Results: WFA treatment significantly blocked glial reactivity and RGC apoptosis by 80% and 60%, respectively. Induced glial reactivity exacerbated the gliosis by 16 fold, and RGC damage by 7 fold. This increased vulnerability in both parameters was completely rescued by WFA treatment. WFA inhibited p38 mediated TNF-α secretion in cultured retinal astrocytes, and significantly reduced injury induced TNF-α immunoreactivity in the inner retina in vivo.

Conclusions: Inhibition of IF dynamics effectively protected the inner retina from excitotoxic damage. Our results suggest this mechanism is regulated through release of TNF-α by retinal astrocytes and Müller glia.

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Program Number: 4189 Poster Board Number: D0208
Presentation Time: 3:45 PM–5:30 PM
The Role of Notch Signaling in the Regenerating Adult Zebrafish Retina
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Purpose: In the damaged zebrafish retina, Müller glia are responsible for regenerating lost cells. We previously showed that Notch signaling is required to maintain Müller glia in a quiescent state, and inhibiting Notch signaling, via intraperitoneal injection of the g-secretase inhibitor RO4929097, is sufficient to induce a regenerative response. Thus, it appears Notch signaling is a negative regulator of retinal regeneration in the zebrafish eye. However, the zebrafish genome encodes four unique Notch receptors; Notch 1a, Notch 1b, Notch 2, and Notch 3. It was unclear if all or only a subset are involved in Müller glia quiescence and if they had any other functions in retinal regeneration. Thus, the purpose of this study was to begin to elucidate the function(s) of the individual Notch receptors in the undamaged and regenerating zebrafish retinas.

Methods: Adult albino zebrafish were placed in complete darkness for 14 days, before being subjected to constant light damage. Retinas were isolated for RNA extraction, and qRTPCR was performed on the four Notch receptor genes using Taqman probes. Dark-adapted albino zebrafish were subjected to morpholino-mediated knockdown of all four Notch receptors, separately, and then subjected to light damage. The retinas were isolated, cryosectioned, and immunolabeled for PCNA, Stat3, and Ascl1a, and analyzed by confocal microscopy.

Results: The qRT-PCR analysis revealed an increase in expression for notch 1a, 1b, and 2, while notch 3 decreased in expression from at from 0 to 16 hours, and increased subsequently throughout the light timecourse. Morpholino-mediated knockdowns of Notch 1a, 1b, and 2 resulted in fewer proliferating Müller glia and neuronal progenitors at 36 and 72hr of light compared to the controls. In contrast, morpholino-mediated knockdown of Notch 3 resulted in increased numbers of proliferating Müller glia at 36 and 72 hrs.

Conclusions: Notch receptors 1a, 1b, and 2, are required for the maximal proliferative response in the light-damaged zebrafish retina. In contrast, downregulation of Notch 3 is necessary for Müller glia to re-enter the cell cycle in response to damage. This suggests that Notch 3 is necessary to maintain Müller glia quiescence and Notch 1a, 1b, and 2 are required for Müller glia and neuronal progenitor proliferation.

Commercial Relationships: Joshua Hobgood, None

Program Number: 4190 Poster Board Number: D0209
Presentation Time: 3:45 PM–5:30 PM
Changes in miRNAs in Müller glia after retinal injury and Dicer deletion
Stefanie G. Wohl, Thomas A. Reh. Biological Structure, University of Washington, Seattle, WA.

Purpose: microRNAs (miRNAs) are negative regulators of gene expression and play roles in retinal development and regeneration (in zebrafish). Less is known about the role of miRNAs in the response to injury in mouse Müller glia (MG). We used NanoString technologies® and quantified miRNAs in 1) mature wild type MG, 2) after light damage (LD), as well as 3) in Dicer conditional knock out (CKO) MG cells.

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