Report for Retina Australia project

Development of regenerative therapy for retinitis pigmentosa using cellular reprogramming

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Project summary:

Photoreceptors are the light-sensing cells in the retina that enable vision. The loss of photoreceptors is a key hallmark of many degenerative eye diseases, including retinitis pigmentosa (RP) which affects >1 million people worldwide. While some options exist to forestall such photoreceptor degeneration, there are no effective means to cure blindness once photoreceptors are lost.

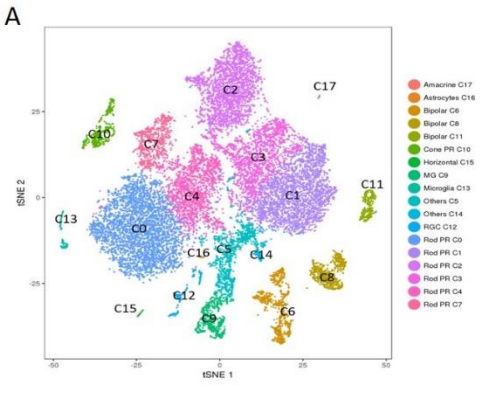
Cell reprogramming technology could change this, restoring vision by regenerating these lost cells. This technology allows us to convert cells from one type into another by activating certain key genes. This gives us a unique opportunity to generate photoreceptors in the lab, and the exciting possibility of regenerating photoreceptors in the patient's eye to restore vision.

To harness this potential, this project aims to develop cell reprogramming technology that targets the stem cells within the retina (the Müller glial cells) to regenerate into photoreceptors. Firstly, we built a world's first human retina gene atlas at single cell resolution. This atlas provided the crucial genetic information to develop cell reprogramming method to target Müller glial cells to regenerate photoreceptors. Using this knowledge, we have successfully developed a cell reprogramming method to generate human photoreceptors in a lab dish. Detailed characterisation of the derived photoreceptors showed that they expressed the right marker genes and proteins and are functional in light detection, providing supporting evidence to the feasibility of our reprogramming method to generate human photoreceptors. Also, we tested the feasibility of our cell reprogramming technology in rodent models. We developed a linage tracing system that allowed us to identify newly regenerated photoreceptors within the rodent's eye, and obtained pilot safety data for application of cell reprogramming technology in rodents. We are in the process of analysing the efficacy of using cell reprogramming for photoreceptor regeneration in rodents.

The team is truly grateful to the generous support from Retina Australia to advance this project. Overall, our findings provided new insights into the genetic signal that enables vision, and pave the way to develop cell reprogramming technology as a novel regenerative therapy to treat photoreceptor losses. This would have direct implications in treating RP, as well as other blinding diseases with photoreceptor losses such as age-related macular degeneration and Stargardt's disease.

Scientific outcomes:

1. We have built a world's first human retina gene atlas at single cell resolution (1,2; Figure 1). This atlas contained the gene expression profile for all major cell types within the human neural retina. Using this crucial genetic information, we have computationally predicted the candidate genes that can be used for cell reprogramming to generate photoreceptors.



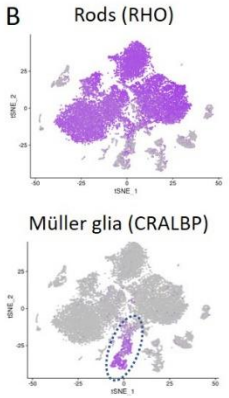


Figure 1: A) Human retina gene atlas with transcriptome of major cell types in the retina, including B) rod photoreceptors (RHO+), and Müller glial cells (CRALBP+) (2).

2. Using a CRISPR activation system that we have optimized (3), we showed that we can efficiently activate up to 9 candidate genes in human Müller glial cells. This system greatly increases our capacity to screen for different gene combinations and enabled us to identify the optimal condition for cell reprogramming to generate human photoreceptors.
3. We developed a novel cell reprogramming method to convert human Müller glial cells into photoreceptors. Immunocytochemical analysis showed that the derived photoreceptors expressed photoreceptor markers RHO and PDE6B. Multielectrode array analysis showed that the derived photoreceptors possessed functional electrophysiology and are capable of light detection.
4. We developed a lineage tracing system using Cre-loxP that allows us to label retinal cells in red and Muller glial cells in green (Figure 2). This lineage tracing represents a crucial tool to trace cell reprogramming in the eye and identify newly regenerated photoreceptors in animal models.

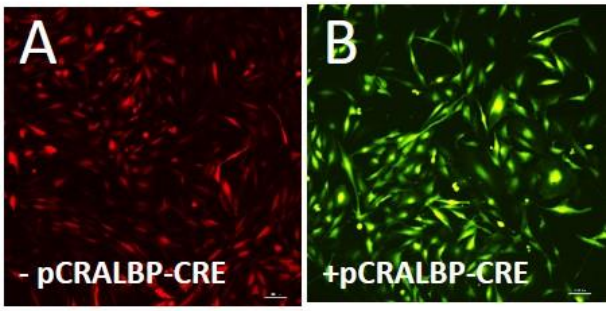


Figure 2: Verification of the two-vector lineage tracing system, which allow colour switch from A) DsRed to B) GFP that is controlled by specific CRE expression in Muller glial cells.

5. Our pilot data showed that viral injection of reprogramming genes does not harm the vision in healthy rats, which support the safety of application of cell reprogramming in vivo.

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