Müller Glia Heterogeneity: the diversity and function of subtypes

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Background

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. In mammals, retinal damage is largely irreversible. Müller glia (MG) are generally responsible for structural and neurotrophic support in the vertebrate retina. In zebrafish however, MG partially de-differentiate into progenitor cells which generate rod photoreceptors throughout adulthood. Additionally, the zebrafish MG respond to retinal injury by producing progenitors that replace any damaged retinal cell types[1] (Figure 1). Humans also possess Müller glia, although unfortunately this regenerative phenotype has been virtually completely lost to mammals

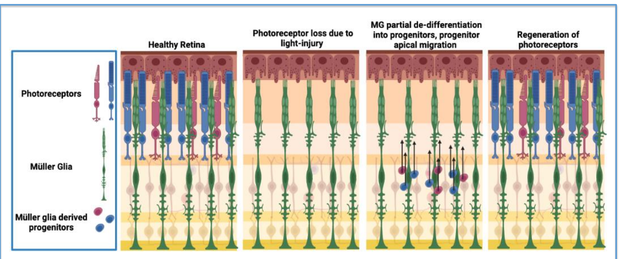
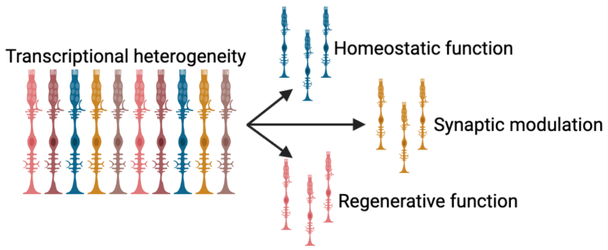


Figure 1. Schematic of Müller glia mediated photoreceptor regeneration following light injury. Release of cytokine and growth factors from damaged photoreceptors triggers MG partial de-differentiation into multipotent progenitors. Following apical migration, the progenitor differentiates into fully functional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

Müller glia heterogeneity

Once thought to be a homogeneous population, recent advances in RNA sequencing technology reveal transcriptionally heterogeneous MG subpopulations in the chick and human[2,3]. Transcriptional heterogeneity may indicate functional heterogeneity, as schematized in the diagram below.



- **Retinitis pigmentosa affects 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]**

Zebrafish as a Model Organism

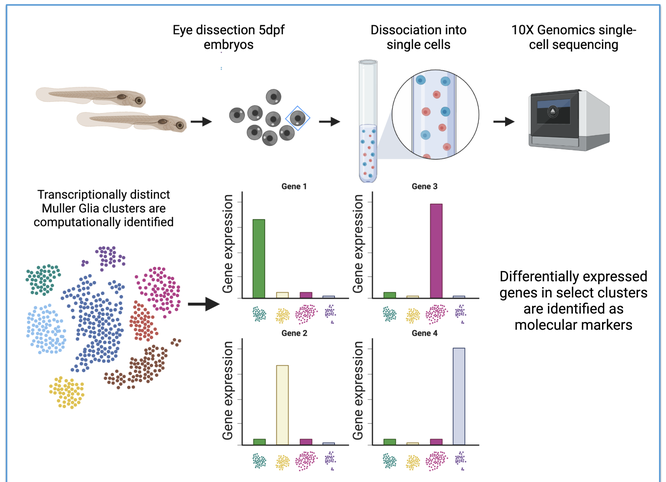
- Amenability to genetic manipulation
- Adult retinal neurogenesis and regenerative response to injury
- Eye structure and cone dominant vision, similar to that of a human
- Transparent in larval form

Hypothesis and Rationale

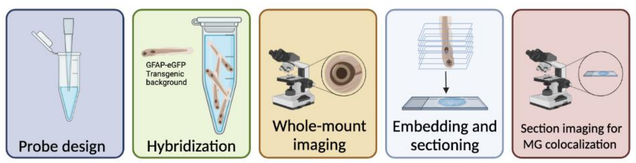
Müller glia in zebrafish can be characterized by a molecular heterogeneity

Exploring MG heterogeneity is essential for identifying functionally distinct phenotypes. The regenerative capacity of the zebrafish makes it possible to identify a regenerative MG phenotype. Identifying the transcriptional signature of a regenerative phenotype may enable the coercion of human MG into a regenerative state through gene therapy. With this approach, novel strategies for regenerative therapies using a patient's endogenous retinal cells may one day be possible.

Methods



Molecular marker probe design for *in situ* hybridization



Results

Transcriptionally distinct MG clusters

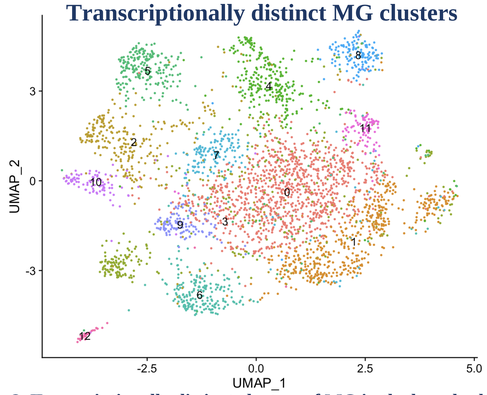


Figure 2. Transcriptionally distinct clusters of MG in the larval zebrafish. Each dot represents a single MG cell. Distance between cells is proportional to transcriptional similarity. Transcriptionally similar cells form discreet clusters, indicated numerically.

Results

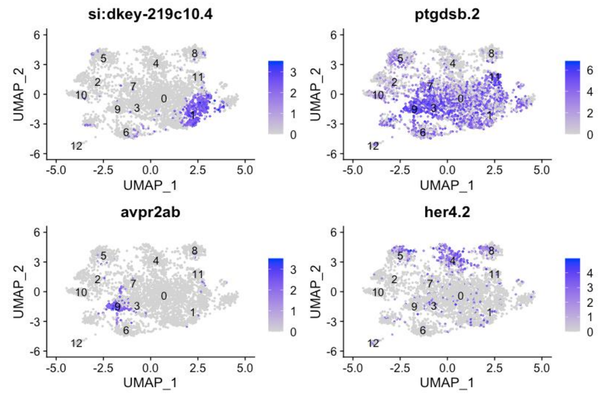


Figure 3. Expression of cluster-specific molecular markers for MG subtypes in the larval zebrafish. Examples of four genes differentially expressed in varying MG clusters shown by uniform manifold approximation plot (UMAP). Purple indicates expression.

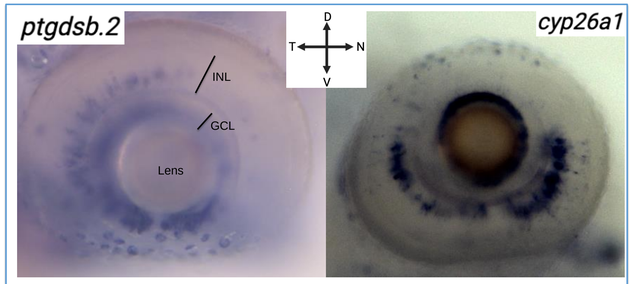


Figure 4. Spatially biased MG heterogeneity in the zebrafish retina. Whole mount expression profiles of genes expressed in MG with spatially biased topology. Purple staining indicates the presence of marker gene RNA. Left: *ptgdsb.2* expressed in the temporal INL. Right: *cyp26c1* expressed in the ventral INL. Markers were identified in MG with by co-expression with MG-specific antibody (glutamine synthetase; data not shown). Abbreviations: INL, inner nuclear layer; GCL, ganglion cell layer.

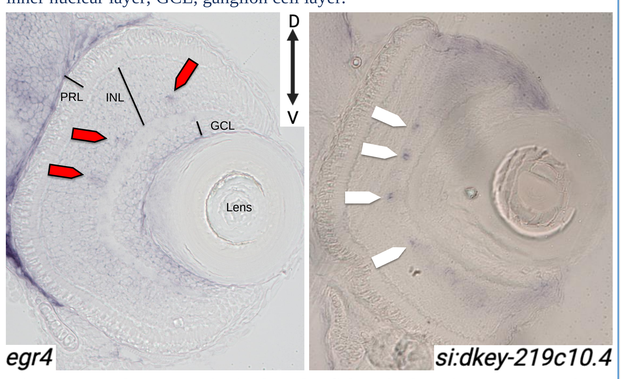


Figure 5. MG heterogeneity in the zebrafish retinal section. Two examples of non-overlapping differentially expressed MG markers exhibiting non-uniform expression. Left: MG expressing *egr4* (red arrowheads). Right:MG expressing *si:dkey-219c10.4* (white arrowheads). Abbreviations: PRL, photoreceptor layer; INL, inner nuclear layer; GCL, ganglion cell layer. Markers were identified in MG with by co-expression with MG-specific antibody (glutamine synthetase; data not shown).

Future Directions

Identify neurogenic and non-neurogenic MG subtypes

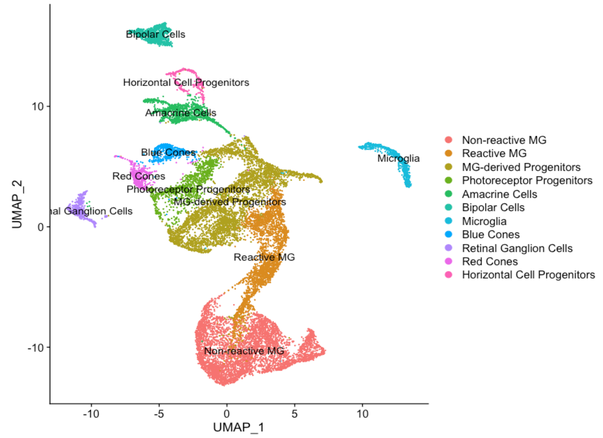


Figure 6. Single cell transcriptome of adult zebrafish MG and MG-derived progeny following light-injury regenerative response over several days[7]

Validate functional capacity of neurogenic subtype through loss of function

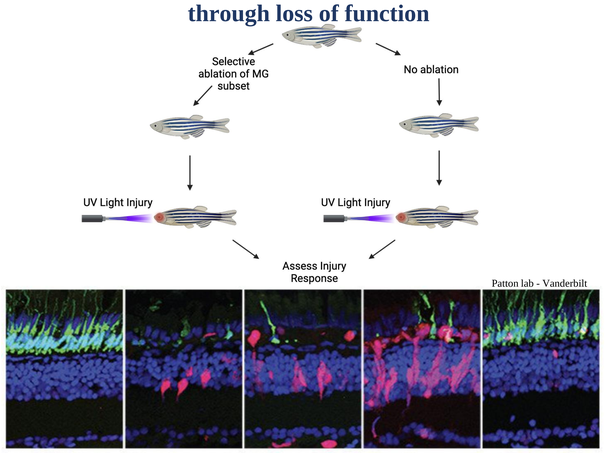


Figure 7. Experimental approach. Schematized light injury approach in subtype-ablated fish (top). MG response (mauve) to photoreceptor (green) death following photoreceptor ablation with UV light (bottom).

References

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4. Fighting Blindness Canada. 2018. Retinitis Pigmentosa. https://www.fightingblindness.ca/eyehealth/eye-diseases/retinitis-pigmentosa/
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