Semaphorin in Retinal Regeneration

Katelyn Shewchuk[1], Sarah McFarlane[1]

Cumming School of Medicine, University of Calgary

Hotchkiss Brain Institute

University of Calgary, Cumming School of Medicine

CIHR IRSC

Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

Background

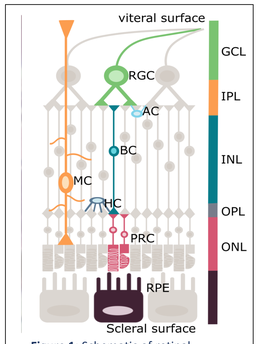


Figure 1: Schematic of retinal organization, circuitry, and cell types.

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) lead to the death of all retinal cell types, depending on the disease[2]
- In the mammalian retina, retinal cells cannot be regenerated
- Loss of retinal cells leads to permanent vision loss in humans, leading to diminished quality of life and a negative impact on mental health[3]

Zebrafish model

Zebrafish can regenerate lost retinal cells through two mechanisms:

1. Ciliary marginal zone (CMZ): a stem cell niche found in the periphery of the eye and proliferates throughout the life of a zebrafish[4]. It also upregulates cell proliferation in response to proximal injury.

2. Muller Glia: resident glial cells that are able to dedifferentiate to produce progenitors in response to injury. These progenitors can then produce any retinal cell type[5].

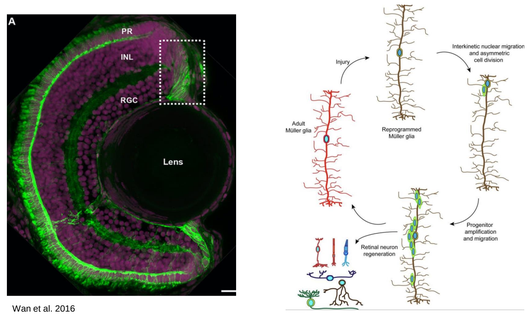


Figure 3: Section of zebrafish retina (left), Rx2+ cells in the CMZ and photoreceptors (green), Kaede fluorescent protein (red) labels other cell types in the retina[4]. Muller glia undergo dedifferentiation to produce new progenitors in response to retinal cell death to produce all retinal cell types (right) [5].

The CMZ does not exist in humans, however there is a similar structure called the ciliary body which expresses progenitor markers and is anatomically very similar to that of the CMZ. However this structure does not actively proliferate post injury. Moreover, mammalian Muller 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 mechanisms such as axonal development. The McFarlane Lab observed the expression of Semaphorin3fa (Sema3fa) mRNA in the CMZ as well as the inner nuclear layer where Muller Glia bodies reside. We have also demonstrated that Sema3fa loss impacts CMZ function and organization during development[6,7].

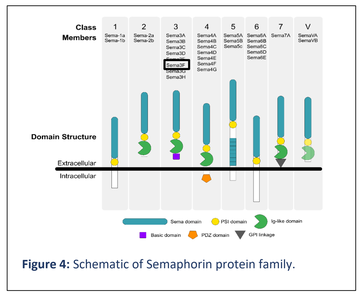


Figure 4: Schematic of Semaphorin protein family.

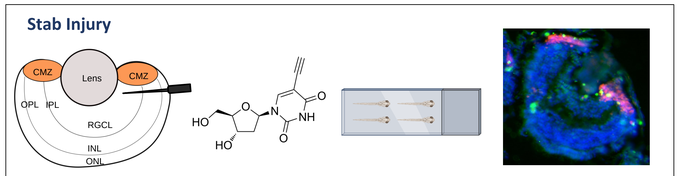
Hypothesis and Rationale

I hypothesize Sema3fa promotes proliferation of the CMZ and Muller Glia in response to retinal cell death and injury. I will investigate the role of Sema3fa in proliferative response to injury through the use of two injury models and a Sema3fa knock out line of zebrafish developed in the McFarlane lab. Stab injury induces CMZ proliferative response as cell death occurs adjacent to the region. Light injury induces Muller glia proliferation as photoreceptors in the central retina are lost. I aim to assess changes due Sema3fa knockout on a cellular and genetic level.

Materials and Methods

Injury Models

Stab Injury

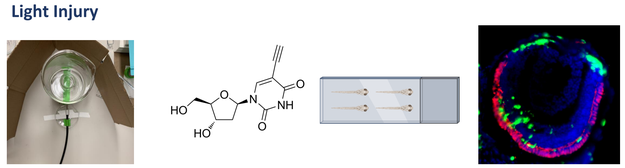


Perform stab injuries on fish at 5 days post fertilization → Perform 8 hour EdU pulse 48 hours post injury (hpi) → Cryosection samples and perform immunohistochemistry → Image and analyze

Figure 5 : Flow chart of stab injury model, EdU labeling method, and tissue processing

Zebrafish are used at 5 days post fertilization (dpf). This injury model provides precision and consistency while maintaining survival. It was also used as it induced significant increase in EdU positive cells in the CMZ (Figure 7).

Light Injury



Perform light injuries on fish at 5 days post fertilization → Perform 8 hour EdU pulse 48 hours post injury (hpi) → Cryosection samples and perform immunohistochemistry → Image and analyze

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 retina, inducing Muller glia proliferative response but not the CMZ.

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.

Results

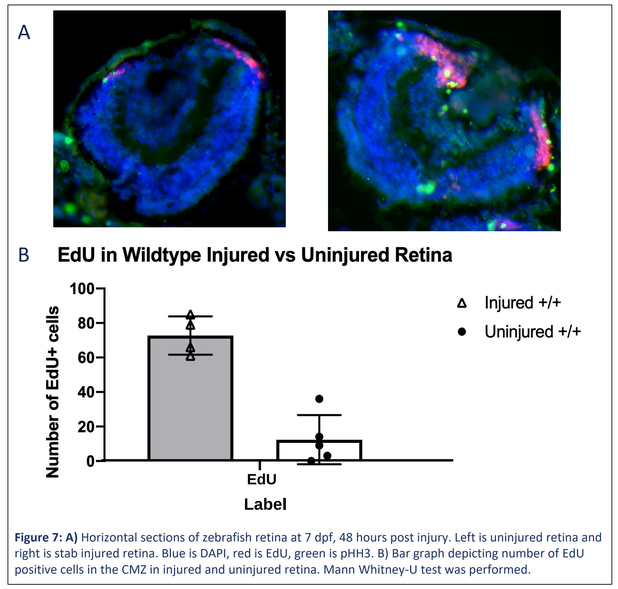


Figure 7: A) Horizontal sections of zebrafish retina at 7 dpf, 48 hours post injury. Left is uninjured retina and right is stab injured retina. Blue is DAPI, red is EdU, green is pHH3. B) Bar graph depicting number of EdU positive cells in the CMZ in injured and uninjured retina. Mann Whitney-U test was performed.

A qualitative analysis showed an increase in EdU positive cells in the CMZ in the injured retina compared to uninjured retina.

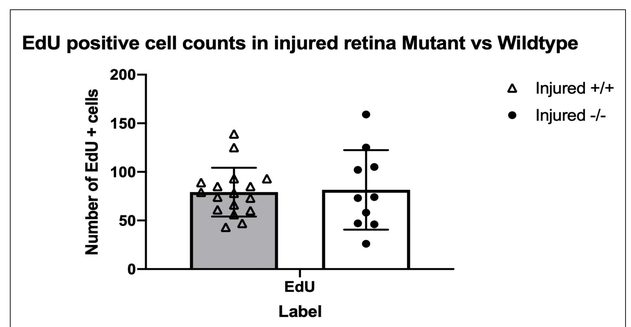


Figure 8: Number of EdU positive cells counted in the CMZ 48 hours post injury between wildtype and Sema3fa knockout zebrafish. Fish were injured at 5 dpf and EdU pulse and imaging was performed at 7 dpf. Mann Whitney-U Test was performed to compare cell counts between groups.

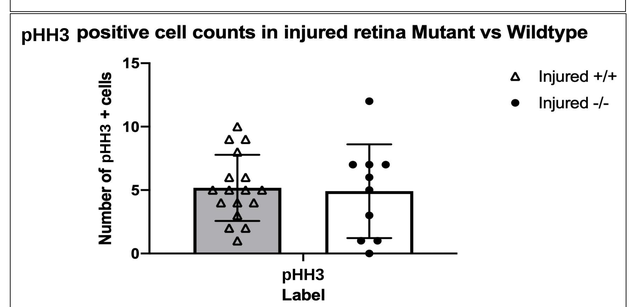


Figure 9: Number of pHH3 positive cells counted in the CMZ 48 hours post injury in wildtype vs Sema3fa knockout zebrafish. Fish were injured at 5 dpf and EdU pulse and imaging was performed at 7 dpf. Mann Whitney-U Test was performed to compare cell counts between groups.

Phosphohistone H3 (pHH3) was as a marker for cells undergoing mitosis while EdU labels cells that have gone through S-phase in the 8 hour EdU pulse therefore labeling all newborn retinal cells as well as potentially activated Muller glia cells and progenitors. Between wildtype and Sema3fa knockout fish, there was no change in EdU or PHH3 positive cells in the CMZ (Figures 8 and 9).

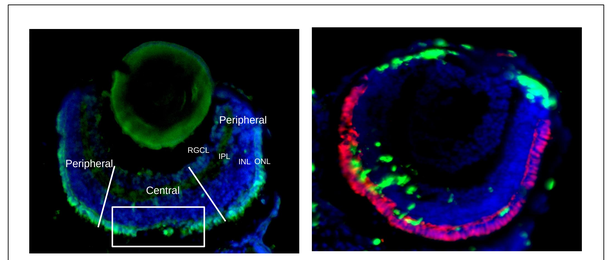


Figure 10: Transverse section of zebrafish retina at 7 dpf, 48 hours post injury. Left is labeled with Zrp1 (green) which labels photoreceptors. In the white box it is visible that photoreceptors have been ablated in the light injury paradigm. On the right is an eye post EdU pulse with EdU positive cells (green) in the CMZ as well as in the central retina where the light injury lead to photoreceptor death. Zrp1 is labeled in red. In both images DAPI labels cell nuclei.

In the future we plan on using a light injury paradigm which injures the central photoreceptors (Figure 6 & 10). This injury model induces Muller glia proliferation and injury response. EdU positive cell counts will be used again as a readout of proliferation.

Conclusions and Future Directions

From these results, we conclude that Sema3fa does not play a role in the initial proliferative response in the CMZ post stab injury. In the future we plan to investigate Muller glia proliferative response via EdU cell counts post light injury. Moreover, it will be valuable to investigate changes in gene expression via RT-qPCR and in situ hybridization post injury. Finally, more time points post injury will be investigated as well as other timepoints during development of zebrafish.

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

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Acknowledgments

University of Calgary, Cumming School of Medicine

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