The Edward N. & Della L. Thome Memorial Foundation Awards Program in Age-Related Macular Degeneration Research

2020 Grant Cycle

Laura Ensign, Ph.D.
Marcella E. Woll Professor of Ophthalmology
Johns Hopkins University

"Novel Gel-forming Eye Drop for Treatment of Age-related Macular Degeneration"

Scientific Abstract

Injectable therapies for blocking VEGF have provided impressive initial benefits to patients with neovascular age-related macular degeneration (nAMD), but long-term outcomes have been disappointing. The short duration of action requires that injections be repeated every 1-2 months. Life circumstances often make it difficult or impossible to return to the clinic as frequently as is required for injections. One solution is to develop a treatment that can be self-administered by patients so that treatment can continue even when patients are unable to return to their retina specialist. Patients are able to apply drops to their eyes, but thus far it has not been possible to deliver adequate amounts of drug to the retina by topical eye drops. We have engineered a novel thermosensitive gelling eye drop (OcuGel) that is liquid at room temperature and spreads to cover the surface immediately and uniformly, and then gels to trap the medication in place against the ocular surface to provide enhanced absorption. The thin gel film is unnoticeable and optically clear. Our preliminary data shows that daily topical dosing of OcuGel containing a small molecule drug with potent anti-angiogenic properties strongly suppressed CNV in mice. Similarly, we have found that daily dosing in rabbits led to significantly higher drug concentration in the choroid and retina than what would be needed to prevent blood vessel growth. This is a key finding, given the prior reports of drugs not reaching the posterior ocular tissues effectively in large animals with eyes more similar in size to that of humans. Here, we propose further characterization and preclinical testing of OcuGel for treatment of nAMD that includes characterization of the gel material properties and drug delivery mechanism, pharmacokinetics, efficacy in large animals, and ocular surface safety.

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Brian Hafler, M.D., Ph.D.
Assistant Professor of Ophthalmology and Pathology
Yale University

"Single-cell Transcriptomic Analysis of the Inflammatory Landscape in Human Age-related Macular Degeneration"

Scientific Abstract
Age-related macular degeneration (AMD) is a neurodegenerative disease that is among the leading causes of blindness in the elderly. The cell types and molecular pathways that promote neuroinflammation and contribute to AMD are not well understood. Our preliminary data based on single cell analysis indicate that the key inflammatory pathways reside in specialized glial cells known as microglia. We hypothesize that functional changes in microglia influence disease pathogenesis. The investigations proposed here will allow the comparison of gene signatures of microglia in patients with varying stages of AMD and healthy individuals, helping elucidate the mechanism of AMD pathogenesis and highlighting novel approaches to prevent permanent neuron loss in the retina and to halt blindness.
Using massively parallel single-cell RNA sequencing of human retinas, our lab reported the first single-cell transcriptomic atlas of the human retina. Based on new data, we hypothesize that dysregulation of innate immune checkpoints and upregulation of inflammatory molecules in microglia lead to neurodegeneration in AMD. To explore this hypothesis, we will identify the molecular signature of AMD microglia with highly parallel single-nuclei transcriptional profiling using a novel enrichment technique for glia from human AMD tissue. We hypothesize that microglia from AMD samples will have a pathogenic disease-associated inflammatory phenotype. We will use lentiviral short hairpin mediated knockdown and overexpression of AMD associated genes in microglia to identify pathways that are targetable to reverse the pathologic chronic inflammation in AMD. We anticipate that our work will give insight into the development of a new therapeutic approach for individuals suffering from AMD by targeting microglia to reverse the chronic inflammation in disease. The positive outcomes of our proposed research will have the immediate impact of directly leading to therapeutic targets that lay the foundation for a novel therapy for AMD that targets inflammation.

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Robert Mullins, Ph.D.
Professor; Martin and Ruth Carver Chair in Ocular Cell Biology
University of Iowa

"Pathophysiology of MMP9 Polymorphisms in Neovascular AMD"

Scientific Abstract
Whereas most of the genetic variants associated with age-related macular degeneration (AMD) identified to date do not distinguish between risk for atrophic vs. neovascular AMD, recent large scale genetic studies have identified variants in the matrix metalloproteinase-9 (MMP9) gene in neovascular AMD. Our research team recently replicated these findings in an independent cohort of AMD patients. The mechanism(s) by which these variants confer increased risk of neovascularization are not understood. In this multidisciplinary research program we will determine the role of MMP9 polymorphisms on expression and function of MMP9; on global gene expression at the single cell level of resolution: on the ultrastructural and biomechanical properties of Bruch's membrane in genotyped human eyes; and on the angiogenic behaviors of iPSC-derived macrophage, RPE, and choroidal endothelial cells that harbor CNV-associated polymorphisms. A better understanding of the transition from intermediate to neovascular AMD is needed and will provide better opportunities for early intervention.

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Aaron Nagiel, M.D., Ph.D.
Assistant Professor of Ophthalmology
Childrens Hospital Los Angeles

"Hippo Pathway Inhibition for the Treatment of Geographic Atrophy"

Scientific Abstract

Geographic atrophy (GA) in dry age-related macular degeneration (AMD) causes visual impairment through progressive and irreversible loss of the retinal pigment epithelium (RPE) and photoreceptors. Although in non-mammalian vertebrates Müller glia and RPE cells respond to injury by reentering the cell cycle to restore lost cell types, these endogenous repair mechanisms are repressed in mammals by Hippo signaling. We have recently identified and characterized first-in-class small-molecule inhibitors of Lats kinases (LKI), the core enzymes in the Hippo pathway. Our preliminary studies have shown that LKI treatment induces regenerative proliferation of Müller glia and RPE in human retinal organoids and in undamaged mouse retina with no evidence of toxicity. Building on these encouraging preliminary results, this proposal will test through two aims whether Hippo inhibition is sufficient to facilitate photoreceptor and RPE restoration in the damaged retina in vivo. In Aim 1, we will ascertain the effect of Lats inhibition on Müller glia in two animal models of dry AMD: the Mertk mutant mouse model and a laser-injury rabbit model. Retinal imaging and correlative histopathologic, immunofluorescence, and RNA-seq analyses will be performed at 1-4 weeks post intravitreal LKI administration to assess Müller cell proliferation and differentiation into photoreceptors. In Aim 2, we will determine whether Lats inhibition facilitates regeneration of the RPE monolayer through an in vitro scratch assay and by analyzing the laser-injured rabbit retinas used in Aim 1. With unique access to these commercially unavailable Lats kinase inhibitors, this collaboration between the Gnedeva and Nagiel laboratories has the potential to identify novel therapies for GA in dry AMD that facilitate innate self-repair of the retina. This holds great promise for a currently untreatable condition that would only require a clinic-based intravitreal injection of a small-molecule drug.

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2020 Grant Cycle

Patsy Nishina, Ph.D.
Professor
The Jackson Laboratory

"Mouse Models Bearing Human AMD GWAs Alleles"

Scientific Abstract

Despite the significant progress made toward understanding the genetic basis of AMD, we still are at early stages of developing therapeutic strategies from this knowledge. Part of the problem is the lack of animal models in which we can directly determine the contribution of the different GWA variants and we can do invasive, longitudinal studies to detect early biomarkers for the disease to use as surrogate end-points in drug development. Another part of the problem is having robust animal models that recapitulate the multigenic nature of the disease in which therapeutic strategies can be tested. In this application, we are directly testing the contributions of GWAs variants, ARMS2A69S and CETPD442G to development of AMD-like features under different modifiable conditions known to increase AMD risk. These variants were selected because their effect on AMD risk has not been resolved in large GWA studies and understanding whether they mediate pathological effects is critical for developing effective therapeutics. We will also be studying these single variants on different genetic backgrounds and in combination with other variants that may interact with them in order to build complex models that more accurately reflect what might be occurring in the highly genetically heterogeneous human population. Our long term goal, beyond the scope of this proposal, is to place the variants generated/characterized in this study on a high risk susceptible collaborative cross background strain that bears as many of the AMD GWAs that affect gene expression (derived by eQTL analysis of retinal tissue in mouse) to reflect human AMD transcriptomic profiles for the 54 AMD GWAs genes. All models generated will be available for distribution so that they can serve as resources for studying gene and environmental interactions that may affect AMD (by combining different alleles under different environmental challenges), and for testing therapeutic interventions.

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Dorota Skowronska-Krawczyk, Ph.D.
Assistant Professor of Physiology and Biophysics; Assistant Professor of Ophthalmology
University of California Irvine

"Aging, Lipid Metabolism, and Vision"

Scientific Abstract

Age is one of the most relevant clinical traits associated with almost all blinding conditions (e.g., glaucoma, diabetic retinopathy and age-related macular degeneration (AMD)). The overarching goal of this project is to determine how age-related changes in membrane composition ("aging membranes") including the tightly packed membranes of the outer segment of photoreceptors, are detrimental to retinal cells and compromise vision in older individuals. We hypothesize that these same events are pertinent to the early stages of the dry form of AMD. In this project, using an established portfolio of biochemical and electrophysiological approaches, we will determine how the disturbed composition of polyunsaturated fatty acids (PUFAs) in photoreceptor cells affects visual cycle kinetics leading to visual impairment (Aim 1) and, using a set of state-of-the-art molecular approaches, identify the molecular pathways contributing to aging membranes in both healthy and disease states (Aim 2). Our novel transgenic mice model will make these questions tractable experimentally. In sum, we propose to explore the concept of the fundamental roles of PUFAs in aging, focusing on age-related changes in lipids composition as a component of etiology of dry AMD. We expect that work performed under this proposal will create a solid foundation for future studies aimed at designing novel therapies for patients suffering from impaired vision due to normal aging, as well as age-related diseases of the eye such as AMD.

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2020 Grant Cycle

David Wu, M.D., Ph.D.
Assistant Professor of Ophthalmology
Massachusetts Eye and Ear Infirmary

"Metabolic Regulation of the Outer Retinal Blood Barrier and Age-related Macular Degeneration"

Scientific Abstract

The Outer Blood-Retinal Barrier (OBRB), spanning the choriocapillaris, Bruch's membrane, and the retinal pigment epithelium (RPE), undergoes pathognomonic changes in Age-related Macular Degeneration (AMD). How these are linked with the earliest known AMD changes, the loss of the perifoveal rod photoreceptors, remains unknown. There is growing awareness that photoreceptors and RPE are mutually dependent members of a tightly regulated metabolic ecosystem. Photoreceptors metabolize glucose from RPE by aerobic glycolysis and shuttle lactate back to the RPE. Rod loss in early AMD may decrease available lactate for the RPE. To understand the implications of this disruption, we performed transcriptome profiling of RPE from mice in which Lactate Dehydrogenase A (LDHA), the critical subunit for lactate production, was floxed out of rods (Rho-Cre:Ldhafl/fl). Severing this metabolic link between photoreceptors and RPE downregulated RPE expression of all three Vascular Endothelial Growth Factor receptors (VEGFRs), key signaling components for OBRB maintenance.

RPE VEGF secretion is critical for choriocapillaris maintenance, but less is known about the role of RPE VEGFRs. We will use biochemical and molecular techniques to probe the RPE VEGF autocrine loop and understand how dysfunction may injure the choriocapillaris and the RPE. We will identify the predominant form(s) of VEGF receptors in RPE, and use RPE-selective AAV constructs to express short-hairpin RNAs (shRNAs) and knockdown RPE VEGFR in wild type mice to recapitulate the Rho-Cre:Ldhafl/fl phenotype. We will rescue Rho-Cre:Ldhafl/fl mice with AAV restoration of RPE VEGFR deficiency. We will reverse RPE lactate deficiency in Rho-Cre:Ldhafl/fl mice and see if this normalizes OBRB changes. Finally, we will examine post-mortem tissue for evidence of LDHA changes in AMD. This proposal will establish a novel link between early rod loss in AMD with subsequent changes in the OBRB, opening new potential treatment avenues for early AMD.

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2020 Grant Cycle

Donald Zack, M.D., Ph.D.
Professor of Ophthalmology
Johns Hopkins University

"Pathways to Inhibit RPE EMT"

Scientific Abstract
Dysfunction and degeneration of the retinal pigmented epithelium (RPE) plays an important role in the pathogenesis of AMD. Environmental factors, including exposure to growth factors, cytokines, hypoxia, and oxidative stress, all of which accumulate with age, can initiate an RPE stress response pathway that ultimately results in induction of epithelial-mesenchymal-transition (EMT) leading to RPE de-differentiation. RPE EMT has been reported to be involved in both atrophic AMD as well as in the scarring associated with neovascular AMD. We have established a platform to study RPE-EMT using human stem-cell derived RPE (hRPE) monolayer cultures. We will use the platform in this project to investigate the gene-expression networks, at the single cell level, that drive RPE-EMT induction. Using both whole single-cell transcriptomic-profiling through the course of RPE-EMT induced by two different mechanisms, as well as single-cell CRISPR-screening for genes, that when expression is reduced, cause cells to maintain RPE integrity upon EMT induction, we will identify pathways involved in the regulation of RPE-EMT. The transcriptomic networks involved in RPE-EMT and the genes that maintain RPE integrity upon EMT induction will implicate biological pathways that could be targeted to modulate RPE EMT/de-differentiation. We will directly test the contribution of these pathways in RPE-EMT by CRISPRi mediated gene knock-down. This project will systematically define the coordinated gene networks and network trajectories that are involved in hRPE EMT/de-differentiation; networks that could potentially be targets for therapeutic intervention.