Microglia and retinal degenerations: Identifying key modulators of inflammation as therapeutic targets

The most common cause of blindness in Australia is **Age-Related Macular Degeneration (AMD)**, costing the Australian economy ~5 billion dollars annually [1] and ~350 billion dollars globally. Current projections indicate that by 2030, 1.7 million people in Australia will suffer vision loss due to AMD, with a major contribution being the current lack of treatment options available for the more prevalent atrophic or 'dry' form of the disease. AMD shares a number of pathophysiological states with other ageing neurodegenerative diseases such as Alzheimer's and Parkinson's disease, including: i) disease prevalence, which increases with age; and ii) neuroinflammation, in which activated microglia, the resident immune cells of the central nervous system (CNS), and blood-borne macrophages are recruited to areas of tissue damage.

Dysregulation of microglia and macrophages is a key pathogenic mechanism underlying many age-related neurodegenerative diseases, highlighting the importance of understanding how these cells respond to ageing and stress.

We have previously demonstrated in both human AMD tissue and animal models that resident microglia and/or recruited macrophages, are key players in the progression of retinal degenerations [2-4], and contribute to AMD pathogenesis (reviewed in [5]). We have demonstrated that reducing microglia/macrophage migration and activation [6] correlates with increased photoreceptor survival and retinal function [7-9]. Following the breakthrough discovery that microglia have a distinct embryonic origin [10] compared to recruited macrophages, the question remains as to whether differences in microglia and macrophage cellular origin influence their responses to stress and their contributions to progressive degeneration.

Funding provided by Retina Australia has allowed us to investigate the role that both microglia and macrophages play in response to retinal degenerations, and identify microRNA (miRNA) as a key player in the regulation of these immune cells. miRNA are small endogenously expressed non-coding RNA molecules that post-transcriptionally regulate gene expression (reviewed in [11]). They are abundant in the CNS (reviewed in [12]), play a key role in regulating inflammation (reviewed in [13]), and are involved in the progression of retinal degenerations [14]. Several miRNAs have been implicated in the regulation of inflammation, including miR-124 and miR-155, which have been suggested as possible therapeutic targets in other neurodegenerative diseases such as Alzheimer's and Parkinson's [15-17]. We have demonstrated that miRNA dysregulation is a key feature of retinal damage, using a model of photo-oxidative-induced retinal degeneration (Figure 1).

Specifically the funding has enabled us to:

1. Establish a culturing technique for retinal microglia in order to study their changes in response to stress and damage (Figure 2).
    a. This work is now complete and a manuscript is being prepared for publication in late 2019.

2. Establish a reporter strain to allow us to visually identify microglia and macrophages [18], allowing for further dissection of the miRNA differences between microglia and macrophages, especially those pertaining to inflammatory pathways (Figure 3).
    a. This work is ongoing and further funding has been obtained to complete this project.
3. Demonstrate that miR-124 provides protection against retinal degenerations and that miR-124 is pivotal in maintaining normal retinal homeostasis.
    a. This project is complete and a manuscript is being prepared for publication.
4. Demonstrate that miR-155 is differentially regulated in microglia and macrophages in response to retinal damage. Reducing the expression of miR-155 can reduce retinal inflammation and degeneration (Figure 3).
    a. Work is almost complete and is being prepared for publication.

All members of my research team would like to thank Retina Australia for their ongoing support to our research. This funding has laid the foundations for a number of developing projects and enabled the procurement of significant secondary funding, including an ANU Translational Fellowship. This work and the growth of my research group would not have been possible without this important contribution from Retina Australia. We would also like to acknowledge the wonderful support from the local members of the ACT Retina Australia Group for their ongoing support to members of the ACT community and in working with the ANU to support local vision research.

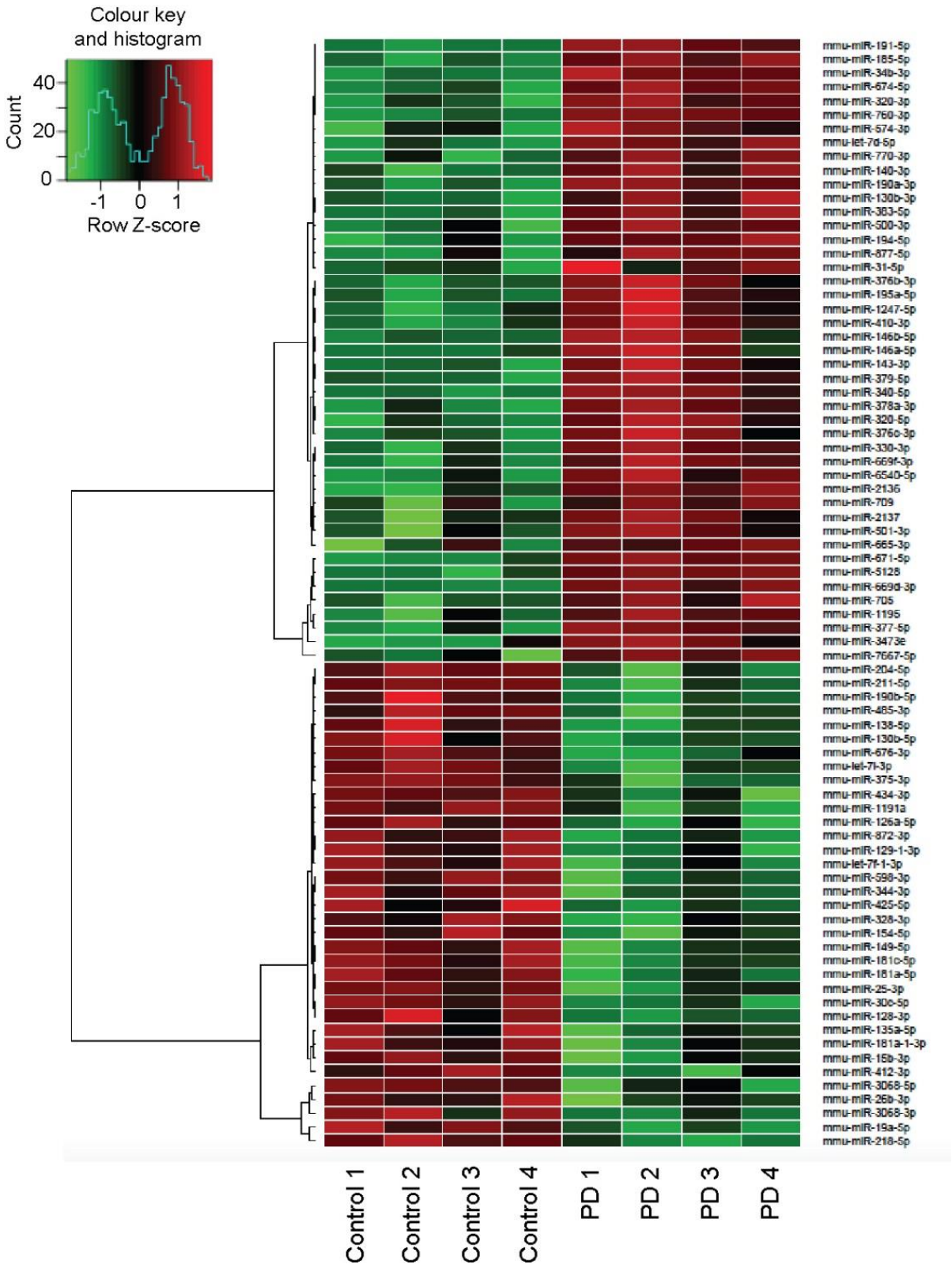

Figure 1. Retinal miRNA changes following photo-oxidative damage (PD). A heat map depicting the expression changes of the top 80 differentially expressed miRNA between control (dim-reared) and photo-oxidative damaged retinas. Strong correlation was observed between the samples of the two variables (green indicates decreased expression, red indicates increased expression).

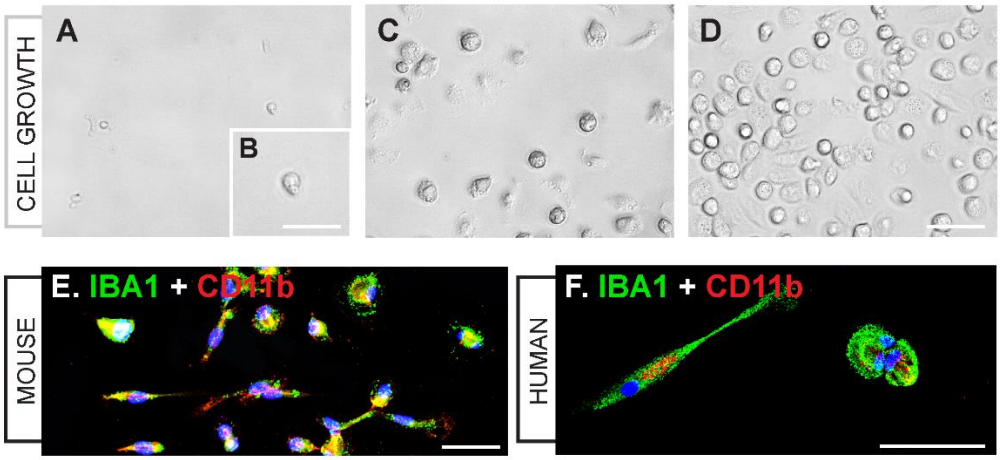

Figure 2. Culturing of primary CD11b^{+} retinal microglia. **A-D:** Microglia were isolated from retinas using FACS, and were supplemented with GM-CSF and M-CSF for 4 weeks. Representative images show cells at day 1 (A-B), 2 weeks (C) and 4 weeks (D) after isolation. **E-F:** Immunolabelling using IBA1 and CD11b markers for microglia/macrophage populations isolated from mouse (E) and human (F) retinas. Scale bar is 50µm (25µm for B).

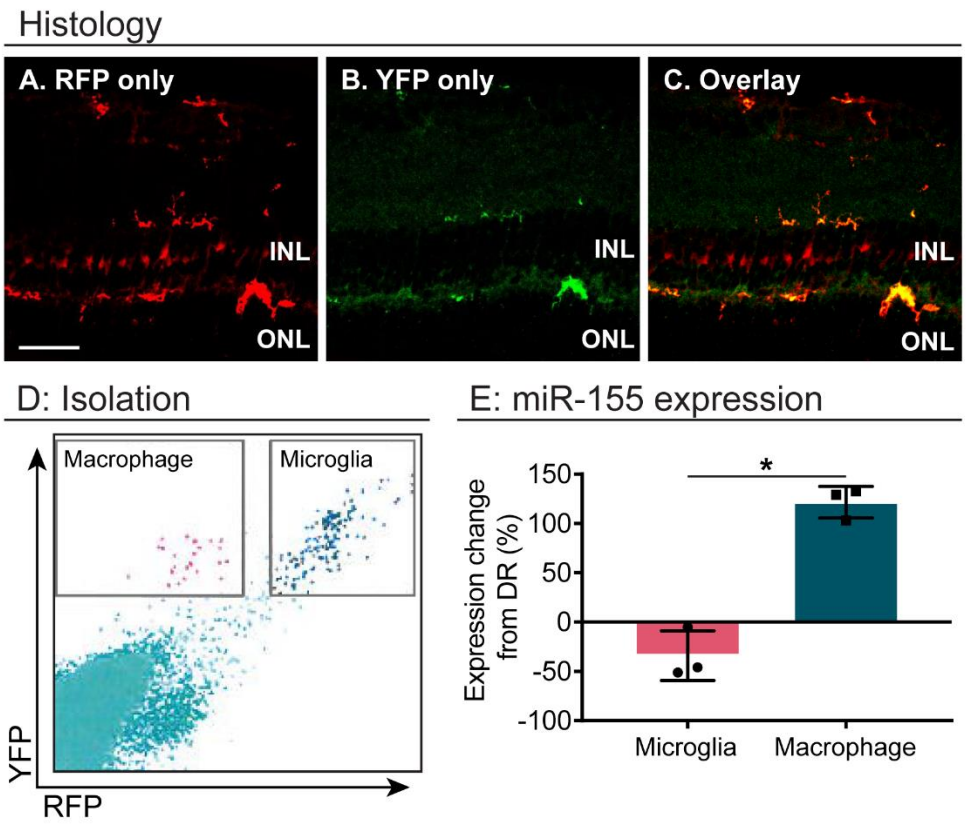

Figure 3. A-C: Establishment of a fate-mapping reporter strain of microglia and macrophages (red RFP^{+}, A) to distinguish microglia (yellow RFP^{+} YFP^{+}, C) vs macrophages (green YFP^{+}, B). **D:** FACS isolation plot of YFP^{+}/RFP^{+} cells show a clear distinction between the microglia and macrophage populations in the retina. **E:** miR-155 expression was significantly different between microglia and macrophage populations. Scale bar is 100µm.

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