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Differentiation and Three-dimensional Organization of Retinal Ganglion Cells using Human Induced Pluripotent Stem Cells

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Retinal Ganglion Cells (RGCs) are a type of neuron which function to relay visual messages between the retina and brain, and are characterized by their long axons which form part of the optic nerve. Dysfunction in this communication pathway is highly implicated in degenerative blinding disorders such as glaucoma. Unique applications using human induced pluripotent stem cells (hiPSCs) offer the ability to model human diseases, and potentially develop novel therapeutic approaches to rescue or replace damaged cells. In order to better understand the progression of degenerative eye diseases, a remaining challenge is to precisely identify the sequence of events which contribute to the diseased state, and how their features differ from non-diseased cells. Efforts were therefore undertaken to visually document the maturation of RGCs by analyzing their morphology and three-dimension organization at varying stages of development. Induced retinal cells were harvested at six different stages of development and fixed in 4% paraformaldehyde (PFA) solution to arrest their development. Cells were then cryoprotected in combinations of sucrose and Optimal Cutting Temperature (OCT) solutions, and frozen using powered dry ice. Following cryostat sectioning, samples were subject to immunocytochemistry staining to visualize for retinal-like organization of cells. Preliminary results have indicated the presence of the RGC marker Brn3, as well as markers for other retinal cell types. Future tests intend to characterize these retinal cell types according to their morphology and three-dimensional organization.

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