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Edward N. and Della L. Thome Memorial Foundation, Bank of America, N.A. Trustee,
Awards Program in Age-Related Macular Degeneration Research

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\$750,000 Awards; December 15, 2009 – December 14, 2012

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2009 Three-Year Award Recipients
Edward N. and Della L. Thome Memorial Foundation, Bank of America, N.A. Trustee,
Awards Program in Age-Related Macular Degeneration Research

Rajendra Apte, M.D., Ph.D.

Assistant Professor, Ophthalmology & Visual Sciences
Washington University School of Medicine

“The Role of Cholesterol in Regulating the Pro-Angiogenic Properties of Senescent Macrophages in Age-Related Macular Degeneration”

Scientific Abstract

The macrophage is a key component of the innate arm of immunity and is critical in regulating initial immune response to tumors, infections and in inflammation. The macrophage is also a central player in sustaining immune privilege in the eye. Immunosenescence is characterized by age-related changes in both the innate and adaptive compartments of the immune system. Innate immunity, specifically macrophage function, has received particular attention in the eye as it can modulate developmental and post-developmental angiogenesis. Choroidal neovascularization plays a central role in visual impairment and blindness in age-related macular degeneration (AMD), the leading cause of blindness in people over 50 years of age in North America.

The work described in this proposal will help elucidate the mechanisms by which senescence induces a functional drift in macrophages towards a deleterious pro-angiogenic phenotype. These questions are especially relevant to the importance of macrophages in neovascular AMD. These goals will be accomplished by demonstrating that abnormal processing of cholesterol, a dominant component of drusen, causes old macrophages to become pro-angiogenic. The experiments outlined in this proposal are specifically designed to identify critical pathways in the regulation of cholesterol that are potentially important in how macrophages regulate choroidal neovascularization. Our results will directly identify specific targets for translational research for which there are currently available therapeutic agents. Such an approach would facilitate rapid development of clinical protocols in order to develop new agents that prevent blindness from this devastating disease.

Peter Campochiaro, M.D.

Eccles Professor of Ophthalmology
Johns Hopkins University School of Medicine

“Sustained Delivery of Antiangiogenic Peptides for Neovascular Age-Related Macular Degeneration”

Scientific Abstract

Ranibizumab, an Fab that binds vascular endothelial growth factor (VEGF), has provided benefit to patients with neovascular age-related macular degeneration (NVAMD), but there is room for improvement. With monthly injections, 34-40% of patients achieve substantial improvement in vision, but 60% do not. In follow-up portions of phase III studies when patients were switched from monthly dosing to prn treatment, most of the visual gains were lost. VEGF antagonists reduce leakage from choroidal neovascularization (CNV) and suppress growth, but do not cause regression of existing CNV; therefore the minority of patients with NVAMD who achieve substantial improvement with ranibizumab may require frequent injections for the rest of their lives to maintain those gains. Thus, new treatments are needed for NVAMD.

Several endogenous proteins have antiangiogenic activity and also cause regression of established CNV, which could provide benefit in patients not helped by ranibizumab and may "reset the clock" and reduce the need for frequent injections in those that respond to ranibizumab. However, these proteins are large and not ideally suited for use as therapeutic agents. Using bioinformatics, short candidate sequences sufficient for antiangiogenic activity have been identified. These sequences are around 12-24 amino acids in length and can be readily synthesized using solid-phase methodology; eventually, this methodology could be used to effectively produce the peptides for clinical applications. Synthetic peptides derived from several of the candidate sequences have been demonstrated to suppress endothelial cell proliferation and migration in vitro and neovascularization in vivo in angiogenesis assays, including corneal and laser-induced choroidal neovascularization. Using the most promising peptides identified in the initial screens and nanoparticle technology, we propose to develop a new treatment for NVAMD. Two models of subretinal NV (with testing for suppression and regression of NV in each) that have been demonstrated to have predictive value for benefit in patients with NVAMD will be used to test individual naked peptides and peptides packaged in nanoparticles to identify an optimal therapeutic agent for testing in clinical trials. These studies could lead to a complementary treatment that will further gains already achieved with VEGF antagonists and improve the lives of patients with NVAMD.

Constance Cepko, Ph.D.

Professor, Department of Genetics and Ophthalmology
Investigator, Howard Hughes Medical Institute
Harvard Medical School

“Promotion of Photoreceptor Survival using HDACs and Gluconeogenic Genes”

Scientific Abstract

Two types of photoreceptors initiate vision in vertebrates: rods, for low light vision, and cones, for bright light and color vision. In humans, cones comprise only 5% of the photoreceptors. However, a specialized central area of the retina, the fovea (within the macula), comprises only cones, and it is this area that is used for all of our high acuity vision. Unfortunately, this is the area which is quite susceptible to degeneration in age related macular degeneration (AMD). There are also many diseases that affect the retina more generally, with 197 genes mapped which lead to loss of vision, most affecting photoreceptors. Gene replacement, using viral vectors, for those diseases where a genetic lesion is known to be causal, is being attempted. However, given the number of genes, and the fact that many of the mutations are dominant, it will be difficult to approach each type of genetic lesion with a specific gene replacement or knock-down. Gene therapy can be envisioned, however, if it is aimed at a common cause for rod and/or cone death, no matter what the underlying cause. This is the basis for this application. We found that the addition of the histone deacetylase 4 (HDAC4) gene to rod photoreceptors promoted their survival in a murine model of retinitis pigmentosa (RP). We more recently found that HDAC4 could promote cone survival when transduced directly to cones in vivo.

We have also investigated the mechanism of cone death in four different murine models for human RP. Following rod death, the cones appear to be nutritionally deprived and undergo self-digestion, or autophagy. We have been able to promote survival and function of cones in one of these animal models by delivering a combination of 3 genes that endowed cones with the ability to make their own glucose. We wish to optimize this approach by using different viral vectors. If successful, HDAC4 and/or gluconeogenic genes may promote the survival of photoreceptors, and perhaps other neurons, in AMD as well as in other blinding illnesses.

Margaret DeAngelis, Ph.D.

Assistant Professor of Ophthalmology
Director, Ocular Molecular Genetics Institute
Massachusetts Eye & Ear Infirmary

“Identifying Underlying Mechanisms of Age-Related Macular Degeneration for the Development of Appropriate Preventive and Therapeutic Interventions”

Scientific Abstract

The overall goal of our research is to elucidate key regulatory components in pathways which are implicated in the development of neovascular age-related macular degeneration (AMD) so that appropriate preventive and therapeutic targets can be developed. We propose to accomplish this goal by analyzing key regulatory components in a defined novel network/pathway of genes that show significant altered expression from our recent studies of patients with neovascular AMD compared to their normal siblings using high throughput ChIP-seq based assays (chromatin immunoprecipitation followed by direct sequencing). Specifically we will determine which genes and their regulatory regions are directly regulated by retinoic acid receptor-related orphan receptor alpha (RORA), an anti-angiogenic transcription factor, that we have identified as a key protective agent against neovascular AMD, in 3 different cohorts (family based; unrelated case-control and prospective nested unrelated case-control), using high throughput ChIP-seq based assays on lymphoblastoid cell lines from our patient cohorts as well as human autopsied eyes with and without neovascular AMD. Examining both patient cell lines and patient ocular retinal tissue will not only help us to determine tissue specificity of RORA binding, but help to elucidate whether or not AMD is a systemic disease or a localized disease, ultimately determining avenues for drug delivery. These studies should help us to 1) determine where in the human genome RORA binds; 2) how the specificity of binding is achieved; 3) how RORA affects gene expression; 4) how this expression is related to genes that function in RORA's pathway(s). We will also evaluate regulatory components in HTRA1/ARMS2, gene(s) from our recent studies that showed epistatic interactions between this gene(s) and RORA. Concurrently with our human in vitro studies, we will characterize the functional consequences of Rora gene expression using a tissue specific conditional knock-out mouse model that lacks Rora (Rora^{-/-}) exclusively in the retina and determining whether Rora or a combination of genes in the Rora pathway are able to rescue retinal phenotypes similar to those found in patients with AMD. We anticipate that this study will lead to the identification of druggable targets for the treatment and prevention of neovascular AMD.

Albert Edwards, M.D., Ph.D.
Senior Research Associate
Institute for Molecular Biology
University of Oregon

“Pathophysiology of AMD”

Scientific Abstract

This proposal addresses two important questions for advancing our understanding of the pathophysiology of age-related macular degeneration (AMD). The first question is what are the biological pathways that mediate the genetic risks conferred by the age-related maculopathy susceptibility locus 2 (ARMS2) on chromosome 10q26. Genetic variants on haplotypes (segments of DNA inherited as a block) in this region alter the risk of developing AMD. These haplotypes span a hypothetical gene called LOC387715 (or ARMS2 by some) and the promoter region of a protease involved in transforming growth factor signaling and blood vessel homeostasis called high temperature requirement A1 (HTRA1). Which exact DNA sequence changes on these haplotypes alter AMD risk is the subject of intense investigation by us and others. Here we propose to determine the differences in RNA expression (transcriptome) within donor retina and retinal pigment epithelium (RPE) between subjects homozygous for either the protective or risk haplotypes.

The second aim to test the hypothesis that increased activation of the alternative complement pathway in blood could contribute to AMD in humans. This hypothesis arises from the observation by us and others that complement activation is increased in the blood of patients with AMD compared to controls. Further, we have shown that many of the genetic risks that contribute to AMD underlie the increased activation of complement in blood. The simplest explanation is that some of the genetic variation in complement pathways leads to increased activation of complement in the fluid phase (e.g., blood, tissue fluid) and this contributes to formation of AMD. We propose to test this hypothesis by creating animal models of increased activation of complement. We will look for AMD-like endpoints in these animals and determine the impact, if any, of systemic activation of complement on the retina, RPE and Bruchs membrane.

The answers to both questions should have immediate implications for future research in AMD and management of patients. The first question will provide insight into the biological pathways altered by variation at 10q26, while the second question will determine if chronic reduction in complement activation should be considered to reduce the incidence of AMD.

Scott Fraser, Ph.D.

Anna L. Rosen Professor, Division of Biology
California Institute of Technology

"Novel Diagnostic for AMD"

Scientific Abstract

Age-related macular degeneration (AMD) is the leading cause of severe visual loss in the elderly in the United States. The principal therapy for "wet" (neovascular) AMD is the intraocular injection of anti-angiogenic agents such as ranibizumab (Lucentis®, Genentech). This stabilizes vision in over 90% of patients and significantly improves vision in approximately 1/3 of them. Because most patients with wet AMD have lost reading and driving vision when therapy is initiated, it is important to screen high-risk patients periodically; however, current imaging modalities are not able to detect reliably the early choroidal neovascularization (CNV) of pre-symptomatic wet AMD.

We have modified optical coherence tomography (OCT) to obtain phase contrast (PC-OCT), which enables small blood vessels to be detected. With this contrast, the motion of blood cells in the smallest capillaries and the Brownian motion of blood cells in leakages can be readily detected. We hypothesize that PC-OCT can visualize CNV at pre-clinical stages of wet AMD, visualize regions of leakage in active CNV, and detect the deposition of lipid (fat) in Bruch's membrane, an important risk factor for development of wet AMD.

Our experimental goals are to refine and adapt PC-OCT to optimally visualize vascular structure in the human eye. PC-OCT will follow the status of the eye longitudinally, and tools will be refined that permit the accurate comparison of data to detect eye changes. Feasibility of PC-OCT for detection of pre-clinical wet AMD will be tested in asymptomatic patients to determine if they have small regions of sub-RPE CNV and subretinal hemorrhage. Feasibility of detecting leakage from CNV will be determined by comparisons between PC-OCT and conventional angiographic imaging. The PC-OCT instrument will also be refined to image lipid accumulation of Bruch's membrane, and high-risk patients will be screened to identify detectable changes occurring before CNV ingrowth takes place.

James Handa, M.D.

Robert Bond Welch Professor, Wilmer Eye Institute
Johns Hopkins University School of Medicine

“Cigarette Smoking Induces Oxidative Damage and an Enhanced Innate Immune Response during Early Age-related Macular Degeneration”

Scientific Abstract

The long-term objectives are to identify causative pathophysiologic sites of AMD on which to develop target-specific treatments. We will study whether cigarette smoking (CS) causes oxidative damage and activates excessive innate immune mediated inflammation, and if Nrf2, the most powerful redoxsensitive transcription factor, activates a comprehensive antioxidant protective response. Nrf2 signaling decreases with smoking, and its decreased activity can be reversed by the triterpenoids. Herein, we will test the hypothesis using the following aims, that chronic cigarette smoking induces persistent oxidative stress in the fundus such that Nrf2 signaling becomes inadequate, and results in oxidative stress and an uncontrolled innate immune response with the development of AMD.

Specific Aim 1: To test the hypothesis that Nrf2 signaling protects against cigarette smoke-induced oxidative stress and excessive innate immune activation in the fundus. We will use a genetic loss- and gain-of-function strategy by placing mice in a smoking chamber or air for up to 6 months. We will test whether loss of Nrf2 in Nrf2 deficient mice results in an insufficient antioxidant response and oxidative damage. Since CS excessively activates complement and Toll-like receptors (TLR), we will determine whether these innate immune response components participate in the development of AMD. The response will be compared to increased Nrf2 signaling in Keap1 deficient mice. Specific Aim 2: To test the hypothesis that Nrf2 signaling has a differential protective effect among critical fundus cell types after cigarette smoke exposure. Protection by Nrf2 in specific fundus cell types will be delineated using the outcomes defined in aim 1 using Cre-loxP mediated, RPE-specific, photoreceptor-specific, and vascular endothelial cell-specific Nrf2 and Keap1 deficient mice. Specific Aim 3: To determine if pharmacological activation of Nrf2 protects the fundus from developing features of AMD. Instead of a genetic approach, we will use pharmacologic activation of Nrf2 with triterpenoids, which have been proven safe in a Phase I trial. We will determine whether triterpenoids given to wild-type mice exposed to cigarette smoke will activate Nrf2 signaling, and protect against oxidative and excessive innate immune mediated damage, and prevent changes seen in early AMD.

Bryan Jones, Ph.D.

Assistant Professor, Ophthalmology

University of Utah School of Medicine Moran Eye Center

“Comprehensive Characterization of the Retina-Choroid Interface in Normal Aging and Late Stage AMD Phenotypes Using an Integrated Approach that Includes Computational Molecular Phenotyping”

Scientific Abstract

Age-related macular degeneration (AMD) affects an estimated 18% of Americans from 65 to 74 and 30% older than 74. While AMD represents one of the best characterized diseases from a genetic perspective, we currently know far less about the mechanisms mediating disease progression, particularly in geographic atrophy (GA) and choroidal neovascularization (CNV).

Therefore, this project will: 1) Define normal human retinal, RPE and choroidal histology, and tissue metabolic identity through the aging process, over three decades (60-90 years) typically associated with onset of AMD; and 2) Document and compare disease progression in AMD tissues to those of normal aging, creating indices of disease-related differences and timelines for early, to late stage [geographic atrophy (GA) and choroidal neovascularization (CNV)] disease.

The pathways and mechanisms through which genetic and non-genetic risk factors modulate development of AMD pathogenesis remain largely unexplored. Moreover, current treatment for AMD is palliative and limited to late-stages or exudative forms of the disease. The long-range goal is to provide a rigorous set of AMD-related pathway and biomarker data that can be employed to develop therapies for various AMD phenotypes. We have designed studies to test the central hypothesis that specific, emergent metabolic pathways index and participate in the etiology and pathogenesis of AMD through two aims. The first aim provides a comprehensive cellular metabolic profiling at the choroid-RPE-retina interface from an unprecedented repository of human donor eyes, characterizing the normal aging eye. The second aim compares, in multivariate space, profiles from AMD eyes, including GA and CNV. These aims directly address that (i) retinas are complex heterocellular tissues composed of over 70 classes of cells and (ii) most tissues are altered in inherited retinal degenerative diseases. Defining disease and stage-specific cytoarchitectural and metabolomic responses in AMD is critical for highlighting cellular targets for intervention.

Patsy Nishina, Ph.D.
Professor
The Jackson Laboratory

“Molecular Analysis of Mouse Models that Develop AMD-like Phenotypic Characteristics”

Scientific Abstract

While notable exceptions exist, for most inherited retinal disorders there are few effective treatments or cures and, in most cases, only a rudimentary understanding of the pathogenic pathway(s) involved. A major goal of the current research, therefore, is to identify gene defects and to understand the cellular pathways and molecular mechanisms that lead to disease. Once disease etiology is understood at the molecular level, pharmaceutical or genetic therapies are more easily designed to delay onset of the disease or provide a cure. The first logical step in understanding the molecular mechanisms underlying a disease process in any genetic disease is to identify the mutated gene(s) underlying the condition, a primary goal of this application in which two newly discovered mouse eye models that develop retinal pigmented epithelial atrophy will be studied. At the successful conclusion of this research proposal, we will identify the two genes that underlie the rpea1 and rpea2 mutations that lead to RPE atrophy and subsequent photoreceptor cell death. Our biochemical and histological studies in the rpea1 and rpea2 mutants will provide critical baseline characterization of pre-clinical alterations (i.e. primary lesions) and of the pathology these mutations cause. Finally, these two well-defined mouse models will be made available to investigators in this field to generate further hypotheses about how RPE atrophy might impact AMD.

In this application we will:

- Aim 1. Identify the basis for rpea1 & rpea2 and the pathways in which they function.
- Aim 2. Identify abnormalities caused by the rpea1 and rpea2 mutations to determine the nature of the pathological changes observed and the affected biological pathways.

Victor Perez, M.D.

Associate Professor, Ophthalmology
Miller School of Medicine, University of Miami

“Adaptive Immunity as the Missing Link between Oxidative Stress and Complement Activation in the Pathophysiology of Age-Related Macular Degeneration”

Scientific Abstract

Age-related macular degeneration (AMD) is the leading cause of legal blindness in elderly individuals in the United States and industrialized countries. One hallmark of the disease is the accumulation of debris (termed drusen) below the retinal pigment epithelium (RPE). AMD occurs in dry and wet forms: dry AMD relates to damage of the macula caused by atrophy whereas wet AMD involves neo-vascularization. Recently, several studies have implicated the immune system in the AMD disease process. Activated complement factor proteins have been found in drusen from AMD patients. Likewise, genetic markers (polymorphisms) within complement factor genes have been associated with development of AMD, suggesting that inflammation is a component of this disease. Oxidative stress is a second major pathway found to play a role in AMD. However, there is limited experimental data linking oxidative damage and complement activation. Our lab has developed a mouse model of dry AMD in which an oxidative stress-induced modification (carboxyethylpyrrole, or CEP) of self antigens leads to AMD-like lesions and complement deposition in the RPE. The research proposed in this application will test the hypothesis that adaptive immune responses generated against retinal products adducted with CEP are the initiating step in the development of AMD. We propose the novel concept that antigen specific T and B cell responses are crucial in the targeting of complement-mediated retinal damage in AMD and their regulation can be modulated to prevent or reverse disease. In addition, new AMD-associated genetic markers within adaptive immunity-related genes will be identified in humans and non-human primates. The immunological characterization of our AMD model will generate new insights into the biology and possible treatment of this serious blinding disease by providing a mechanistic link between oxidative stress and complement activation.

Our specific aims are:

Specific Aim 1: To test the hypothesis that development of T and B cell responses against CEP are important early events in the onset of AMD.

Specific Aim 2: To develop a non-human primate model of dry AMD for translational research.

Specific Aim 3: Identification of novel AMD-associated polymorphisms within adaptive immunity-related (AIR) genes in humans as possible targets for treatment.

Janet Sparrow, Ph.D.

Anthony Donn Professor of Ophthalmic Science
Columbia University

“Limiting RPE Lipofuscin Accumulation by Harnessing Enzyme-Mediated Degradation”

Scientific Abstract

Autofluorescent bisretinoid pigments accumulate with age as the lipofuscin of retinal pigment epithelial (RPE) cells in the eye. These pigments originate in photoreceptor outer segments from reactions of visual cycle retinoid and are deposited in the RPE secondarily. There has long been speculation of a link between RPE lipofuscin accumulation and the pathogenesis of age-related macular degeneration (AMD). Our recent work indicates that products of the photooxidation of RPE bisretinoids serve as activators of the complement system. This finding is significant given genetic association studies indicating that complement dysregulation underlies the pathogenesis of AMD in a significant proportion of cases.

Efforts in my laboratory are directed toward understanding the composition of RPE lipofuscin, the biosynthetic pathways by which the bisretinoids forms and the mechanisms by which RPE cells are adversely affected by lipofuscin accumulation. The long-term goal of this work is to prevent vision loss in age-related and monogenic forms of macular dystrophy by developing therapies to retard the accumulation of RPE lipofuscin.

Since bisretinoids of RPE cells accumulate over time, it is apparent that these compounds are not degraded by the lysosomal enzymes of the cell. The goal of the proposed research is to develop a therapy to reverse the accumulation of the bisretinoids of RPE lipofuscin. We will test the hypothesis that exogenous enzymes can be delivered to RPE cells for the purpose of safely degrading the bisretinoid constituents of RPE lipofuscin. This approach is particularly relevant for AMD patients who are usually diagnosed later in life and perhaps long after other lipofuscin-targeted therapies could be effective. The specific aims are to i) use non-cellular systems to test the efficacy with which selected groups of enzymes can cleave the known RPE bisretinoids including A2E, all-trans-retinal dimer and A2-DHP-PE; ii) develop approaches for introducing exogenous enzymes to the lysosomal compartment of RPE cells and test for degradation of intracellular bisretinoids; iii) ascertain whether enzyme-mediated cleavage of bisretinoids such as A2E has adverse effects on the cell. These studies involve techniques of cellular and molecular biology along with chromatographic and mass spectrometry analysis.

2009 One-Year Award Recipients

Edward N. and Della L. Thome Memorial Foundation, Bank of America, N.A. Trustee,
Awards Program in Age-Related Macular Degeneration Research

Christine Curcio, Ph.D.

Professor of Ophthalmology
University of Alabama at Birmingham

"Improved Anatomical Endpoints for Treatments of Age-related Macular Degeneration"

Scientific Abstract

Non-invasive imaging of retinal cross-sections at near-histological detail is revolutionizing the clinical management of patients with age-related macular degeneration (ARMD). The newest clinical instruments use spectral domain optical coherence tomography (SD-OCT), based on physical principles similar to ultrasound. Points of in vivo identification essential for maculopathy management and research, such as accurate identification of chorioretinal laminar boundaries and pathologic features, have not been systematically validated. Using a unique resource of human donor eyes, this translational research project will obtain ex vivo SD-OCT images of human macula and optic nerve, followed by high-resolution, wide-field histological cross-sections. Project MACULA (Maculopathy Unveiled by Laminar Analysis) will use 10 donor eyes in each of 5 groups (young adult, normal aged adult, early ARMD, geographic atrophy, and neovascular ARMD) to achieve the following objectives:

1. Compare laminar boundaries visible in ex vivo SD-OCT to those seen in histological cross-sections of the same eyes.
2. Compare specific pathologies seen in ex vivo SD-OCT to those seen in histological cross-sections of the same eyes.
3. Measure for each specimen the thickness of each retinal layer, Bruch's membrane (BrM), and choroid across the macula.
4. Assemble a library of ARMD pathology viewed en face to facilitate interpretation of SD-OCT volume reconstructions and other technologies that reveal that plane.
5. Using whole slide scanning, establish an online atlas of ARMD pathology that can inform next-generation tomographic imaging.

Improved knowledge about macular anatomy will help inform assessment of risk for choroidal neovascularization and assessment of treatments. We anticipate that data will be used by opinion leaders in clinical imaging, instrumentation engineers, ophthalmic educators and illustrators, and developers of new animal models of ARMD.

Patricia D'Amore, Ph.D.

Professor and Senior Scientist, Ophthalmology and Pathology, Harvard Medical School
Co-Director of Research, Senior Scientist, & Ankeny Scholar of Retinal Molecular Biology,
Schepens Eye Research Institute

“An In Vitro Model of Disrupted Retinal Pigment Epithelial Cell-Matrix Interactions”

Scientific Abstract

The hallmark phenotype of AMD is the presence of acellular deposits, drusen, located between the retinal pigment epithelium (RPE) and Bruch’s membrane (BrM). This proposal aims to test the hypothesis that disruption of RPE -extracellular matrix interactions by drusen deposits initiates a cascade of events that leads to an altered differentiation of RPE cells and to the progression of AMD. The studies aim to develop and validate an in vitro model in which RPE interactions with the extracellular matrix are disrupted by drusen-like microparticles and to use the model to investigate the effects of disrupted RPE cell-extracellular matrix interactions on the RPE phenotype and function. To develop a reproducible in vitro model of disrupted RPE cell-extracellular matrix interactions, drusen-like microparticles with surface features and composition similar to human drusen will be fabricated. ARPE-19 cells and primary RPE will be cultured on transwells for four wk to generate an extracellular matrix, removed non-enzymatically, the drusen-like particles distributed across the matrix, and ARPE-19 cells re-plated. Cell-cell and cell-matrix interactions will be examined by SEM. The effect of disrupted RPE cell-extracellular matrix interactions on RPE phenotype and functions will be investigated including, attachment, survival, phagocytosis, junctional complexes, actin cytoskeleton, ultrastructure, and transepithelial resistance as well on the expression level of RPE-secreted molecules including, growth factors, metalloproteinases, and integrins. Results of these studies will provide insight into the pathogenesis of AMD.

Douglas Vollrath, M.D., Ph.D.

Associate Professor of Genetics and Ophthalmology
Stanford University School of Medicine

“Retinal Degeneration in Mice Due to Loss of RPE Mitochondrial Energy Production: a Model of AMD”

Scientific Abstract

Retinal pigment epithelial (RPE) cell dysfunction and death are widely believed to play central roles in age-related macular degeneration (AMD). Evidence indicates that reduction of RPE mitochondrial function is among the mechanisms that contribute to AMD pathogenesis, but the epithelial changes that result from mitochondrial deficiency are largely unknown and no suitable animal model of a postnatal primary RPE degeneration has been described. To gain insight into the RPE response to diminished mitochondrial function and the consequences for the adjacent choroid and photoreceptors, we created mice with postnatal loss of RPE mitochondrial respiratory chain function through an RPE-specific knockout of a nuclear gene essential for mitochondrial DNA stability. Our model has several features that make it suitable and practical for investigating AMD pathogenesis. The onset of degenerative changes is gradual, but becomes severe before one year of age. While most RPE cells lose mitochondrial function, a minority do not, modeling the heterogeneity of cell damage seen in AMD. The retinas of affected animals eventually display a number of features seen in the geographic atrophy form of AMD including: 1) abnormal RPE morphology 2) attenuated RPE cells 3) RPE cytoplasmic inclusions 4) RPE autofluorescence 5) loss of RPE epithelial integrity 6) RPE cell migration and 7) progressive photoreceptor degeneration that starts in the posterior retina. We propose to characterize the retinal response to loss of RPE oxidative phosphorylation through deep sequencing of RPE and neural retina mRNA populations and targeted biochemical and cell biological studies. We further propose to determine the effect of dietary restriction on RPE and photoreceptor survival in our model. When thoroughly characterized, our model could serve as a recipient for RPE cell transplantation studies and provide a testing ground for small molecules and growth factors that preserve RPE and/or photoreceptor function in the context of decreased RPE energy production. Dietary restriction has been found to be beneficial not only for extending life span, but also in slowing degenerative changes in many mammalian organs, including the central nervous system. Success of this pilot project could therefore lead to new approaches to the treatment of AMD.