Determining the Therapeutic Effect of Human Neuritin 1 on the Restoration of Degenerated Retinal Ganglion Cells from Glaucoma Patients

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Background and Hypothesis: Glaucoma is a group of optic neuropathies that affects approximately 76 million people worldwide. The main risk factor is elevated intraocular pressure (IOP) which predominantly affects retinal ganglion cells (RGC), resulting in cell death and permanent vision loss. Current therapeutics for glaucoma involve reducing IOP and halting progression of disease, but no current treatments can revive degenerated RGCs. Our project aims to evaluate the therapeutic effect of human Neuritin 1 (NRN1) in regenerating and protecting RGC loss in glaucoma. By administering NRN1 to the RGCs in culture, we hope to elucidate the efficacy in helping glaucomatous RGCs recover from cell death.

Project Methods: Immunofluorescence (IF), gene expression, and karyotyping experiments were performed on iPSCs to confirm they were successfully reprogrammed from donor keratocytes. The iPSCs were differentiated to retinal organoids (RO) to generate RGCs *in vitro*. After around 30 days of differentiation, the ROs were dissociated to isolate RGCs. The RGCs were seeded at one end of three different *in vitro* collagen scaffolds. The first received no NRN1 treatment, the second received NRN1 at the cell body, and the third received NRN1 at the opposite end. IF was done on RGC-seeded scaffolds with RBPMS and NEFL antibodies to confirm RGC marker expression and neurite growth.

Results: The iPSCs were successfully reprogrammed from donor keratocytes. We successfully generated RGCs from both non-glaucomatous and glaucomatous donor iPSCs. The RGCs effectively integrated within the collagen scaffolds. After NRN1 treatment, non-glaucomatous and glaucomatous RGCs demonstrated differential expression of RGC specific markers.

Conclusions and Potential Impact: Our study demonstrates that NRN1 exhibits a therapeutic effect on glaucomatous RGCs. This study lays the foundation that NRN1 could potentially restore vision in glaucoma patients. Additionally, iPSC-derived RGCs can successfully be obtained from human donor eyes and cultured for future research for testing therapeutics.