Developing a retinal ganglion cell organoid enrichment protocol for glaucoma research and therapeutics

Tahani, Baakdhah[1], MD, MSc, PhD, Jeremy, Sivak[1], PhD

[1]Donald K Johnson, Eye Institute, University of Toronto

Purpose: Glaucoma is a neurodegenerative eye disease that involves damage to the optic nerve, which is formed of fibers projected from retinal ganglion cells (RGCs) to transmit electrical signals to the brain. Glaucoma is the second leading cause of blindness in North America and the leading cause of permanent blindness worldwide. Unfortunately, RGCs lost in glaucoma cannot be generated or replaced within the eye due to a lack of proliferative capabilities in adult tissues. There is a lot of interest in the field to study and replace lost RGCs by growing them in vitro using 2D cultures of pluripotent stem cells (PSCs). Yet, the development of a differentiation protocol with an efficient yield has been faced with many challenges, such as relatively small numbers and a particular vulnerability to injury. In this project, we are taking advantage of recent advances in culture techniques to establish and refine a novel differentiation protocol to generate RGC-enriched organoids from human PSCs.

Method: The protocol is being optimized and adapted from currently established whole-retina organoid methods, with several improvements to increase the proportion of RGCs and associated inner retinal cell types. Whole retinal organoids provide an excellent starting place because they recapitulate the developmental timing and spatial organization of the human retina. Because of their three dimensional organization, organoids allow direct cell-cell and cell-matrix interactions, producing cells with relevant physiological and pathological responses.

Results: Preliminary data using our modified protocol show substantially higher expression RGC markers (Atoh7 and Brn3b) compared to control organoids.

Conclusion: Ongoing studies are profiling the function, abundance, and subtype of RGCs produced by this novel method. This technique will be a powerful platform for studying RGC function and glaucoma pathogenesis, testing of therapeutics, and cell replacement.