Semaphorin in Retinal Regeneration

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Background

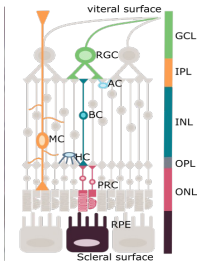


Figure 1: Schematic of retinal organization, circuitry, and cell types.

The Retina

- The retina is responsible for the detection and transmission of visual information to the optic nerve[1]
- Contains a wide variety of cells types
- Complex circuitry that is highly organized

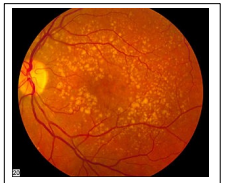


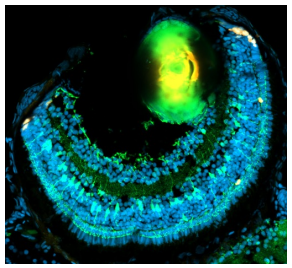
Figure 2: Retina undergoing age-related macular degeneration. White spots are areas where retinal cells have died.

Retinal Degenerative Disease

- Retinal degenerative diseases (RDDs) induce death of all retinal cell types, depending on the disease type[2]
- The mammalian retina cannot replace lost cells
- Loss of retinal cells causes permanent vision loss in humans, leading to diminished quality of life and a negative impact on mental health[3]

Müller glia driven repair

Müller Glia are resident glial cells that are able to dedifferentiate to produce progenitors in response to injury. These progenitors can then produce any retinal cell type[4]. In the healthy retina, Müller glia perform homeostatic functions such as maintaining ionic balance, recycling neurotransmitters, and providing nutrients to surrounding neuronal cell types.



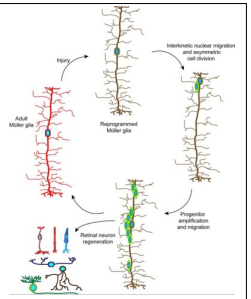


Figure 3: Section of zebrafish retina (left) immunofluorescence. Green glutamine GFAP for Müller, blue is DAPI and red is EdU. Müller glia undergo dedifferentiation to produce new progenitors in response to retinal cell death to produce all retinal cell types (right) [4].

Mammalian Müller glia undergo reactive gliosis which only produces scarring of the retina. This scar is only transiently protective and over time can lead to even more extensive damage and cell death. It is speculated that differences in cell signaling and the extracellular environment between mammals and zebrafish is what drives this stark difference between proliferative capacities in the retina.

Semaphorin3fa

Semaphorins are a large family of signaling proteins involved in many processes such as axonal development. Published single cell RNA sequencing data demonstrates *sema3fa* mRNA expression in resting Müller glia and a downregulation of its expression in activated cells[5]. This downregulation points to a potential role of the protein in Müller glia driven repair.

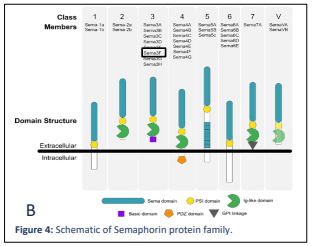


Figure 4: Schematic of Semaphorin protein family.

Hypothesis and Rationale

I hypothesize that Sema3fa controls Müller glia response to injury. I will investigate the role of Sema3fa in proliferative response to injury through the use of a light injury model and a Sema3fa knock out line of zebrafish developed in the McFarlane lab. I aim to assess changes due Sema3fa knockout on a cellular and genetic level.

Materials and Methods

Injury Model

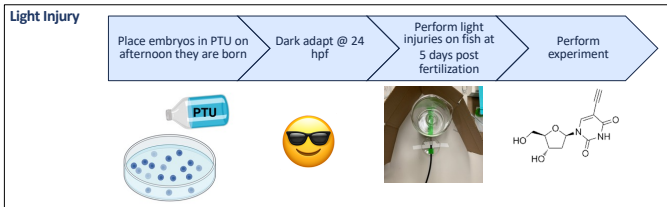


Figure 6: Flow chart of light injury model, EdU labeling method, and tissue processing

Zebrafish were injured at 5 dpf. This injury model is used as it induces injury in the central photoreceptors, inducing Muller glia proliferative response. Statistical analysis was performed using GraphPad Prism. Mann Whitney-U Tests were performed to compare cell counts as it is a non-parametric test and the distribution of values is unknown.

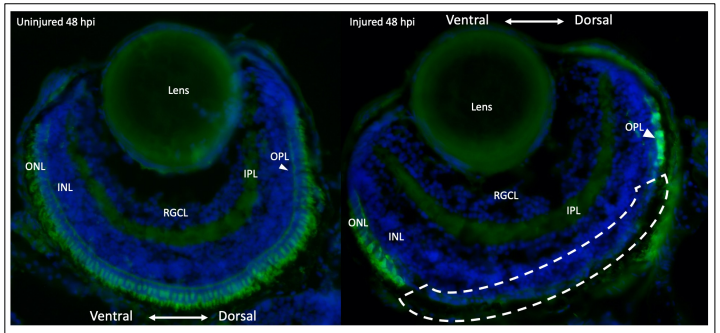


Figure 7: Light ablation method induces loss of photoreceptors. At 48 hours post injury, zpr1 staining (green) in the injured retina indicates ablation of photoreceptors as shown in the white dotted line.

Results

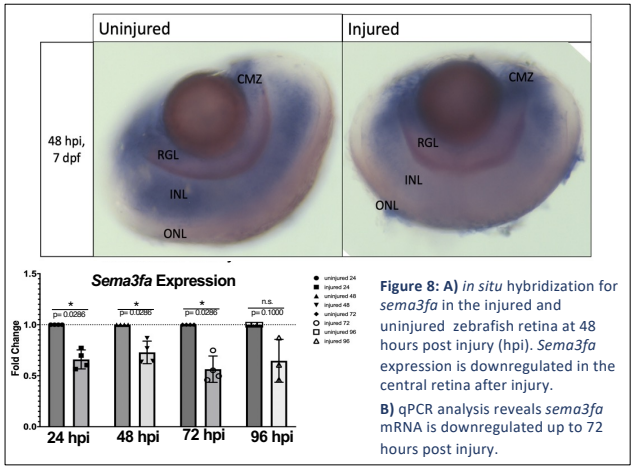


Figure 8: A) *in situ* hybridization for *sema3fa* in the injured and uninjured zebrafish retina at 48 hours post injury (hpi). *Sema3fa* expression is downregulated in the central retina after injury.

B) qPCR analysis reveals *sema3fa* mRNA is downregulated up to 72 hours post injury.

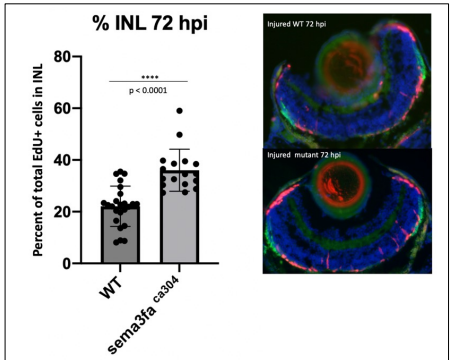


Figure 9: Loss of Sema3fa protein leads to increased proliferation in the inner nuclear layer (INL) at 72 hour post injury (hpi). Green stains zpr1 for photoreceptors, red is EdU which labels cells in s-phase or after. Blue is DAPI.

Loss of Sema3fa protein in the retina results in prolonged proliferation on the INL (Figure 9). These findings point to a potential role of Sema3fa protein in the proliferative response during Müller glia directed repair.

Conclusions and Future Directions

From these results, it is indicated that *sema3fa* may play a potential role in Müller glia directed repair. With these findings we hope to uncover new mechanisms behind regeneration in the zebrafish eye, which could be harnessed in future clinical treatment of retinal degenerative diseases.

References

1.Hoon M, Okawa H, Della Santina L, Wong RO. Functional architecture of the retina: development and disease. Prog Retin Eye Res. 2014;42:44-84.

2.Veleri S, Lazar CH, Chang B, Sieving PA, Banin E, Swaroop A. Biology and therapy of inherited retinal degenerative disease: insights from mouse models. Dis Model Mech. 2015;8(2):109-29.

3. Wan Y, Almeida AD, Rulands S, Chalour N, Muresan L, Wu Y, et al. The ciliary marginal zone of the zebrafish retina: clonal and time-lapse analysis of a continuously growing tissue. Development. 2016;143(7):1099-107.

4. Goldman D. Müller glial cell reprogramming and retina regeneration. Nat Rev Neurosci. 2014;15(7):431-42.

5. Hoang T, Wang J, Boyd P, Wang F, Santiago C, Jiang L, et al. Gene regulatory networks controlling vertebrate retinal regeneration. Science. 2020;370(6519).

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