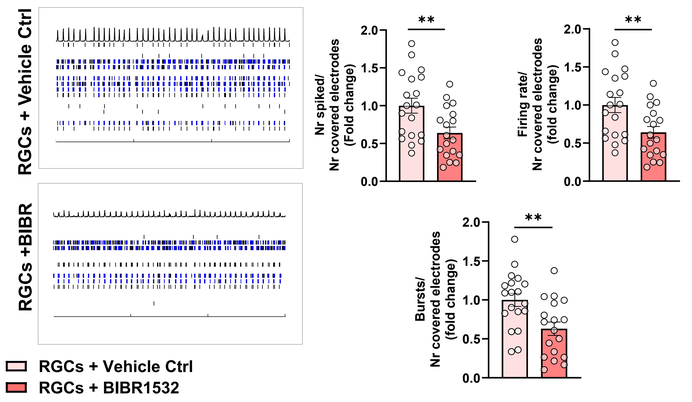


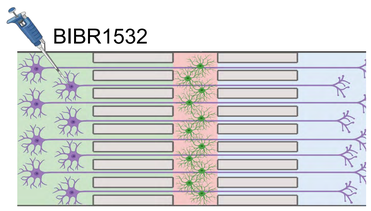
Figure 5. RGCs incubated with BIBR exhibited functional changes inclusind decreased number of spikes as well as decreased levels of firing rate and number of bursts.

Conclusions

- BIBR is able to induce an aging related phenotype in RGCs derived from hPSCs, including:
  - (i) Increased levels of yH2AX
  - (ii) DNA damage
  - (iii) Increased expression of β-Galactosidase as well as several aging/senescence related markers
  - (iv) Morphological and functional changes
- This model more accurately reflects glaucoma in an in vitro human model, furthering the knowledge of this disease and opening new avenues for glaucoma research

Future Directions

- Investigation of the effect of reactive astrocytes on the axonal compartment of BIBR-induced aged RGCs



- Utilize our single cell RNAseq data to compare to human patients with glaucoma

Acknowledgments

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