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 excitoxic damage. Our results suggest this mechanism is regulated through release of TNF-α by retinal astrocytes and müller glia.
Commercial Relationships: Jeremy M. Sivak, Genentech (F); **Darren Chan**; **Xiaoxin Guo**, None; **Izzy Livne-Bar**, None
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Program Number: 4188 **Poster Board Number:** D0207
Presentation Time: 3:45 PM–5:30 PM
Effect of TNF-α on the expression of glial fibrillary acidic protein (GFAP) and on the photoreceptor differentiation of human Müller glial stem cells *in vitro*.
Erika Aquino, Karen Eastlake, Angshumonik Angbohang, Peng T. Khaw, G. Astrid Limb. NIHR Biomedical Research Centre for Ophthalmology, UCL Institute of Ophthalmology and Moorfields Eye Hospital, London, United Kingdom.
Purpose: During retinal gliosis, biochemical and physiological changes occurring in the retina lead to Müller glia proliferation and overexpression of glial fibrillary acidic protein (GFAP), as well as the release of pro-inflammatory factors such as TNF-α. However, the exact role and regulation of GFAP in human Müller glia is not understood, and the role of TNF-α on the neural differentiation of these cells has not been examined. This study investigated the *in vitro* effect of TNF-α on GFAP expression by the Müller glial stem cell line MIO-M1 as well as on the photoreceptor differentiation of these cells.
Methods: MIO-M1 cells were cultured with TNF-α in the presence or absence of FGF-2, taurine, retinoic acid and IGF-1 (FTRI), known to induce photoreceptor differentiation in these cells. RNA was extracted, cDNA produced and GFAP gene expression examined by RT-PCR. Protein expression of GFAP and NR2E3, a marker of photoreceptor differentiation, were examined by western blot analysis. Optical density of bands obtained from PCR and western blot were quantitatively measured and statistically analysed by paired t-test.
Results: MIO-M1 cells cultured with increasing concentrations of TNF-α showed that the mRNA and protein expression of GFAP decreased in a dose-dependent manner ($p<0.05$). Upon culturing cells with FTRI to induce photoreceptor differentiation, there was a significant increase in NR2E3 protein expression ($p=0.003$). Addition of TNF-α to cells cultured with FTRI did not modify NR2E3 expression as compared to FTRI alone. Moreover, FTRI caused a significant downregulation of GFAP mRNA expression ($p<0.0001$) in these cells.
Conclusions: The present results that a decrease in GFAP expression occurs in MIO-M1 cells cultured with FTRI indicates that the neural differentiation process itself may prevent the development of gliosis. They also suggest that TNF-α may have a protective effect on gliosis in human Müller glia. Further understanding of the mechanisms by which TNF-α may prevent GFAP upregulation may aid in the design of therapeutic approaches to prevent or control retinal gliosis.
Commercial Relationships: Erika Aquino, None; **Karen Eastlake**, None; **Angshumonik Angbohang**, None; **Peng T. Khaw**, None; **G. Astrid Limb**, None
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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 qRT-PCR 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

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Changes in miRNAs in Müller glia after retinal injury and Dicer deletion
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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.