Müller Glia Heterogeneity: Diversity and Function of Subtypes

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Introduction

The vertebrate retina and Müller glia-mediated regenerative mechanisms

The vertebrate retina is a laminated structure at the back of the eye responsible for the phototransduction of light into electrical signals. Damage to the retina either from injury or disease leads to visual impairment or blindness and in mammals, retinal damage is largely irreversible. With their cell body located in the retinal inner nuclear layer, Müller glia (MG) are generally responsible for structural and neurotrophic support in the vertebrate retina. In zebrafish however, MG partially de-differentiate to produce progenitor cells which constantly produce rod photoreceptors throughout adulthood. Additionally, zebrafish MG respond to retinal injury by producing progenitors that replace any damaged retinal cell types[1]. Humans also possess Müller glia although unfortunately this regenerative phenotype has been virtually completely lost to mammals.

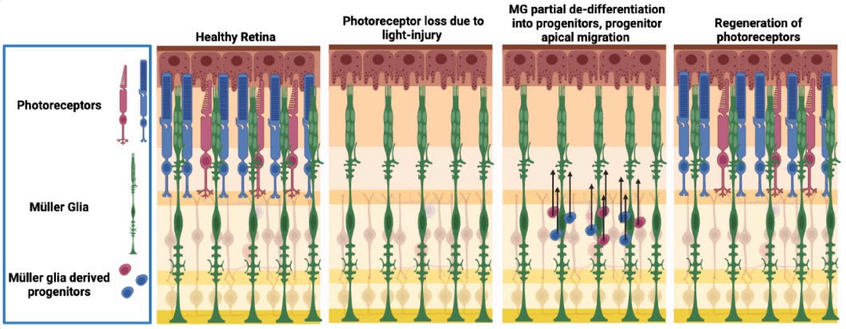
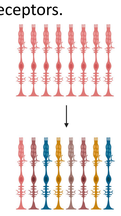


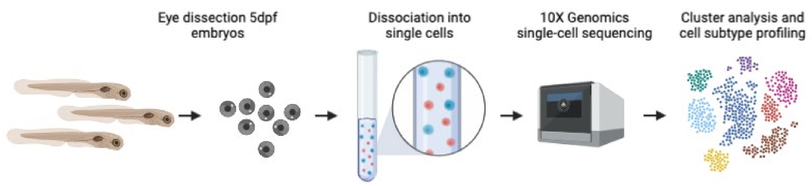
Figure 1. Schematic of Müller glia mediated photoreceptor regeneration following light injury. Release of cytokine and growth factors from damaged photoreceptors triggers MG partial de-differentiation into multipotent progenitor. Following apical migration, the progenitor differentiates into fully functional photoreceptors.

Müller glia heterogeneity

Once thought to be a homogeneous population, recent advances in analytical sequencing technology reveal transcriptionally heterogeneous Müller glia subpopulations in the chick and human[2,3].



Single-cell RNA sequencing



20,609 cells from 40 whole eyes of 5 dpf zebrafish embryos included for preliminary analysis

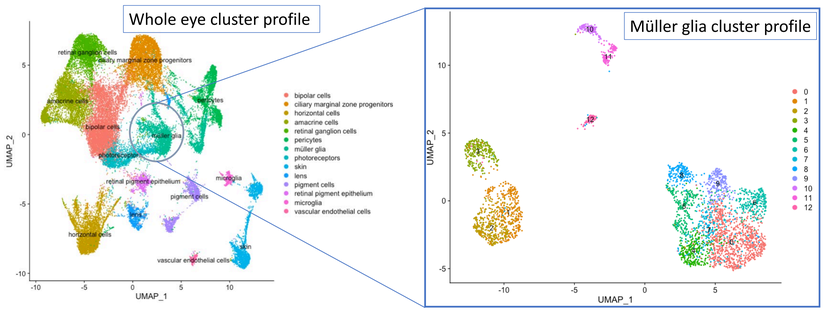


Figure 2. Single-cell RNA sequencing (scRNAseq) provides transcriptional profiling at the resolution of the single cell for thousands of individual cells in a given tissue type. Computational plotting of scRNAseq data displays each cell as a single dot. The distance between any two given dots is proportional to the transcriptional similarity between two cells, resulting in clusters of transcriptionally similar cells. Clusters of cells at the resolution of the whole tissue represent different cell types, whereas clusters of cells at the resolution of a single cell type represent transcriptionally distinct cell subtypes.

Hypothesis

Müller glia in zebrafish can be characterized by a molecular heterogeneity

Individual subtypes have a distinct functional role in photoreceptor genesis

Purpose

Retinitis pigmentosa: inherited retinal disease affecting between 1:3500 to 1:4000 Canadians[4]

Macular degeneration affects approximately 2.5 million Canadians[5]

Vision loss is expected to cost Canadians $30.3 billion by 2032[6]

Exploring Müller glia-mediated neurogenesis in a regenerative model such as the zebrafish is essential for identifying the transcriptional signature of a regenerative phenotype. If transferred to humans, the induction of this regenerative phenotype presents therapeutic potential to those suffering from ocular damage, disease or blindness.

Aim 1: Identify molecular markers to define specific subtypes

Preliminary Findings

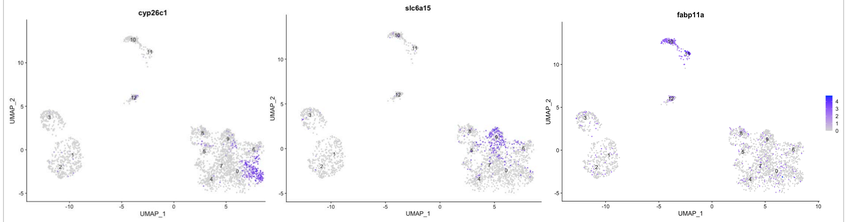
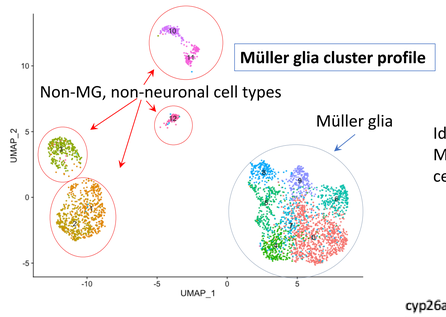
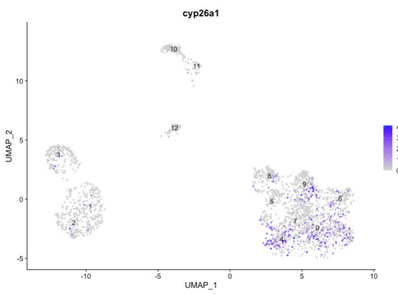


Figure 3. Molecular markers with selective cluster representation. Three examples of different markers each with selective cluster expression. cyp26c1 (left) with selective expression in cluster 0, slc6a15 (middle) with selective expression in cluster 9 and fabp11a (right) with selective expression in clusters 10, 11 and 12.



Identification of cluster subset representing Müller glia amongst additional non-neuronal cell types



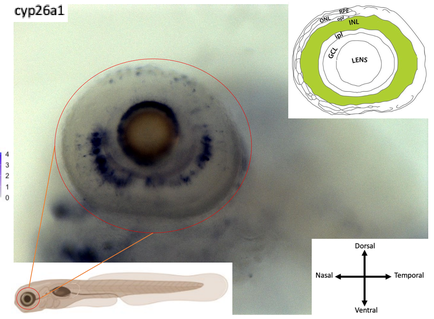


Figure 4. *in situ* hybridization of molecular marker mRNA within the zebrafish inner nuclear layer. cyp26a1 scRNAseq expression data within the zebrafish Müller glia (left). Whole-mount *in situ* hybridization of cyp26a1 mRNA specifically expressed within the ventral zebrafish inner nuclear layer (right).

Aim 2: Evaluate inter-species loss of MG neurogenic identity

Evolutionary regression of neurogenic identity analyzed by cross-species scRNAseq integration

Müller glia mediated regenerative response to retinal injury occurs in fish and to some extend frogs and birds but not mammals. This would suggest an inverse relationship between cortical development and Müller glia neurogenic identity. Integration of zebrafish data with publicly available datasets for chick, mouse and primate models will allow for identification of transcriptional signatures present in zebrafish Müller glia but lost to higher order species.

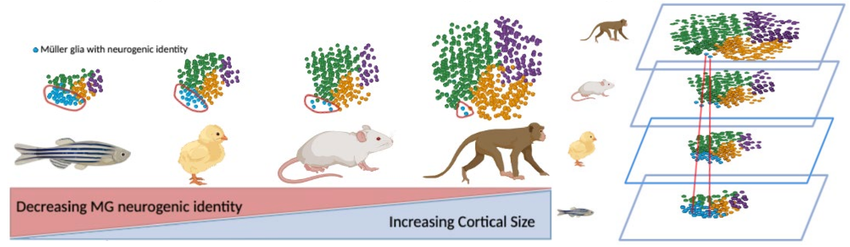
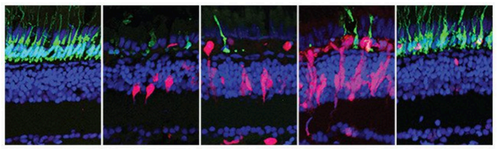


Figure 5. Rationale for integration of zebrafish data with publicly available scRNAseq datasets. Schematic representing evolutionary regression of Müller glia regenerative phenotype with increased cortical size (left). Schematic detailing integration and computational superimposition of Müller glia scRNAseq datasets for the zebrafish, chick, mouse and primate (right).

Future Directions

Identify functional role of MG subtypes on normal rod genesis and in response to injury

Live imaging of subset-derived progeny following injury



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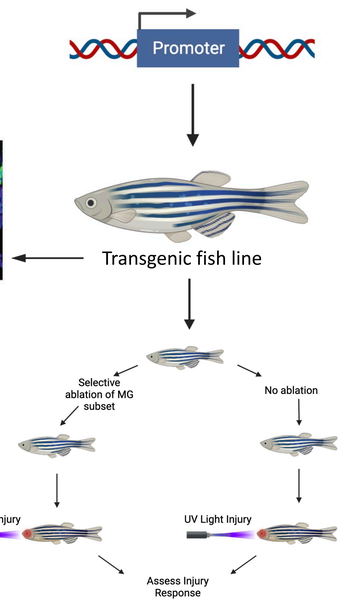

Figure 6. MG subtype functional roles. Establishing a promotor region for a molecular marker of specific Müller glia subtypes enables creation of transgenic lines. Transgenic lines with selective fluorescent expression in subtypes allow for live imaging of subtype-derived progeny in normal rod genesis. Additionally, these techniques can be used for generation of subtype-ablation models to infer MG subtype role in response to injury.

References

1. Powell, C., Cornblath, E., Elsaeidi, F., Wan, J. & Goldman, D. Zebrafish Müller glia-derived progenitors are multipotent, exhibit proliferative biases and regenerate excess neurons. Sci. Rep. 6, 24851 (2016)..
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