Harnessing the Potential of Mammalian Retinal Müller Glia to Regenerate Cone Photoreceptors

Seyedeh Sara Fooladi[1], MSc, Marek Pacal[2], PhD, Rod Bremner[3], PhD,

[1]Department of Laboratory Medicine and Pathobiology, University of Toronto
[2]Tanenbaum Research Institute, Mount Sinai Hospital
[3]Department of Ophthalmology and Vision Sciences, Faculty of Medicine, University of Toronto

Introduction: Cones are photoreceptors, crucial for visual acuity. Their loss leads to blindness in diseases such as Retinitis Pigmentosa (RP). Zebrafish can regenerate their retina and restore their vision upon injury. More specifically, Müller glia (MG) undergo dedifferentiation into a progenitor-like state, then divide and differentiate to replace the missing cells. Contrary to Zebrafish, Mammals cannot fully regenerate their retina. Mammalian MG can regenerate certain inner retinal neurons but to date, no study has reported high-yield regeneration of mature cones. Unlike murine cones, human cones have high levels of MYCN oncoprotein. However, the reason behind this high expression in post-mitotic neurons is unknown. Murine cones arise in the embryonic stage and post-natal progenitors cannot differentiate into cones. Our preliminary data suggests that overexpression of MYCN in postnatal murine retinal progenitors can induce cone lineage. Progenitors and MG are transcriptionally similar therefore, I will investigate whether induced expression of MYCN can facilitate the conversion of MG into cones. Additionally, as MG convert into cones, their population declines thus I will investigate whether MG division can be achieved by removing cell cycle inhibitors such as RB or p53. I hypothesize that manipulating the expression of MYCN and various cell cycle inhibitors will facilitate a full cone regenerative program.

Methods: To target MYCN to MG we will use Glast-CreERT2 transgenic mice, with tamoxifen-inducible, MG-specific Cre. At birth, these mice are electroporated with a Cre-dependent plasmid containing MYCN-GFP. Tamoxifen treatment leads to MG-specific expression of MYCN and GFP in adult mice. To mimic the loss of photoreceptors in humans, we will use rd1 mice which display severe retinal degeneration. To facilitate MG cell cycle entry, we will use Glast-Cre and Rb/p53 floxed alleles to conditionally delete Rb or p53 in MG. Changes in retinal development and cell cycle will be assessed by immunostaining, RNAscope and Single cell RNA sequencing (scRNA-Seq). We'd also perform CRISPR screens to study the underlying genetic circuitry and identify clinically relevant targets.

Results: We have been deciphering the mechanism driving the MYCN-dependent fate switch using cutting edge techniques such as scRNA-Seq. We have already identified several promising candidates that we'd like to explore in our regeneration project.

Conclusions: This study explores MYCN's potential in driving cone genesis. Achieving MG division and cone genesis would offer a clinically applicable solution to retinal regeneration thus revolutionizing available treatments for vision loss.