CORAL GABLES, FLORIDA // SEPTEMBER 5, 2014

HOPE FOR VISION SCIENCE SYMPOSIUM

A one day conference dedicated to research into treatments and cures for retinal degenerative diseases

HOPE for VISION
www.hopeforvision.org

HOPE FOR VISION SCIENCE SYMPOSIUM

"Dwell in possibilities."
– emily dickinson

It is an honor to most cordially welcome you to the second Hope for Vision Science Symposium. There will be a series of Symposia presented on cutting edge research and technology that aims to treat, cure, or restore vision loss due to retinal degenerative diseases.

You are amongst the most eminent scientists in the diverse and exciting areas of retinal degenerative research. Accept our heartfelt gratitude and support as you endeavor in the areas ranging from stem cell studies, gene therapy, microchips and implants to restore or preserve vision, neuroprotective factors, as well as novel drug therapies.

The meeting place is generously donated by the Institute for Cuban and Cuban-American Studies [ICCAS] located on the beautiful campus of the University of Miami in Coral Gables, Florida. Hope for Vision is a 501c3 non-profit organization dedicated to raising funds to advance ground-breaking scientific and medical research for the treatments and cures of blinding genetic retinal diseases.

Hope for Vision distributes over 87% of the funds raised for sight saving research at leading institutions whose scientists are working tirelessly for cures and treatments of blinding retinal diseases. We aim to foster communication between those at the forefront of research on retinal degenerative diseases ,and preservation and restoration of vision.

We extend an opportunity for you to present your latest research findings, meet with colleagues, and discuss your work in a welcoming environment. The presentations will be followed by a period of open discussion at the end of the day. Enclosed are the abstracts that will be presented today. We appreciate your participation greatly. It is our hope that this Scientific Research Symposium will foster mutual relationships, and a foundation for cooperation among researchers across countries and disciplines.

On behalf of Hope for Vision and all those challenged by vision loss and blindness, we would like to once again welcome you to this important Science Symposium. We hope that it will be a productive worthwhile meaningful enriching experience for you. YOU ARE OUR HEROES. We hope you can feel how our hearts see you…

With gratitude and love,

betti lidsky
HOPE FOR VISION

SCIENTIFIC SYMPOSIUM 2014 AGENDA

HOPE for VISION
www.hopeforvision.org

9:00 AM
WELCOME BY DR. ALEX COHEN
Chairman, Hope for Vision Board of Visionary Scientists

9:30 AM
DR. JOHN FLANNERY
Directed Evolution of AAV vectors to target functional subclasses of retinal neurons and glia

9:45 AM
DR. SHANNON BOYE
Expanding the AAV vector toolkit

10:00 AM
DR. MICHELLE HASTINGS
Antisense oligonucleotides for the treatment of Usher syndrome

10:15 AM
DR. JOHN ASH
Developing new therapies to protect retinal neurons and the retinal pigmented epithelium from degeneration

10:30 AM
DR. KIRILL MARTEMYANOV
Molecular mechanisms of low light vision

10:45 AM
DR. BRIAN MCKAY
Pigmentation and Retinal Disease

11:00 AM
DR. BETTY FINI
When is the Eye Like the Prostate? Neuroendocrine Processes and Orphan Receptor GPR158.

11:15 AM
Break

11:30 AM
DR. RONG WEN
Abnormal ERG and retinal degeneration in the DHDDS$^{K42E/K42E}$ mouse

11:45 AM
DR. BYRON LAM
Aberrant Dolichol Chain Length distribution as Biomarkers for Retinitis Pigmentosa Associated with DHDDS Genotypes

12:00 PM
DR. STEPHEN TSANG
Stem Cells and Personalized Medicine

12:15 PM
DR. SHI-JIANG (JOHN) LU
Retinal Cells from Human Pluripotent Stem Cells: Clinical Potential

1:00 PM
Lunch Break

2:00 PM
DR. PETR BARANOV
Human retinal progenitor cells for photoreceptor replacement

2:15 PM
DR. DOUG FENG CHEN
Stimulating endogenous neuroregeneration as a strategy for reversing blindness

2:30 PM
DR. STEPHAN ZÜCHNER
Whole exome sequencing in patients with rare neurogenic disorders uncovers a missing mitochondrial fusion-like protein

2:45 PM
DR. SAMUEL JACOBSON
Early-onset retinal degeneration caused by Tubby-like protein 1 (*TULP1*) gene mutations

3:00 PM
DR. ALEX COHEN
Moderated Open Discussion

5:00 PM
Meeting Closed

ABSTRACTS

DIRECTED EVOLUTION OF AAV VECTORS TO TARGET FUNCTIONAL SUBCLASSES OF RETINAL NEURONS AND GLIA

John G. Flannery, Ph.D.

Clinical gene therapy has been increasingly successful owing both to an enhanced molecular understanding of human disease and to progressively improving gene delivery technologies. Among these technologies, delivery vectors based on adeno-associated viruses (AAV) have emerged as safe and effective. New approaches to engineer and improve AAV vectors are helping to permit broader use of these vectors for both basic research and therapeutic applications. Recent innovations in vector engineering, including rational design and directed evolution, have created novel AAV variants that can specifically target subsets of retinal neurons, epithelia and glia. This lecture will demonstrate some applications of novel AAV vectors to treat animal models of blinding conditions. In early stage retinal diseases, where patients retain photoreceptors the best option is likely gene replacement. For late stage disease, after the photoreceptors have been lost, the intersection of optogenetics and gene therapy has potential for adding a new, light receptive function to surviving retinal interneurons.

Feasibility of this concept was first demonstrated in mice by gene transfer of the optogenetic activator, channelrhodopsin-2 (ChR2), to ON bipolar cells and retinal ganglion cells. Since mammalian retinas have two major functional subclasses of bipolar cells that are activated and inhibited by light (ON and OFF bipolar cells), we hypothesize that this therapeutic strategy will be enhanced by specific delivery of an optogenetic activator to ON bipolars rather than the retinal ganglion cells. In this way, significantly more of the intrinsic retinal signal processing would be harnessed to provide improved vision restoration. Using a process of directed evolution and synthetic biology we have engineered an AAV delivery system to target optogenetic GPCRs bipolar cell subclasses and tested this system to treat blindness in mouse models of retinal degeneration. The light sensing molecules are designed to separate the light sensing molecules from the ion channel effectors. This concept is distinct from the published optogenetic approaches to vision restoration in which the light sensing molecule (chr2, halorhodopsin, LiGluR) is also the ion channel effector. The single molecule approaches have an inherent sensitivity restriction due to the limited surface area of retinal neurons and the lack of an amplifying cascade. We have engineered light-sensitive mammalian G-protein coupled receptors that when expressed in ON-bipolar cells activate a native ion channel via an amplifying signaling cascade that is triggered by an endogenous G-protein. The cascade is normally activated by glutamate release from photoreceptor cells, which activates mGluR6 in ON bipolar cells, in turn activating the ion channel TRPM1. Our preliminary data indicates that by separating the light sensor from the ion channel effector and activating the existing G-protein cascade to interconnect the two, we increase the light sensitivity by ~100 fold and introduce two log units of adaptation into the system. This concept is fundamentally more similar to the phototransduction cascade in photoreceptors than the 'single component' microbial opsins. We are evaluating four light sensing molecules; rhodopsin, m-cone opsin and light-sensitive iGluR we have generated. We have previously shown that the modified glutamate receptor system works to restore light sensing and visually-driven behavior to blind mice, where the receptor (iGluR6 = LiGluR) is an ion channel. Those studies use a 'single component' approach, with LiGluR acting as the light sensor and ion channel "actuator" together. In that study, LiGluR expressed in ganglion cells produced vision restoration, bur required very bright illumination, as there was no significant amplification or adaptation. We have evaluated the efficacy of the switches by electrophysiological and behavioral testing in rodent and canine models of retinal degeneration. Ongoing work in rd1 and rd10 mice include multielectrode array studies to measure the efficiency of driving multiple ganglion cell responses in the absence of photoreceptors. We are testing in vivo, the restoration of ERG responses, cortical responses, behavioral studies (Morris water maze) and free-running mouse optokinetic response tests.

EXPANDING THE AAV VECTOR TOOLKIT

Shannon E. Boye, PhD

Gene replacement therapy for inherited retinal disease has moved from the conceptual to the applied. However, one key issue yet to be resolved is how to safely deliver genes to photoreceptors/RPE in an already fragile, compromised retina. The current standard practice is subretinal injection. Results from clinical trials where subretinal injection was performed under the fovea reveal a loss of retinal thickness (i.e. photoreceptor degeneration) and loss of visual acuity. Damage to the cone-rich fovea can be devastating to patients as this is the area of the retina responsible for acute, daylight vision. This talk will summarize efforts to develop novel Adeno-associated viral (AAV) vectors that are capable of delivering therapeutic protein to photoreceptors (namely foveal cones) following a safer, surgically less-invasive intravitreal injection. Related to this, we have now identified AAV vectors and cellular promoter combinations capable of robust and highly selective transduction of middle retinal cells. The ability of one such vector/promoter combination's ability to restore retinal function to a mouse model of congenital stationary night blindness will be discussed. One limitation of AAV is its limited packaging capacity. I will conclude with a summary of efforts to overcome this hurdle through the use of dual AAV vector technologies that are capable of treating retinal diseases associated with mutations in large genes, such as Usher syndrome and Stargardt's disease.

ANTISENSE OLIGONUCLEOTIDES FOR THE TREATMENT OF USHER SYNDROME

Michelle L. Hastings, PhD

CO-AUTHORS:
Jennifer J. Lentz[1], Francine M. Jodelka[2], Anthony J. Hinrich[2], Frederic Depreaux[2], Abhilash Ponnath[1], Russell Amato[1], Mette Flaat[1], Frank Rigo[3]

Usher syndrome (Usher) is the leading genetic cause of combined deafness and blindness. Type 1 Usher (Usher 1) is the most severe form of the disease and is characterized by profound hearing impairment and vestibular dysfunction from birth, and the development of retinitis pigmentosa (RP) in early adolescence that progresses to blindness. We developed an antisense oligonucleotide (ASO) class of drug called ASO-29, which recognizes the Acadian Usher syndrome mutation c.216G>A (216A) in the *USH1C* gene. ASO-29 is designed to block the deleterious effects of the mutation and partially restores *USH1C* gene expression. ASO-29, and ASOs in general, are an ideal drug platform due to their high stability, deliverability and target specificity.

ASO-29 was tested for its ability block the 216A mutation and restore gene expression in mice that have the *Ush1c*.216G>A mutation. These mice have profound hearing impairment, exhibit circling behavior indicative of severe vestibular dysfunction and have retinal dysfunction early in life. We found that mice treated with a single dose of ASO-29 shortly after birth had normal vestibular function and could hear for up to six months of age. The treated mice also have a modest improvement in visual function.

Our results demonstrate that ASO-29 can effectively correct an *Ush1c* mutation. Current work focuses on developing ASO-29 for the treatment of human Usher syndrome including the optimization of delivery and dosing. We are also designing ASOs to target other mutations that cause

ABSTRACTS

Usher syndrome in order to expand the repertoire of this class of drug for the treatment of diseases of the eye and ear.

[1]Neuroscience Center, LSUHSC, New Orleans, LA; [2]Rosalind Franklin University of Medicine and Science, North Chicago, IL; [3]Isis Pharmaceuticals, Carlsbad, CA.

DEVELOPING NEW THERAPIES TO PROTECT RETINAL NEURONS AND THE RETINAL PIGMENTED EPITHELIUM FROM DEGENERATION.

John D. Ash, PhD

Inherited retinal degenerations are often the result of genetic mutations that cause retinal neurons to undergo some form of cell death. Mitochondria are known to play a key role in production of reactive oxygen species (ROS), and are known to be damaged in disease. However, mitochondria are also a major source of ROS detoxification enzymes and energy production. Our study was designed to determine the role of mitochondria in retinal degeneration and to determine whether drugs that augment mitochondrial activity and energy balance are protective. We used RD10 mice, which are homozygous for a mutation in the beta subunit of the cyclic GMP phosphodiesterase gene, as an inherited model of retinal degeneration, and we used exposure to damaging light (1000 to 1200lux for 4 hours) as an acute model of retinal degeneration. Mice were given daily injections of the drug, metformin. We used peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1α) and beta (PGC-1β) knockout mice to determine the role of mitochondrial gene expression in regulating cell survival in light damage. Metformin led to activation of AMPK in the retina, and preserved retinal photoreceptors in RD10 mice, and protected from light damage. Under normal lighting conditions, PGC-1α and PGC-1β knock-out mice had normal light responses by ERG and normal retina morphology by SD-OCT imaging. Light damage reduced retinal function and thickness in both PGC-1α and wild type mice, but PGC-1α knock-out mice were less sensitive to light damage than wild type mice. In contrast, PGC-1β knock-out mice were more sensitive to light damage than wild type mice. Our data show that Metformin treatment induced mitochondrial DNA replication and induced mitochondrial gene expression in response to stress. This resulted in increased resistance to cell injury and death. In addition, loss of a major transcriptional cofactor for mitochondrial gene expression (PGC-1β knock-out mice) had the opposite effect of increasing sensitive to cell death. Combined, these results suggest that therapies designed to target mitochondria may be an effective tool to promote broad-spectrum neuroprotection.

MOLECULAR MECHANISMS OF LOW LIGHT VISION

Kirill A. Martemyanov, PhD

Signaling in the retina plays essential role in our vision. Light is detected by rod and cone photoreceptors that convert it to the electrical response further propagated through the retina circuitry by means of synaptic communication between neurons. To be able to see at low light levels, highly sensitive rods must faithfully transmit the signal that they generate to downstream rod bipolar cells (RBC). Disruption of synaptic transmission between rods and RBC leads to congenital forms of night blindness. Understanding the mechanisms of synaptic communication between rods and RBC will be needed not only for curing poor vision at low light but also for designing innovative strategies for improving detection sensitivity when photoreceptors are lost due to degeneration.

The efforts in my laboratory are focused on studying molecular players and signaling events at the first visual synapse between rods and RBC neurons. Signal transmission at this synapse is mediated by the G protein pathway where mGluR6

receptor is responding to changes in glutamate released by the photoreceptors to control the activity of the TRPM1 channel which mediates a depolarizing response of RBC. We used proteomics approaches to characterize composition and functional interaction between components of this signal transduction cascade. We found that synaptically enriched protein nyctalopin, that is mutated in a majority of subjects with night blindness, binds to both mGluR6 receptor and TRPM1 channel, possibly scaffolding two principal components of the signaling cascade. We further found that timely activation of TRPM1 and, as a result, scotopic vision require contributions of two G protein signaling regulators: RGS7 and RGS11 that act by promoting G protein deactivation. The search for binding partners of RGS proteins revealed that they are scaffolded by the orphan receptor GPR179 that plays an essential role in targeting of RGS proteins and is indispensible for synaptic transmission.

In summary, our studies reveal how multiple elements of the synaptic signaling cascade in RBC neurons are organized and scaffolded together to ensure proper transmission of signal generated by rods enabling vision at low light.

PIGMENTATION AND RETINAL DISEASE

Brian S. McKay, PhD

Both age-related macular degeneration and glaucoma, the two most common forms of retinal degeneration, are tied to race and pigmentation characteristics in some unexplained way. Age-related macular degeneration (AMD) is much more common in the white population and among those with lighter pigmentation characteristics (blond hair and blue eyes). Thus, darker pigmentation may protect from AMD. Pigmentation and race are complex traits, and drawing a connection from population-based studies as to *how* pigmentation could be involved is not straightforward. In an effort to understand how retinal pigment epithelial cell (RPE) pigmentation might be involved in neurosensory retina health and survival, we have investigated GPR143, a G-protein coupled receptor that functions in the pigmentation pathway and is solely expressed by pigmented cells. Our data suggest that there may be a linkage between GPR143 signaling in RPE and AMD. We have shown that the ligand of GPR143 is L-DOPA, a byproduct of melanin pigment synthesis, and that dopamine acts as an antagonist of the receptor. The receptor is on the apical surface of RPE and both L-DOPA and dopamine are present in the subretinal space. We now have determined that GPR143 functions as a control point for RPE:retinal communication through exosomes. Exosomes are small extracellular vesicles derived from the endocytic compartment, produced and stored in the multivesicular body (MVB). Exosome release is a signal transduction dependent event in which the MVB fuses with the plasma membrane. Exosomes carry miRNA, proteins and lipids and function in intra-tissue communication in which one tissue impacts the physiologic activity of another. In studies of RPE *in situ* using nanoparticle tracking analysis, we show that RPE constitutively release high levels of three populations of exosomes (50, 70, and 90nm diameter). Activation of GPR143 with L-DOPA halts RPE exosome release suggesting tight control of this pathway in RPE. Discovering the role of exosomes in RPE:retina communication is emerging as critical next step in understanding retinal health and diseases, and our identification of GPR143, a pigmentation related GPCR, as a control point will provide a new tool to further our understanding . Of interest with respect to pigmentation and ocular disease, is that the exosomes RPE release carry myocilin, and during receptor-mediated endocytosis of activated GPR143 cytoplasmic myocilin is recruited to this pigment pathway receptor. Taken together, our data suggest GPR143 signaling may tie both AMD and glaucoma to signal transduction events in the pigmentation pathway.

ABSTRACTS

WHEN IS THE EYE LIKE THE PROSTATE? NEUROENDOCRINE PROCESSES AND ORPHAN RECEPTOR GPR158.

M. Elizabeth Fini, PhD

CO-AUTHORS:
Nitin Patel, PhD, Tatsuo Itakura, PhD, and Shinwu Jeong, PhD [1]

Members of the large of G protein-coupled receptor (GPCR) clan represent the most successful targets for pharmaceutical intervention to date. GPR158, a member of the glutamate family, is an "orphan", with functional roles and physiological/pharmaceutical ligands still to be discovered. GPR158 is expressed at high levels throughout the brain, and is also expressed in the retina. Recently, mutations in the closely related orphan GPR179 were shown to cause autosomal-recessive complete congenital stationary night blindness, however a vision-related role for GPR158 remains to be determined. At last year's Hope for Vision meeting, we reported our characterization of GPR158's activity in the neuroendocrine (NE)-like trabecular meshwork cells of the aqueous outflow pathways and our findings implicating GPR158 in ocular hypertension (OH)[2]. This year we report our identification of a role for GPR158 in another NE organ, the prostate[3]. GPR158 over-expression promotes, and siRNA knockdown inhibits, cell proliferation in a range of prostate cancer (PCa) cell lines. Mutagenesis experiments demonstrate that this activity is dependent on translocation of GPR158 to the nucleus, thus indicating a non-canonical GPCR mechanism. GPR158 over-expression also stimulates androgen receptor (AR) expression in both androgen-dependent and androgen-insensitive PCa cell lines. Androgen treatment stimulates endogenous GPR158 expression in LNCaP cells by two-fold; however, androgen depletion stimulates GPR158 expression by 20-fold. The latter effect is accompanied by transdifferentiation, to the NE phenotype, associated with aggressive forms of androgen-independent PCa. Interestingly, we observe that a very high level of GPR158 over-expression alone can induce markers of NE differentiation. This suggests that GPR158 has two different activities, depending on expression level. Subcellular fractionation indicates that GPR158 is primarily nuclear in both normal and transdifferentiated LNCaP cells, however, the very high level of GPR158 expression in the transdifferentiated cells means there is considerably more protein at the plasma membrane. We hypothesize that the new activity observed at the highest GPR158 expression levels represents a canonical GPCR activity, an idea we are now investigating. We find GPR158 protein levels are elevated in human PCa. Very significantly, our analysis of publically available data indicates that increased GPR158 expression is significantly associated with decreased disease-free survival. Taken together, our findings strongly suggest that pharmaceuticals targeting GPR158 activities could represent an innovative approach to the prevention and management of PCa, which may also be of value for treating eye diseases.

[1] USC Institute for Genetic Medicine, Keck School of Medicine, University of Southern California

[2] Patel N, Itakura T, Gonzalez JM, Jr, Schwartz SG, Fini ME. **Structure, Expression, and Functional Characterization of GPR158: An Orphan Member of G Protein-Coupled Receptor Family C: Glucocorticoid-Stimulated Expression and Novel Nuclear Role.** *PLoS One* 2013;8(2):e57843.

[3] Patel N, Itakura T, Jeong S, Liao C-P, Pinski J, Gross ME, Fini ME. **Orphan Receptor GPR158 Regulates Prostate Cancer Growth and Progression via Dual Pathways, in preparation.**

ABNORMAL ERG AND RETINAL DEGENERATION IN THE DHDDS$^{K42E/K42E}$ MOUSE

Rong Wen, MD, PhD

CO-AUTHORS:
Byron L. Lam[1], Ziqiang Guan[3], Mateusz J. Graca[2], Jeffery S. Prince[2], Yiwen Li[1]

The K42E mutation in the DHDDS gene encoding dehydrodolichol diphosphate synthase (DHDDS) was recently identified to be responsible for 12% of autosomal

recessive retinitis pigmentosa (arRP) in patients of Ashkenazi Jewish origin. To further study retinal degeneration associated with this mutation, we created a transgenic mouse model that harbors the DHDDS K42E mutation by the knock-in technology. The mutant mice appear normal and fertile. Measurement of blood dolichols in the plasma of DHDDS$^{K42E/K42E}$ mice by liquid chromatography-mass spectrometry (LC-MS) showed a characteristic shortening of dolichol length distribution. In DHDDS$^{K42E/K42E}$ mice, dolichol 17 (D17) became the dominant species instead of dolichol 18 (D18) in wild-type (wt) littermates. As a result, the DHDDS$^{K42E/K42E}$ mice have much higher plasma D17/D18 ratio. The shortening of dolichol profile indicates abnormal dolichol biosynthesis, similar to what was found in patients. Scotopic full-field ERGs showed significant smaller a-wave and a significant decrease in the b-wave amplitude, as compared to the wt controls. However, the decrease in the b-wave amplitude was disproportionally more than the a-wave, making the b- to a-wave amplitude ratio close to 1 in mutant animals, as compared to the ratio of more than 2 in the wt controls. Morphologically in 3 months old mutant mice, the rod outer segments were about half of the length of those in the wild-type controls, and the outer nuclear layer thickness is about 60% of the normal thickness in the wild-type controls. In addition, abnormally large vesicles are found frequently in photoreceptor inner segments in mutant retina, suggesting impaired vesicle formation. These results demonstrate that the DHDDS$^{K42E/K42E}$ mouse recapitulates the human retinal degeneration phenotype.

[1]Bascom Palmer Eye Institute; [2]Department of Biology, University of Miami, Miami, FL; [3]Department of Biochemistry, Duke University Medical Center, Durham, NC.

Supported by NIH grants R01EY018586, P30-EY014801, LIPID MAPS Collaborative Grant GM-069338, Department of Defense grant W81XWH-09-1-0674, Adrienne Arsht Hope for Vision fund, and an unrestricted grant from Research to Prevent Blindness, Inc.

ABERRANT DOLICHOL CHAIN LENGTH DISTRIBUTION AS BIOMARKERS FOR RETINITIS PIGMENTOSA ASSOCIATED WITH DHDDS GENOTYPES.

Byron L. Lam, MD, PhD

CO-AUTHORS:
Ziqiang Guan, Potyra R. Rosa, Yiwen Li, Rong Wen

In search of quantitative biomarkers for retinitis pigmentosa (RP), we characterized urinary and plasma dolichol profiles in autosomal recessive RP (arRP) patients and carriers with mutations in the DHDDS gene encoding dehydrodolichol diphosphate synthase, a key enzyme in dolichol biosynthesis. Dolichols are long chain polyisoprenoid alcohols composed of 17-21 isoprene units.

Urine and/or plasma were collected from 9 DHDDS arRP patients, 35 DHDDS arRP carriers, 19 normals, and 34 arRP patients with wild-type DHDDS. Lipids were extracted and dolichols were measured by liquid chromatography-mass spectrometry (LC-MS). Dolichol length distribution was quantified as the ratio of dolichol 18 and dolichol 19 (D18/D19 ratio), the two most abundant dolichol species in human.

The mean plasma D18/D19 ratio of arRP K42E/K42E DHDDS patients (2.84±0.38, mean±SD, n=8) is significantly higher than K42E carriers (1.56±0.11, n=25, P<0.001) and normals (0.82±0.12, n=16, P<0.001). The mean urinary D18/D19 ratio of K42E/K42E patients (4.00±0.45, n=7) is also significantly higher than K42E carriers (1.27±0.19, n=30, P<0.001) and normals (0.47±0.06, n=13, P<0.001). Receiver-operating-characteristic (ROC) analysis shows both plasma and urinary D18/D19 ratios discriminate K42E/K42E patients from K42E carriers, and K42E carriers from normals with 100% sensitivity and 100% specificity. The predictive power of the D18/D19 ratio was tested by screening plasma samples from 36 arRP patients with unknown genotypes, and two were identified to carry the K42E/K42E DHDDS mutation. In

ABSTRACTS

addition, an arRP patient with novel compound heterozygous T206A/K42E DHDDS mutations was found to have high D18/D19 ratios similar to those of K42E/K42E patients, and the mean plasma and urinary D18/D19 ratios of T206 carriers (1.31±0.06, 1.04±0.04, n=5) were similar to K24E carriers, indicating similar functional defects of these two mutations.

Our results indicate mutations in the DHDDS gene lead to a characteristic shortening of plasma and urinary dolichols, which can be used as a functional readout of the enzyme. Urinary and plasma D18/D19 ratios reliably determine if a DHDDS genotype is disease-causing. D18/D19 ratio is a viable objective functional biomarker and can be readily adapted as a clinical test for arRP diagnosis and carrier screening with DHDDS or other genetic mutations that impair dolichol biosynthesis.

STEM CELLS AND PERSONALIZED MEDICINE

Stephen Tsang, MD, PhD

In the current era of personalized medicine, we have identified a large number of genetic variants in patients with various diseases using next-generation sequencing. To prove that genetic variants cause disease, we have traditionally relied on animal models. However, substantial differences exist between mice and humans. One obvious example is drastic difference in lifespans. Less obviously, the dopaminergic neuron projections in mice are shorter than humans compared to overall body length, and the alpha synuclein *Snca* knockout mouse does not develop Parkinson disease. With reprogramming technology, generation of patient-specific stem cell lines to use for validation of sequence variants, elucidation of pathophysiology, and targeted drug screening have become feasible. Recently developed high-efficiency genetic editing tools make it possible to correct the mutation, enabling transplantation back into the patient as an autologous approach to regenerative medicine.

RETINAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS: CLINICAL POTENTIAL

Shi-Jiang (John) Lu, PhD, MPH

Human pluripotent stem cells (hPSC) promise to provide an unlimited and reproducible source of replacement cells for human diseases. Various ocular diseases often result in blindness due to the loss of post-mitotic retinal neurons and other supporting cells. Among these diseases, retinitis pigmentosa (RP) and age-related macular degeneration (AMD) are characterized by loss of photoreceptor cells in the outer nuclear layer and retinal pigment epithelium (RPE); whereas glaucoma is characterized by optic nerve damage and death of retinal ganglion cells (RGC) that lead to irreversible loss of vision. We have developed a robust platform to generate photoreceptor progenitors, retinal ganglion cell progenitors and RPE from multiple hPSC lines. Photoreceptor progenitors derived from hPSCs expressed specific molecular markers for photoreceptor and integrated into outer nuclear layer after subretinal injection in mice. After further maturation in vitro, hPSC-ganglion progenitors differentiated into ganglion cells. RPE derived from hPSCs were capable of extensive photoreceptor rescue in both the RCS rat and Elov14 mouse. Improvement in visual performance was 100% over untreated controls and near-normal functional measurements were recorded at >60 days in RCS rats.

ACT is currently carrying out clinical trials at multiple sites (4 in the US and 2 in the UK) to establish the safety and tolerability of subretinal transplantation of hESC-RPE in patients with Stargardt's macular dystrophy and dry-AMD. hESC-RPE cells showed no signs of hyperproliferation, tumorigenicity, ectopic tissue formation, or apparent rejection after transplantation. Structural evidence confirmed hESC-RPE had attached and continued to persist, and visual improvement was observed in many of the treated eyes. These results suggest that hESC-RPE may provide a potentially safe source of cells for the treatment of a variety of retinal degenerative diseases.

HUMAN RETINAL PROGENITOR CELLS FOR PHOTORECEPTOR REPLACEMENT

Petr Baranov, MD, PhD

Loss of photoreceptors due to retinitis pigmentosa, age-related macular degeneration and other age, trauma and genetic-related retinal degenerative disorders leads to progressive loss of vision, and in some cases complete blindness. The successful allotransplantation of mouse photoreceptor precursors, isolated from developing retina or derived from pluripotent cell lines demonstrates the feasibility of restoration of retinal function through cell therapy. We have also shown that pig retinal progenitor allografts are able to survive and differentiate in pig retina. Human photoreceptor precursors, differentiated from pluripotent cell lines (ES, iPSC) also have the capacity to survive and form photoreceptors after transplantation into murine models of retinal degeneration. However, time- and resource-consuming protocols for photoreceptor differentiation from pluripotent cells, as well as the potential for tumor formation, currently limit the translational potential of this approach.

An alternative strategy is to isolate mitotically active human retinal progenitor cells (hRPC) from developing retina and expand them in vitro to the quantities required for both for preclinical characterization and clinical application. We have overcome the "supply" problem of this approach, by expanding in low oxygen conditions, allowing us to generate trillions of cells from one isolation. We have, in collaboration with a British stem cell company (ReNeuron ltd.), identified the optimal timeframe for hRPC isolation, determined the conditions for scale-up expansion, and generated three banks of GMP isolated and expanded hRPCs. We have demonstrated that the population continues to proliferate and express markers specific for stem cells and eye field development. In vitro differentiation studies confirmed that hRPC are capable to form photoreceptors as they express rod-specific proteins, such as rhodopsin and rod outer segment membrane 1.

Our xenotransplantation studies show the ability of hRPC to survive for up to 12 weeks, form rod photoreceptors after the transplantation into retinal degenerative hosts (rhodopsin knockout mice) and rescue vision in pigmented Royal College of Surgeons rats.

As a conclusion we submit that human retinal progenitor cell transplantation is a promising approach for photoreceptor cell replacement, and may be of use in retinal degenerative disorders such as dry AMD or Retinitis Pigmentosa.

STIMULATING ENDOGENOUS NEUROREGENERATION AS A STRATEGY FOR REVERSING BLINDNESS

Dong Feng Chen, MD, PhD

Recent progress in clinical trials of using human embryonic stem cell-derived cell transplantation therapy for age-related macular degeneration and in animal models of retinal diseases suggests the feasibility of stem cell strategy in preserving sight or reversing vision loss. This has opened the door to more treatments using cell replacement strategy to attack a problem common to various forms of retinal neuron damage or degeneration. Particularly, a chemical- or drug-based strategy of mobilizing endogenous stem cells to repair neural damage may present many advantages including less concern over immune rejection, neuron integration, and tumor formation. Recently, my laboratory has discovered a small molecule compound alpha-aminoadipate that converts mouse and human Müller cells—dormant stem-like cells found throughout the retina—into retinal progenitors and generate different retinal cell types *in vivo* and *in vitro*. We have now shown that subretinal injection of alpha-aminoadipate in adult mice with retinal degeneration rescues degenerating photoreceptors and leads to restoration of light-induced response of the retina. The retina has long served a model of the central nervous system (CNS),

ABSTRACTS

including the brain and spinal cord. This finding thus may have a broad impact on the development of a regenerative and cell replacement therapy for CNS injury and disorders.

WHOLE EXOME SEQUENCING IN PATIENTS WITH RARE NEUROGENIC DISORDERS UNCOVERS A MISSING MITOCHONDRIAL FUSION-LIKE PROTEIN

Stephan Züchner, MD, PhD

CO-AUTHORS:
Alexander Abrams[1], Michael Gonzalez[1], Adriana Rebelo[1], Julia Dallman[2]

Inherited optic atrophy and axonal neuropathy are primarily caused by mutations in the canonical mitochondrial fusion genes *OPA1* and *MFN2*, respectively. Interestingly, some patients may present symptoms of both diseases, however it remains to be elucidated for over 10 years, why defects in mitochondrial fusion mechanisms cause these disorders. We identified families with axonal neuropathy and optic atrophy phenotypes with recessive mutations in the putative mitochondrial carrier gene *SLC25A46*. Phylogenetic and functional evidence shows that SLC25A46 is the closest and thus far elusive equivalent to Ugo1p, a fungal specific protein that couples the fusion of outer and inner mitochondrial membranes. We further demonstrate SLC25A46 interaction with the mitofilin protein complex, which quickly becomes a hub for neurodegenerative diseases. In a zebrafish model, we observed neuronal degeneration, altered mitochondrial distribution in motor neurons, as well as abnormal mitochondrial fusion intermediates. Gene silencing in mammalian cells, in congruence with patient fibroblasts, causes the formation of an interconnected and hyperfilamentous mitochondrial networks. Our results underline the unique opportunities of identifying and characterizing rare disease genes in order to build trusted biological networks of disease and for targeted therapy.

[1] Dr John T. Macdonald Foundation Department of Human Genetics and John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine; [2] Department of Biology, University of Miami

EARLY-ONSET RETINAL DEGENERATION CAUSED BY TUBBY-LIKE PROTEIN 1 (*TULP1*) GENE MUTATIONS

Samuel G. Jacobson, MD, PhD

Fifteen years ago, an autosomal recessive retinal degeneration, first described as RP14, was identified as caused by *TULP1* gene mutations. What have we learned about the disease and mechanism in the interval from discovery to the present day? A cohort of *TULP1*-RD patients (ages 5-36 years) were studied with clinical examinations, kinetic and dark-adapted chromatic perimetry, electroretinography, and optical coherence tomography. There were longitudinal data in some of the patients. Visual acuities were no better than 20/80 (no shared refractive error). Nystagmus was present to varying degrees in most patients. A relatively rapid loss of peripheral visual function occurred in the first two decades of life; ERGs to all stimuli were not detectable. Residual function was in the central retina and was mediated by cone photoreceptors. Photoreceptor lamination by OCT was detectable, albeit abnormal, in the very central retina. *TULP1*-RD is a relatively rare early-onset and rapidly progressive disease of rods and cones. The relationship of these human results to those in studies of a Tulp1-deficient mouse model will be discussed.

NOTES

SPEAKERS

JOHN D. ASH, PHD
Francis M. Bullard Eminent Scholar
Chair in Ophthalmic Sciences
Associate Professor of Ophthalmology
University of Florida
Gainesville, FL
jash@ufl.edu

SHANNON E. BOYE, PHD
Assistant Professor
Retinal Gene Therapy Group
Department of Ophthalmology
University of Florida
Gainesville, FL
shaire@ufl.edu

DONG FENG CHEN, MD, PHD
Associate professor of Ophthalmology
Harvard Medical School
Associate Scientist
Schepens Eye Research Institute
Boston, MA
dongfeng_chen@meei.harvard.edu

M. ELIZABETH FINI, PHD
Vice Dean for Research
Professor and Director
USC Institute for Genetic Medicine
Keck School of Medicine
Los Angeles, CA
efini@med.usc.edu

JOHN FLANNERY, PHD
Professor of Neurobiology
Department of Molecular and Cell Biology
University of California, Berkeley
Berkeley, CA
flannery@berkeley.edu

MICHELLE L. HASTINGS, PHD
Assistant Professor
Chicago Medical School
Rosalid Franklin University of
Medicine and Science
Chicago, IL
michelle.hastings@rosalindfranklin.edu

SAMUEL G. JACOBSON, MD, PHD
Center for Hereditary Retinal Degenerations
Scheie Eye Institute
Philadelphia, PA
jacobsos@mail.med.upenn.edu

BYRON L. LAM, MD, PHD
Bascom Palmer Eye Institute
University of Miami School of Medicine
Miami, FL
blam@med.miami.edu

SHI-JIANG (JOHN) LU, PHD, MPH
Senior Director for Research
Advanced Cell Technology Inc
Marlborough, MA
sjlu@advancedcell.com

KIRILL A. MARTEMYANOV, PHD
Associate Professor
Department of Neuroscience
The Scripps Research Institute
Jupiter, FL
kirill@scripps.edu

BRIAN S. MCKAY, PHD
Associate Professor
Department of Ophthalmology and
Vision Science
University of Arizona
Tucson, AZ
bsmckay@eyes.arizona.edu

STEPHEN TSANG, MD, PHD
Associate Professor of Pathology and
Cell Biology and Ophthalmology
College of Physicians & Surgeons
Columbia University
New York, NY
phosphodiesterase.gamma@gmail.com

RONG WEN, MD, PHD
Professor of Ophthalmology
Bascom Palmer Eye Institute
University of Miami
Miller School of Medicine
Miami, FL
rwen@med.miami.edu

PETR BARANOV, MD, PHD
Schepens Eye Research Institute
Massachusetts Eye and Ear,
an affiliate of Harvard Medical School
Boston, MA
petr_baranov@meei.harvard.edu

STEPHAN ZÜCHNER, MD, PHD
(Interim) Chair, Dr. John T. Macdonald
Foundation Department of Human Genetics
Professor for Human Genetics and Neurology
John P. Hussman Institute for
Human Genomics
University of Miami
Miller School of Medicine
Miami, FL
szuchner@med.miami.edu

WHO WE ARE

HOPE for VISION
www.hopeforvision.org

BOARD OF VISIONARY SCIENTISTS

ALEX W. COHEN, MD, PHD
Chairman, Board of Visionary Scientists
Assistant Professor
Dean A. McGee Eye Institute
Oklahoma City, OK

EDUARDO ALFONSO, MD
Chairman, Bascom Palmer Eye Institute
University of Miami Miller School of Medicine
Miami, FL

JEAN BENNETT, MD, PHD
University of Pennsylvania
F.M. Kirby Center for
Molecular Ophthalmology
Scheie Eye Institute and
Children's Hospital of Philadelphia
Center for Cellular and
Molecular Therapeutics
Philadelphia, PA

THOMAS W. CHALBERG, PHD
Founder & CEO
Avalanche Biotech
San Francisco, CA

DEBORA B. FARBER, PHD
Jules Stein Eye Institute
UCLA Center for Health Science
Los Angeles, CA

JOE G. HOLLYFIELD, PHD
Cole Eye Institute
Cleveland Clinic Foundation
Cleveland, OH

SAMUEL G. JACOBSON, MD, PHD
Center for Hereditary Retinal Degenerations
Scheie Eye Institute
Philadelphia, PA

NANCY J. MANGINI, PHD
Northwest Center for Medical Education
Indiana University School of Medicine
Gary, Indiana

CARMEN PULIAFITO, MD, MBA
Dean, Keck School of Medicine
University of Southern California
Los Angeles, CA

VAL SHEFFIELD, MD, PHD
Roy J. and Lucille A. Carver
College of Medicine
University of Iowa
Iowa City, IA

STEPHAN ZÜCHNER, MD, PHD
Institute for Human Genomics
University of Miami Miller School of Medicine
Miami, FL

BOARD OF DIRECTORS

BETTI LIDSKY
Chairperson

JAIME EDELSTEIN, MD
President

ALEX COHEN, MD, PHD
Vice President

ABE KOSS, PA
Treasurer

DARIA ZAWADZKI, ESQ
Secretary

DANIEL BISGEIER, MBA
Member

SCOTT KLEIN, MBA
Member

HELEN LIN, MBA
Member

SANDRA REUS, MBA
Member

{ MY HEART TO JACQUE, LAURA, JANIS AND ROZI. YOU ARE THE LOVE THAT MAKES ALL THINGS POSSIBLE }

WITH OUR HEARTFELT GRATITUDE FOR YOUR CARING HEARTSIGHT AND COMMITMENT TO OUR MISSION:

Arnie Michael Films
Bacardi
Barker Animation Art
Chikovsky and Schafer, PA
The Continental Group
Continental National Bank
Fine Food Events
FirstService Residential
Gables Pediatrics
E. Gorin & E. Hanabergh, MD PA
ICCAS
The Karp Family
The Koss Family
Leon Medical Centers
The Lidsky Family
MediaMath
The McGuinn Family
Parent Petroleum
Sabadell United Bank
The Schwartz Family
South Motors Automotive Group
Southern Wine and Spirits
Sunshine Gasoline Distributors
Tabacalera Perdomo

...and our founding angel, Adrienne Arsht

SAVE THE DATE:

2015 Hope for Vision Science Symposium

September 10-11, 2015

Location TBD

HOPE for VISION
www.hopeforvision.org