

Retina Australia – 2005 Report

Name of grantee: Prof PE Rakoczy

Institute: Lions Eye Institute / Centre for Ophthalmology and Visual Science

Project title: Can the process of advanced retinal degeneration be prevented or slowed down in the *Rpe65*^{-/-} models of LCA?

Project summary:

The retinal pigment epithelium (RPE) is the outermost layer of the retina. It is made up of a single layer of pigmented cells, known as RPE cells. The RPE cells play a key role in normal retinal functioning, including several actions that assist, support and maintain the photoreceptors. Genetic mutations and malfunctions in the RPE cells are the cause of many ocular diseases including retinal degenerations, retinitis pigmentosa, Leber's congenital amaurosis (LCA) and inherited macular degeneration.

A possible treatment for these genetic diseases of the retina is the use of gene therapy. Gene therapy is based on overcoming the negative effects caused by a mutated, non-functioning gene, by inserting a normal, functioning copy of a gene into the diseased retinal cells. Our particular focus has been on using "virus-mediated" gene therapy, where a laboratory virus, that has been experimentally modified to become harmless (a "recombinant" virus), is used as a vehicle to deliver the normal, functioning gene to inside the retinal cells. We have been one of the few research groups internationally to show that virus mediated gene therapy is able to stop some of the disease effects caused by retinal gene mutations (Lai et al, 2004; Narfström et al 2003; Lai et al, 2005).

The success of our virus mediated gene therapy work is very exciting, and suggests that this therapy may, in the future, provide a treatment for patients with genetically based retinal diseases. Unfortunately, our work also showed us the limitations of the virus-mediated gene therapy technology, for example, the restricted length of time over which the treatment worked (Lai et al, 2004). If virus-mediated gene therapy is going to be used to treat patients in the future it is critical that this technology is improved. One area where the virus-mediated gene therapy can be improved is to ensure that the normal, functioning gene is only activated, or more precisely "expressed", within the correct type of retinal cell, in our case the RPE cells.

This current project is focussed on developing a technology that will allow us to express our normal, functioning gene only within the RPE cells of the retina. We have been achieving this aim by producing by engineering a unique segment of DNA, a "gene control fragment", that has the ability to control the expression of our gene, and limit it solely to the RPE cells (the "hR65CE-GV" promoter and control element are described in detail in Zhang et al, 2004; Sutanto et al, 2006). So far we have designed and produced our control fragment and tested its function within RPE cells in the laboratory. This work has shown us that the control fragment we produced works well, with good gene expression amounts and expression only occurring in RPE cells (Zhang et al, 2004; Sutanto et al, 2006). The 2005 funding from Retina Australia allowed us to then move forward to the next stage of the project, that is, to insert our control fragment into our engineered gene therapy virus, in preparation for testing out new technology in laboratory animals *in vivo*.

Retina Australia 2005 - results

The work achieved on the project in 2005 involved the construction and production of the new gene therapy virus containing our control fragment, and also the thorough testing and documentation of the virus and its gene expression that is needed before any in vivo work can be performed. We produced two new gene therapy viruses this year, both containing our gene control fragment, but with differing genes to be expressed. The first virus contained a control gene in the form of a fluorescent marker (green fluorescent protein, GFP) that will allow us to easily track the location and gene expression from our gene therapy viruses. The other contained a normal, functioning copy of the RPE65 gene, an important retinal gene that is found in RPE cells and whose mutation leads to Leber's congenital amaurosis (LCA) in children. The RPE65 gene was chosen because we have an ideal test model for this gene in the form of a mouse strain (*Rpe65*^{-/-} mice). These mice have a mutated, non-functioning copy of the RPE65 gene, and succumb to a retinal degenerative disease very similar to LCA.

The production of the two gene therapy viruses, the GFP and RPE65 viruses, was performed in two stages; firstly the genetic engineering of the gene therapy viruses to insert our control fragment into their DNA; and secondly, to actually make the complete viruses, a method that is performed in the laboratory using specialised cell strains. Once the two gene therapy viruses were produced, the rest of the year was spent on testing and examining them in terms of their ability to express the GFP and RPE65 genes; their ability to only express these genes in RPE cells; and a series of safety tests including checking for damage and/or mutations, and confirming that they are unable to reproduce themselves outside our controlled laboratory environment. Large-scale production of the gene therapy viruses is now underway, with the hope of obtaining further funding to test them in laboratory animals in vivo. The results produced during this project will lead to at least one scientific publication (Zhang et al, 2006), and form the basis of our current NHMRC application (submitted 21st Apr, 2006 for funding commencing Jan 2007). We sincerely thank Retina Australia for supporting this project.

References:

Lai CM, Yu MJ, Brankov M, Barnett NL, Zhou X, Redmond TM, Narfstrom K, **Rakoczy PE**. Recombinant adeno-associated virus type 2-mediated gene delivery into the *Rpe65*^{-/-} knockout mouse eye results in limited rescue. *Genet Vaccines Ther*. 2004, 2:3.

Narfstrom K, Katz ML, Bragadottir R, Seeliger M, Boulanger A, Redmond TM, Caro L, Lai CM, **Rakoczy PE**. Functional and structural recovery of the retina after gene therapy in the RPE65 null mutation dog. *Invest Ophthalmol Vis Sci*. 2003, 44: 1663-72

Lai C-M, Shen W-Y, Brankov M, Lai YKY, Lee S-Y, Yeo IYS, Mathur R, Pineda P, Barathi A, Ang C-L, Constable IJ, **Rakoczy EP**. Long-term evaluation of AAV-mediated sFlt-1 gene therapy for ocular neovascularization in mice and monkeys. *Mol Ther* 2005, 12:659-68.

Zhang D, Sutanto EN, **Rakoczy PE**. Concurrent enhancement of transcriptional activity and specificity of a retinal pigment epithelial cell-preferential promoter. *Mol Vis*. 2004, 10: 208-14.

Sutanto E, Zhang D, Lai YKY, Shen W-Y, **Rakoczy PE**. Potential use of cellular promoter(s) to target RPE in AAV-Mediated delivery. In: *Retinal Degenerative Diseases* (JG Hollyfield, RE Anderson, MM La Vail, Eds) 2006, Chapter 37, 572:267-273

Zhang D, Lai C-M, **Rakoczy PE**, Recombinant adeno-associated virus 2 mediated cell-specific gene expression in the retinal pigment epithelium. 2006, Manuscript in preparation