**Minnesota Regenerative Medicine**
**FINAL Progress Report**
**Date: June 6, 2017**
**Grant Title: Toward Autologous Retinal Cell Replacement Therapy for Age-related Macular Degeneration**
**Grant Number: MRM 2015 5337**
**Principal Investigator: Deborah Ferrington, PhD**
**Co-Investigators: James Dutton, PhD and Ching Yuan, PhD**
**Project Timeline: May 1, 2015 - April 30, 2017**

**Overview of project:**

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly. This disease causes individuals to lose their central vision, which affects activities such as reading, driving, and face recognition, and has a significant negative impact on daily function and quality of life. AMD is especially prevalent among individuals of Northern European heritage and thus of great importance in Minnesota since 32% of the population are of Scandinavian ancestry. A key feature of AMD is the loss of retinal pigment epithelial (RPE) cells. The goal of the proposed work is to develop new clinical strategies that either protect the RPE and slow disease progression, or repair the damage created by the disease. Taking a personalized medicine approach, we envision using induced Pluripotent Stem Cell (iPSC)-derived RPE developed from patient biopsies to either identify drugs that promote RPE survival or as an autologous transplant therapy for RPE cell replacement.

**Progress to Date:**

**Aim 1: Differentiate iPS cell lines from AMD and healthy donor conjunctival cells into RPE**

Results from this work have been published this year in *PLOS ONE* (Geng et al., 2017). In this publication, we report about the successful generation of iPSC-derived RPE from conjunctival biopsies of five different human donors; two controls (no disease) and three with AMD. The process involved transducing conjunctival cells with iPSC reprogramming factors using Sendai virus based vectors (Cytotune 2.0™ Invitrogen). The method used to generate RPE from the iPSC-lines included a defined 14-day induction of RPE, followed by expansion of iPSC-derived RPE that form pigmented monolayers. These results indicate that we have been successful in generating iPSC-derived RPE from donors with and without AMD. The RPE induction protocol consistently provides RPE that closely matches the phenotype and gene expression of primary RPE. Furthermore, we have developed these cells under cGMP conditions so that the transition to clinical application should be seamless.

**Aim 2: Compare primary cultures of RPE with iPSC-derived RPE from the same donor**.

As a first step in completing this aim, we had to establish the validity of our RPE primary cultures. Our first manuscript characterizing the primary RPE cultures was recently accepted for publication in *Redox Biology* (Ferrington et al., Redox Biology, in press). In this paper, we asked whether RPE from donors with AMD differ in their metabolic profile compared with healthy age-matched donors. Analysis of gene expression, protein content, and RPE function showed that these cultured cells replicated many of the cardinal features of RPE *in vivo*. Using the Seahorse Extracellular Flux Analyzer to measure bioenergetics, we observed RPE from donors with AMD exhibited reduced mitochondrial and glycolytic function compared with healthy donors. RPE from AMD donors were also more resistant to oxidative inactivation of these two energy-producing pathways and were less susceptible to oxidation-induced cell death compared with cells from healthy donors. Investigation of the potential mechanism responsible for differences in bioenergetics and resistance to oxidative stress showed RPE from AMD donors had increased PGC1α protein as well as differential expression of multiple genes in response to an oxidative challenge. Based on our data, we proposed that cultured RPE from donors phenotyped for the presence or absence of AMD provides an excellent model system for studying “AMD in a dish”. Our results are consistent with the ideas that (i) a bioenergetics crisis in the RPE contributes to AMD pathology, and (ii) the diseased environment *in vivo* causes changes in the cellular profile that are retained *in vitro*.

We are preparing an additional manuscript that compares primary RPE to iPSC-derived RPE from the same donor. Since we observed the greatest difference in mitochondrial function between diseased and

control cells, we have focused on that functional assay for our comparison. Mitochondrial function measured in iPSC-derived and primary RPE from two different donors using a Seahorse XFe96 Extracellular Flux Analyzer showed that iPSC-derived and primary RPE had similar mitochondrial function under basal conditions. However, when we measured mitochondrial function under conditions of oxidative stress, the primary RPE were more resistant to peroxide-induced changes. These results have important implications for future use of iPSC-derived RPE in transplantation therapy. It suggests that iPSC-derived RPE would benefit from a pre-conditioning program prior to their transplantation so that they can better withstand the harsh environment of the diseased retina.

**Aim 3: Evaluate the efficacy of compounds that protect the RPE from physiologically relevant challenges.**

In our previous work, we showed that RPE from human donor's with AMD had significantly more mtDNA damage compared with age-matched non-diseased donors and in cultured cells, mitochondrial function was reduced. These results suggest protecting or enhancing mitochondrial function as a potential treatment modality for AMD. Maintenance of mitochondrial function is also essential for RPE cell health and in the ability of RPE to adapt to changing cellular conditions. We have tested three different FDA-approved compounds that protect mitochondria from damage (N-acetylcysteine, NAC), increase mitochondrial biogenesis (pyrroloquinoline, PQQ), or enhance removal of damaged mitochondria through mitophagy (rapamycin, Rapa). All of these compounds were effective in improving mitochondrial function, although the response, i.e., kinetics and magnitude of change, varied between donors. We also observed that there was good synergy between some of the drugs when they were administered together. As observed with the response to single drugs, the response varied between donors. These results support the need for a "personalized medicine" approach to treating AMD.

**The following that have resulted from the Minnesota Regenerative Medicine grant funding:**

**Publications and/or manuscripts submitted for publication:**

Geng Z, Walsh PJ, Truong V, Hill C, Ebeling M, Kapphahn RJ, Montezuma SR, Yuan C, Roehrich H, Ferrington DA, Dutton JR (2017) Generation of retinal pigmented epithelium from iPSCs derived from the conjunctiva of donors with and without age related macular degeneration. *PLOS One* 12 (3), e0173575.doi:10.1371

Ferrington DA, Ebeling MC, Kapphahn RJ, Terluk MR, Fisher CR, Polanco JR, Roehrich H, Leary MM, Geng Z, Dutton JR, Montezuma SR (2017) Altered Bioenergetics and Enhanced Resistance to Oxidative Stress in Human Retinal Pigment Epithelial Cells from donors with Age-related Macular Degeneration. *Redox Biology*, in press.

**Disclosures/patents**: NONE

**Grant applications and/or awards**:

**RO1 EY028554**, submitted to NIH in February, 2017. (Reviewed June 19-20, 2017)
Deborah Ferrington and James Dutton (Multiple-PI), "Mitochondrial defects in the retinal pigment epithelium and the CFH risk factor for age-related macular degeneration".