**Retina Australia Research Report 2023**

**Project Title:** Using RNA-silencing to tackle neuroinflammation in retinal degeneration.

**Lay description:** Recent research indicates that the progression of age-related macular degeneration (AMD) is closely linked to the mismanagement of neuroinflammatory pathways, leading to the death of retinal neurons and the vital photoreceptors that enable central vision. The retina, like other parts of the central nervous system, benefits from a specialized immune status, managed by retinal glial cells including microglia and Müller glia. When photoreceptors are damaged, microglia are summoned to the injury site, becoming overactive and exacerbating the damage through inflammation. Meanwhile, Müller glia shift away from their supportive roles, further fuelling the inflammation that guides microglia to the distressed photoreceptors.

Diminishing this neuroinflammation necessitates silencing of immune responses from both microglia and Müller glia simultaneously. ***The challenge faced in the development of therapies against neuroinflammation stems from the fact that neuroinflammation in both microglia and Müller glia is controlled by complex biological pathways that requires simultaneous suppression.***

***The innovation in our approach lies in targeting the transcription factors that oversee these extensive gene networks, a strategy that holds promise due to its success in cancer research.*** This path, unexplored in the realm of retinal neuroinflammation, offers a modern avenue to control the multitude of genes that orchestrate neuroinflammation, potentially revolutionizing AMD treatment.

**Scientific Outcomes**

1. **We identified a family of three transcription factors (TFs) that drive retinal inflammation.**

Through combined studies in animal models of retinal degeneration and human AMD retinas, we demonstrate a robust upregulation of three transcription factors in response to retinal damage (Fig.1). Consistent expression patterns between human and mouse data enhances the credibility of the mouse model as a representative tool for human disease, indicating that the biological processes observed in the mouse model are likely to be similar in humans.

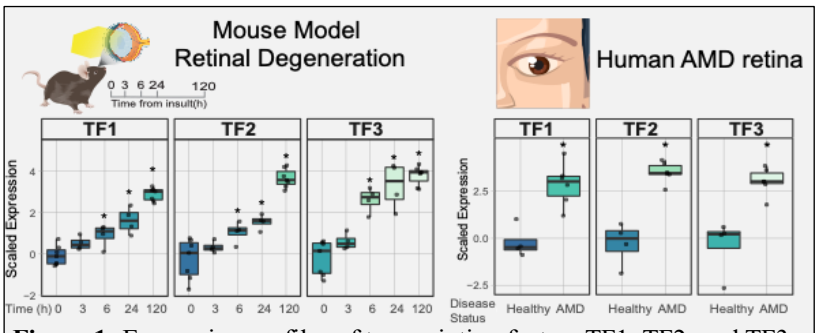


**Figure 1:** Expression profiles of transcription factors TF1, TF2, and TF3 post-retinal damage. Left panel: Time-course expression in an animal model, with treated groups (blue) compared to controls (black) over 72 hours. Right panel: Expression levels in human subjects, contrasting healthy individuals with AMD patients.

2. **We profiled the expression of these three transcription factors across major retinal cell types and show their localisation to retinal glia.**

The left panel in Fig.2 is a clustering map displaying various cell types distinguished by their unique gene expression profiles. Each dot represents a single cell retinal cell whose complete gene expression can be accessed. The right panel is a dot plot showing the relative expression levels and prevalence of TF1, TF2 and TF3 across the cell types. The size of the dots indicates the percentage of cells expressing

the factor (prevalence), and the color intensity represents the expression level (with darker colors indicating higher expression). Together, these visual data imply that for TF1, TF2, and TF3 are predominantly located in retinal Muller glia and microglia. By identifying these transcription factors in glia, we pinpoint the crucial influence these cells have in the inflammatory cascade that leads to retinal degeneration. Such insights are pivotal for devising therapeutic strategies aimed at modulating glial activity, offering the possibility to mitigate retinal inflammation with minimal neuronal interference. This precision in targeting not only enhances the potential efficacy of treatments and also minimizes the risk of adverse effects.

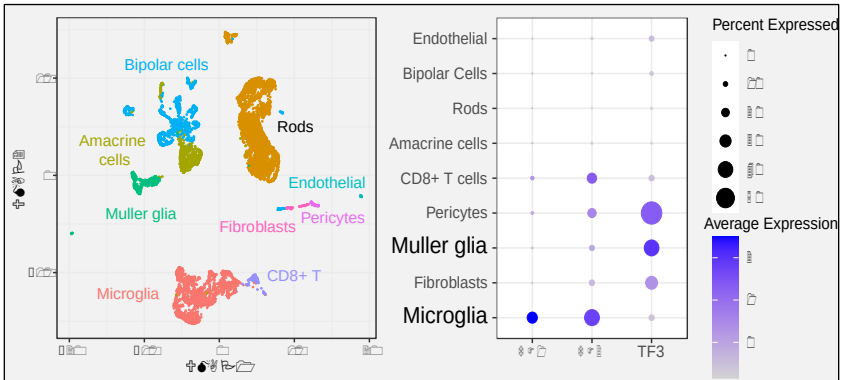


**Figure 2:** Single cell gene expression in the retina and cellular location of TF1, TF2 and TF3.

3. **The suppression of these three transcription factors reduces inflammation and increases cell survival.**

Using our mouse model of retinal degeneration, we investigated whether inhibiting three key transcription factors could mitigate inflammation and protect against retinal cell death. In Figure 3A, we observe a heatmap displaying reduced expression of genes known to regulate inflammation in animals treated with a siRNA cocktail targeting all three transcription factors, suggesting effective inflammation control. Figure 3B depicts an enhanced response to light stimuli in treated animals, indicating preserved retinal function. Lastly, Figure 3C demonstrates that the retinal layer where photoreceptors reside is notably thicker in treated mice, implying decreased cell death and heightened neuronal survival, which may correlate with improved visual outcomes.

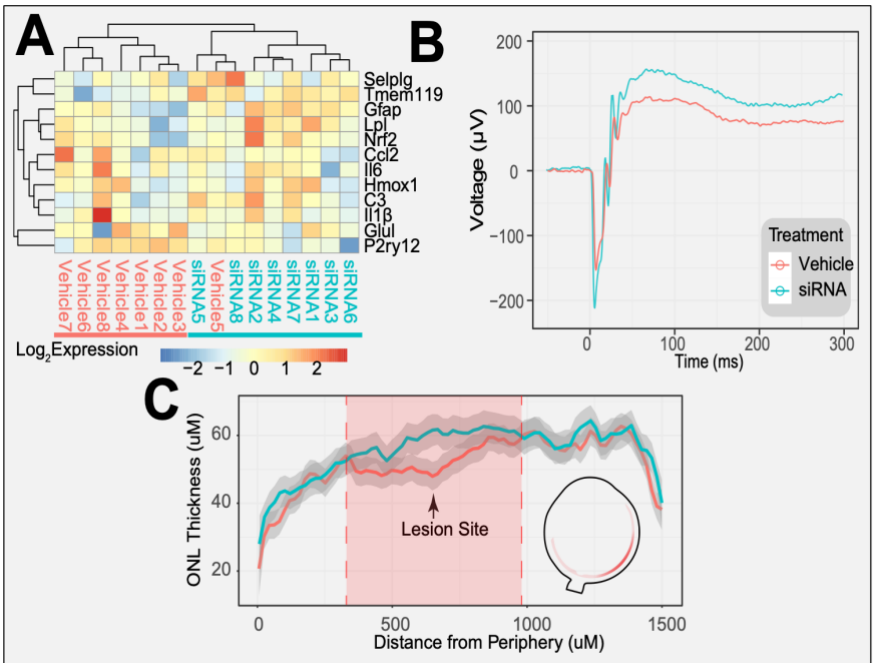


**Figure 3: Inflammation and retinal function in mice treated of TF1, TF2 and TF3 siRNA inhibitors.**

**Significance**

This project addresses a critical gap in AMD treatment by targeting the inflammatory processes at their molecular roots. By focusing on transcription factors in Müller glia and microglia, this research has the potential to yield a novel class of therapeutics for AMD, offering hope to millions suffering from this debilitating condition.