Testing the mTOR pathway in Semaphorin3fa signaling-mediated regulation of the retinal regenerative response in zebrafish larvae

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BACKGROUND[1]

- Retinal degenerative diseases (RDDs) lead to permanent vision loss in mammals who cannot regenerate lost cells
  - Age-related macular degeneration alone is projected to affect 288 million individuals by 2040, worldwide[1]
- Zebrafish Müller glia are able to produce a proliferative niche following retinal insult, which replaces lost cells
  - Mammalian target of rapamycin (mTOR) activity is increased in activated Müller glia

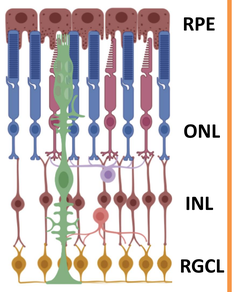


***Figure 1.** Schematic of retinal layers. RPE, retinal pigmented epithelium; ONL, outer nuclear layer; INL, inner nuclear layer; RGCL, retinal ganglion cell layer

Steps of Müller Glia-Directed Repair[1]

1. **Müller Glia Reprogramming.** Interkinetic nuclear migration and asymmetrical cell division
2. **Progenitor Proliferation and Migration**
3. **Cell Cycle Exit and Neuronal Differentiation**

Semaphorin3fa and Müller Glia (MG)

- There is a high conservation between the human and zebrafish retina so the capacity of fish but not humans to regenerate cells may lie in extracellular signals such as Semaphorin3fa (Sema3fa) to promote Müller glia reprogramming[2]
- The McFarlane Lab has observed the expression of *sema3fa* in the inner nuclear layer of the zebrafish retina where Müller glia cell bodies reside
- Published scRNAseq studies have shown a downregulation of *sema3fa* in activated Müller glia following light injury in the adult zebrafish, but the consequences of this has not been explored[3]

OVERALL OBJECTIVE

- Determine whether Sema3fa is involved in the upregulation of mTOR activity in Müller glia during reprogramming, to influence regenerative capacity

HYPOTHESIS AND PROJECT AIM

HYPOTHESIS: after retinal injury, Sema3fa signaling prevents the upregulation of mTOR signaling activity during Müller glia reprogramming

AIM: determine if Sema3fa signaling regulates the activity of proteins involved in MG-directed regeneration of the zebrafish retina, by examining if the phosphorylation state of key proteins that control MG reprogramming are impacted by Sema3fa loss

METHODS

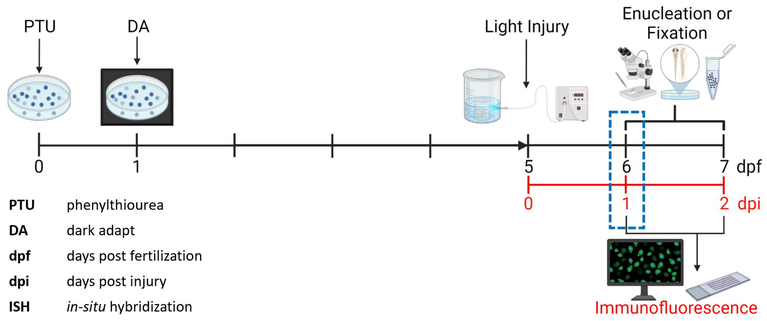


***Figure 2.** Schematic of Methods. At 0 days post fertilization (dpf), embryos are put in 1% phenylthiourea (PTU) to prevent pigment formation. Fish to be injured and their counterpart controls are dark adapted at 1 dpf until the time of injury at 5 dpf. Injured and non-injured fish are fixed at 1- and 2 days post injury (dpi) for immunofluorescence staining targeting mTOR activity marker, pS6

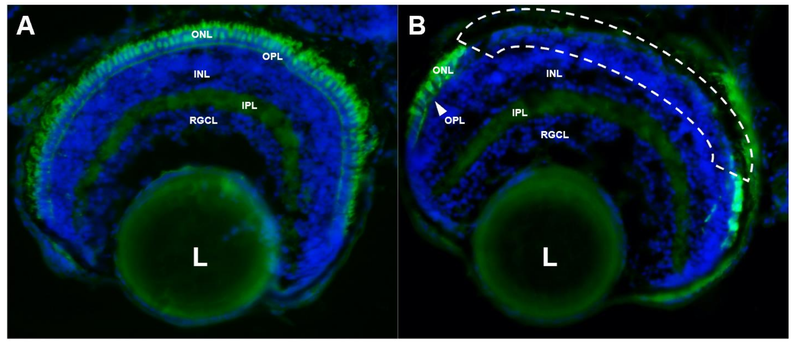


Figure 3. The acute light injury model is robust and consistent. Transverse section of a (A) non-injured zebrafish retina and (B) a light-injured retina. (A) non-injured retina of 7 dpf WT zebrafish embryo. B, light-damaged retina of 7 dpf zebrafish embryo 2 dpi. Each eye was stained for Zpr-1 (green) for photoreceptors and Hoechst (blue) for cell nuclei. (B) Clear ablation of long double-cone photoreceptors resulting from light damage is shown in dashed border validating the light injury model.

DISCUSSION

Sema3fa may be involved in Müller glia reprogramming after light injury. mTOR is essential for MG-directed retinal regeneration following stab injury[3]. SEMA3F/PLXNA1/NRP2 action in mammalian cell lines impedes mTOR activity[4]. Thus, I aimed to determine whether Sema3fa loss would reduce mTOR inhibition, measured by S6 protein activation (phosphorylation). Immunofluorescence showed that Sema3fa MUT have a significantly lower proportion of MG that were pS6 positive in the basal INL after injury (Figure 4). Perhaps, the loss of Sema3fa in MUT impairs the ability of MG to upregulate or respond to factors that upregulate pS6 after injury, which may impede reprogramming.

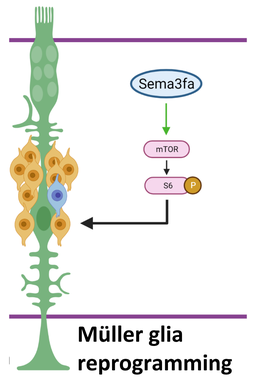


Figure 5. Sema3fa signaling may be involved in MG reprogramming through the upregulation of mTOR signaling. At 1dpi, Sema3fa may be essential to activate mTOR to promote asymmetrical cell division during reprogramming

LIMITATIONS

- Lack of biological replicates
- Does not have absolute number of pS6+ MG

CONCLUSION

In contrast to my hypothesis, the loss of Sema3fa negatively impacts the ability of MG to accumulate pS6 during reprogramming at 1dpi

FUTURE DIRECTIONS

Determine which receptors Sema3fa acts through in MG to produce effects observed in this study

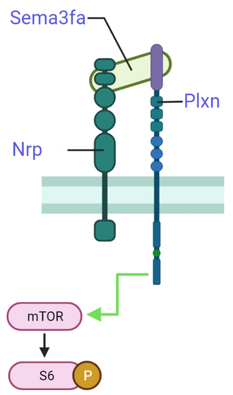
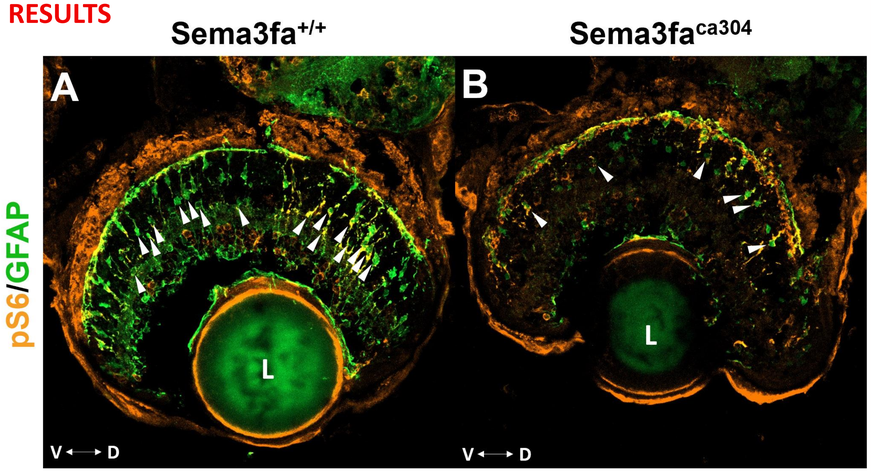


Figure 6. Sema3fa signaling occurs through NRP/PLXN signaling. Sema3F signaling has only been studied in cultured mammalian cells expressing NRP2/PLXNA1; however, effects in mammalian cells are not consistent with the results of my study.

RESULTS



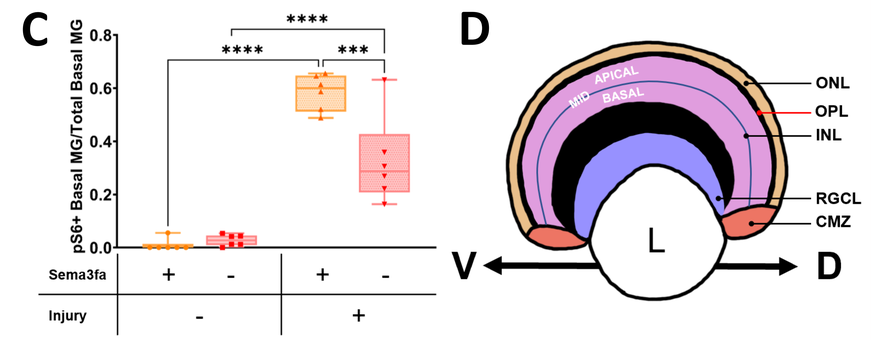


Figure 4. pS6 is significantly less upregulated in MG located at the basal INL of Sema3fa mutants compared to WT at 1 dpi. Transverse sections of injured (A) Sema3fa wildtype and (B) Sema3fa mutant zebrafish larvae at 1 dpi. Each eye was stained for GFAP (green) to visualize Müller glia and pS6, a marker of mTOR activity. (A) and (B) show that pS6 is upregulated after injury in both WT and mutants, respectively; and that this upregulation occurs in MG (white arrows). White arrows only show pS6 positive MG in the basal region of the INL. (C) is a histogram visualizing minimum and maximum proportion of MG that are also pS6 positive; data points represent individual embryos (n = 6 fish for each condition per genotype). A significant difference in the proportion of MG cells in the basal region of the INL that were pS6 positive was found between WT and mutants at 1 dpi. All comparisons were made using an ANOVA followed by a Šidák post-hoc. (D) Each transverse section is shown with the lens pointing down, ventral left, and dorsal right. The INL was split into apical, mid, and basal sections.

***p < 0.0005; ****p < 0.0001

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2. 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
3. Zhang Z, Hou H, Yu S, Zhou C, Zhang X, Li N, et al. Inflammation-induced mammalian target of rapamycin signaling is essential for retina regeneration. Glia. 2020;68(1):111-27.
4. Nakayama H, Bruneau S, Kochupurakkal N, Coma S, Briscoe DM, Klagsbrun M. Regulation of mTOR Signaling by Semaphorin 3F-Neuropilin 2 Interactions In Vitro and In Vivo. Sci Rep. 2015;5:11789.

*Made in BioRender

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