ADDENDUM — ORAL PRESENTATION UPDATES

Monday, 21 July 2014

08:00 – 10:00 RP01 – RPE Replacement and Tissue Engineering — Bayview A

CHANGE IN PRESENTER **– To be presented by Juan Amaral**

09:37 **SURGICAL ASPECTS OF STEM CELL-DERIVED RETINAL PIGMENT TRANSPLANTATION IN ANIMALS**

JUAN AMARAL, Kapil Bharti and Arvydas Maminiskis

NATIONAL EYE INSTITUTE

08:00 – 10:00 RN01 – Gene Regulation and Protein Modulation in Retinal Development — Seacliff B

TITLE UPDATE

09:42 **FUNCTIONAL ROLES OF RAX HOMEOPROTEIN IN MOUSE RETINA DEVELOPMENT**

TAKAHISA FURUKAWA

13:00 – 15:00 LE02 – Can Crystallins Be Used as Therapeutic Agents? — Seacliff A

CHANGE IN PRESENTER, TITLE and ABSTRACT **– To be presented by Natik Piri**

13:49 **CRYSTALLINS PROTECT RETINAL GANGLION CELLS FROM AXONAL INJURY**

NATIK PIRI

JULES STEIN EYE INSTITUTE, UNIVERSITY OF CALIFORNIA, LOS ANGELES

CHANGE IN PRESENTER **– To be presented by Michael Kurnellas**

14:33 **SMALL HEAT SHOCK PROTEINS ARE EFFECTIVE IN NEUROINFLAMMATORY AND ISCHEMIC DISEASE MODELS**

MICHAEL KURNELLAS

STANFORD UNIVERSITY

13:00 – 15:00 RP02 – Phagosomes, Endosomes, and Lipofuscin in the RPE — Seacliff C

PRESENTATION CANCELLED

14:25 **PHAGOSOME MATURATION IN RETINAL PIGMENT EPITHELIAL CELLS IN HEALTH AND RETINAL DEGENERATIVE DISEASE**

CLARE FUTTER, SIlene Wavre-Shapton, Ingrid Meschede, Tanya Tolmachova, Thomas Burgoyne, Emily Eden, Hannah Mitchison, Miguel Seabra

13:00 – 15:00 RC02 – Retinal TRP Channels and Visual Function — Bayview B

CHANGE IN PRESENTER, TITLE and ABSTRACT **– To be presented by David Krizaj**

14:02 **THE ROLE OF TRPC1 CHANNELS IN RETINAL CALCIUM HOMEOSTASIS AND VISUAL SIGNALING**

DAVID KRIZAJ, Peter Barabas, Daniel Ryskamp, Tam Phuong, Tünde Molnar

OPHTHALMOLOGY AND VISUAL SCIENCES, MORAN EYE INSTITUTE, UNIVERSITY OF UTAH

16:30 – 18:30 CO03 – Corneal Stem Cells and Niches — Seacliff D

CHANGE IN PRESENTER **– To be presented by Ursula Schlotzer-Schrehardt**

16:30 **SOX9 AND PPAR GAMMA AS POTENTIAL REGULATORS OF CORNEAL EPITHELIAL DIFFERENTIATION**

URSULA SCHLOTZER-SCHREHARDT

UNIVERSITY OF ERLANGEN-NÜRNBERG, DEPARTMENT OF OPHTHALMOLOGY

Tuesday, 22 July 2014

08:00 – 10:00 LE04 – Communication in the Eye: Channels and Transporters — Seacliff A

PRESENTATION CANCELLED

09:42 **HUMAN OCULAR TRANSPORTERS: TRANSPORTER MEDIATED INTRAOCULAR DRUG DELIVERY**

SUNIL VOOTURI

13:00 – 15:00 RC05 – Photoreceptor Cell Biology I — Bayview A

CHANGE IN MODERATORS **– To be moderated by Joe Besharse and David Williams**

Wednesday, 23 July 2014

08:00 – 10:00 LE07 – Lens Development — Seacliff A

CHANGE IN PRESENTATION TIMES

08:00 **DNA METHYLTRANSFERASES IN MOUSE LENS DEVELOPMENT**

MICHAEL ROBINSON, Thanh Hoang, Devin Bruney, Savana Rosalez, Blake Rasor, Blake Chaffee, Evan Horowitz

09:20 **THE STORY OF LENS: REGENERATION AND EVOLUTION**

PANAGIOTIS TSONIS

08:00 – 10:00 IM04 – Ocular Infection and Host Defense — Golden Gate

CHANGE IN PRESENTER **– To be presented by Justine Smith**

09:32 **MECHANISMS OF TOXOPLASMA GONDII INFECTION OF HUMAN RETINA?**

JUSTINE SMITH

FLINDERS UNIVERSITY

Thursday, July 24, 2014

08:00 – 10:00 RC08 – Photoreceptor Cell Biology II — Bayview B

CHANGE IN MODERATOR **– To be moderated by Orson Moritz**

13:00 – 15:00 CO09 – Corneal Tissue Engineering and Gene Therapy — Seacliff D

CHANGE IN PRESENTER **– To be presented by Nicole Qiaozhi Lu**

14:00 **BIOMATERIALS FOR REPAIR AND REGENERATION OF EYE TISSUES**

NICOLE QIAOZHI LU

JOHNS HOPKINS UNIVERSITY

ADDENDUM — ORAL PRESENTATION REPLACEMENT ABSTRACTS

O268

08:00 – 10:00 Bayview A

RP01 – RPE Replacement and Tissue Engineering

SURGICAL ASPECTS OF STEM CELL-DERIVED RETINAL PIGMENT EPITHELIUM TRANSPLANTATION IN ANIMALS

JUAN AMARAL, Kapil Bharti, Arvydas Maminisquis

OCULAR AND STEM CELL TRANSLATIONAL RESEARCH UNIT, NATIONAL EYE INSTITUTE, NATIONAL INSTITUTES OF HEALTH

Purpose: To develop a reliable method for transplantation of scaffold based autologous iPS cell-derived RPE tissue in a pig model of micropulse laser induced RPE injury.

Method: Polarized fully matured iPS cell-derived RPE tissue is generated using biodegradable PLGA scaffolds. A 532nm micropulse laser is used to denude the host pig RPE with subsequent impairment of the retina. Following pars plana Vitrectomy, a retinal detachment is induced in the lasered area and a sheet of iPS cell-derived RPE tissue is introduce in the subretinal space using a custom made injector. Post laser and surgical follow up is done using mfERG, OCT and fundus autofluorescence.

Results: Several animal models were compared. Mature iPS cell-derived RPE fully integrates into the scaffold to form a polarized tissue. Micropulse laser is able to damage host RPE cells sparing the neural retina. Retina is reattached over the iPS cell-derived RPE scaffold as confirmed by OCT and fundus imaging.

Conclusion: Using a swine model, we show that the use of autologous iPS cell-derived RPE scaffolds is a feasible method for the treatment of retinal degenerations like atrophic Age Related Macular Degeneration.

IRB Status: None

Disclosures:
JUAN AMARAL, MD: No financial relationships to disclose.

O369

13:00 – 15:00 Bayview B

RC02 – Retinal TRP Channels and Visual Function

14:02

THE ROLE OF TRPC1 CHANNELS IN RETINAL CALCIUM HOMEOSTASIS AND VISUAL SIGNALING

DAVID KRIZAJ, Peter Barabas, Dan Ryskamp, Tam Phuong, Tünde Molnar

OPHTHALMOLOGY AND VISUAL SCIENCES, MORAN EYE INSTITUTE, UNIVERSITY OF UTAH

Canonical TRPC1 channels are the closest vertebrate homologs to the primordial TRP (transient receptor potential) channel identified in Drosophila photoreceptors. RT-PCR showed strong expression of Trpc1 transcripts in the mouse retina, with prominent in situ hybridization signals in all three nuclear layers. Genetic ablation of Trpc1 showed no changes in the photocurrent, scotopic or photopic b-wave phenotypes, indicating that the channel does not participate in light-dependent transduction and neurotransmission at rod and cone synapses. However, TRPC1$^{-/-}$ mice exhibited pronounced scotopic/photopic visual acuity phenotypes. Electrophysiological and optical imaging recording from Muller glia and retinal ganglion cells (RGCs) revealed that TRPC1 channels participate in store-operated signaling and Ca homeostasis in both proximal neurons and astroglia. Loss of TRPC1 resulted in disappearance of store depletion-activated, nonselective cation current and a reduction in the amplitude of store-operated [Ca2+]i overshoots. The remaining conductance exhibited the inward rectification characteristic of Orai1 channels. The spatial distribution of the store-operated channels within retinal cells was highly non-uniform, indicating compartmentalized function. In summary, we found that nonsynaptic TRPC1 channels, synergistically with Orai channels, mediate store depletion-mediated Ca signals in RGC and Muller glia but also regulate the excitability of retinal output neurons. Asha Vision has licensed the patents associated with some of the work presented by Daniel Ryskamp.

IRB Status: None

Disclosures:
DAVID KRIZAJ: No financial relationships to disclose.

O010

13:00 – 15:00 Seacliff A

LE02 – Can Crystallins Be Used as Therapeutic Agents?

13:49

CRYSTALLINS PROTECT RETINAL GANGLION CELLS FROM AXONAL INJURY

NATIK PIRI

JULES STEIN EYE INSTITUTE, UNIVERSITY OF CALIFORNIA, LOS ANGELES

Upregulation of alpha crystallins in response to a multitude of stress stimuli or to tissue damage is a commonly observed phenomenon. Based on their anti-apoptotic effect, the upregulation of these proteins was suggested to be a cellular response mechanism against environmental and metabolic insults. Expression of several members of the crystallin superfamily, including alpha A and alpha B, were found to be downregulated in experimental glaucomatous retinas two weeks after IOP elevation (~8% RGC loss), at both mRNA and protein levels. By 5 weeks after an increase in IOP (~20% RGC loss), the transcriptional levels of crystallin genes were elevated to the levels of control or even higher. However, at the protein levels, their expression was still lower than in controls. The observed downregulation of these proteins could undermine cellular defense response to high IOP-induced stress and consequently be associated with or responsible for RGC death. To address this question, we evaluated the cell protective effect of alpha A and alpha B crystallins in a rat optic nerve axotomy model, which is characterized by rapid and specific RGC degeneration. Overexpression of alpha A and alpha B led to an increase in the number of survived RGCs by approximately 95% and 75%, respectively.

Support: NIH/NEI EY018644 (NP)

IRB Status: None

Disclosures:
NATIK PIRI: No financial relationships to disclose.

Monday, 21 July 2014

POSTER SESSION 1

Anterior Segment

Viewing: 10:00 – 10:30; 12:00 – 13:00

Session with Authors: 15:00 – 16:30

P158 (Board 58)

NUCLEI RETENTION AND SUPPRESSED DNASE II ACTIVITY IN THE LENSES OF GLUTAREDOXIN 2 KNOCKOUT MICE

WEI ZHANG[1], Sumin Li[1], Hongli Wu[2], Marjorie Lou[1]

SCHOOL OF VETERINARY MEDICINE AND BIOMEDICAL SCIENCES REDOX BIOLOGY CENTER, UNIVERSITY OF NEBRASKA-LINCOLN[1]; DEPARTMENT OF PHARMACEUTICAL SCIENCES, COLLEGE OF PHARMACY, UNIVERSITY OF NORTH TEXAS HEALTH AND SCIENCE CENTER[2]

Glutaredoxin (Grx) is a key redox regulating enzyme that specifically dethiolate the glutathionylated proteins/enzymes (PSSGs). It is present in the cytosol as Grx1 and in mitochondria and nuclei as Grx2 that may closely associate with maintaining the transparency of a lens. Our laboratory has reported that the Grx2 isozyme protects lens epithelial cells from oxidation-induced mitochondrial malfunction and apoptosis. We have also found that Grx2 knockout (KO) mice display an early on-set and faster progression of lens opacity than the wild type (WT) littermates during aging. The opacity initiates at the nucleus and spreads outwards into cortical and posterior subcapsular regions. The immunohistological study of newborn lens from KO mouse shows higher nuclear density at the germinal zone than that of the WT mice. Nuclei can be seen in the inner fibers in KO lenses, but not the WT lenses. In the immunochemical staining of the lens cryo sections, high nuclei retention is also revealed in KO lenses but not in age-matched WT lenses. DNase II is a key enzyme to breakdown fiber cell nuclei in the lens. Others have reported that DNase II-null mice develop nuclear cataract associated with persistent nuclei in the innermost lens fibers. In our current study, we have found that the activity of DNase II decreases with age in Grx2 KO lenses. The DNA degradation rate decreases by 30-50% in 30 to 37-day-old KO mice compare to the age-matched WT mice. DNase II is known to be a target protein for Grx2, thus the decreased DNase II activity in the lenses of Grx2 KO mice may associate with the delayed denucleation and nuclear cataract development. The ability of Grx2 to dethiolate and reduce lens PSSGs may contribute to the preservation of DNase II activity.

IRB Status: Verified as IRB exempt

Disclosures:
WEI ZHANG, MD: No financial relationships to disclose.

P159 (Board 59)

GLUTAREDOXIN 2 GENE DELETION INCREASES SENSITIVITY TO UV RADIATION (UVR)-INDUCED CATARACT FORMATION IN MICE

XIAOLI TIAN, Wei Zhang, Marjorie Lou

SCHOOL OF VETERINARY MEDICINE AND BIOMEDICAL SCIENCES, REDOX BIOLOGY CENTER, UNIVERSITY OF NEBRASKA-LINCOLN

Ultraviolet light (UVB) radiation is a major risk factor for age-related cataract formation in humans. To study the association of thiol redox regulation with UVB cataractogenesis, we use glutaredoxin 2 (Grx2) gene Knockout (KO) mice as a model. Grx2 is a mitochondrial isozyme of the Glutaredoxin family, which specifically dethiolates and reduces S-glutathionylated proteins, or protein-GSH mixed disulfides (PSSGs) to restore proteins/enzymes function. It is known that Grx2 can protect mitochondrial function for ATP generation, and to prevent cell apoptosis. In the present study, we used 1-1.5m old Grx2 KO and age-matched wild type (WT) mice to expose them to UVB (300 nm) radiation in vivo for 15 min. The lenses were enucleated 2 days post-UV radiation (UVR) and used for morphological and biochemical analyses. Age-matched WT littermates and Grx2 KO non-exposed mice served as the controls. Morphological changes were photographed under dissecting microscope in a dark field. Lens homogenates were analyzed for glutathione (GSH) level by Ellman's reagent and the S-Glutathionylated lens proteins (PSSGs) by immunoblotting using specific anti-GSH antibody. The lenses of WT mice were clear. After exposure to UVR, some anterior sub capsular (ASC) light scattering could be seen in the pupil area. The age-matched Grx2 KO mice showed light scattering in the nuclear region, but ASC opacity appeared both in the pupil area and the peripheral region after UVR. GSH levels were decreased 20-30% while PSSGs were elevated in both UVB-exposed groups but more so in the KO mice. Lens proteins with sizes of 20-25 kDa and 46 kDa were the major PSSG bands. These morphological and biochemical results suggest that Grx2 gene deletion enhances UVB-induced oxidative damage to lens proteins, rendering higher sensitivity for cataract formation. Therefore, regulators for thiol redox homeostasis such as Grx2 may play a role in preserving lens transparency.

Supported by NIH RO1 10595 (MFL)

IRB Status: Verified as IRB exempt

Disclosures:
XIAOLI TIAN, PHD: No financial relationships to disclose.

P160 (Board 60)

BAICALIN INHIBITS EXPERIMENTAL AUTOIMMUNE UVEITIS BY SUPPRESSING VARIOUS CYTOKINES IN MOUSE

SU AH KIM, Suk Woo Yang

DEPARTMENT OF OPHTHALMOLOGY, SEOUL ST. MARY'S HOSPITAL, COLLEGE OF MEDICINE, THE CATHOLIC UNIVERSITY OF KOREA

The aim of this study is to investigate whether baicalin (a flavonoid derived from Scutellaria baicalensis) is effective in inhibiting ocular inflammation in a mouse experimental autoimmune uveitis (EAU) model. C57BL/6 mice (6-8 week old) were divided into three groups: a control group, an EAU group (with sham treated) and baicalin treated group (1mg/kg or 10mg/kg). Mice were immunized with 100 mg interphotoreceptor retinoid binding peptide (IRBP) emulsified in complete freund's adjuvant (CFA) and 0.1ml of this emulsion was injected subcutaneously into the left thigh. Clinical (TEFI: topical endoscopic fundus imaging) and histopathological (H&E staining) scoring of EAU were performed in each groups. Interleukin-1 alpha (IL-1a), interleukin-2 (IL-2) and interleukin 17 (IL-17) concentrations in the eyes of each groups were quantified using enzyme linked immunosorbent assay (ELISA). Intracellular expression of IL-17 in the activated CD4 positive T cells was assessed by flow cytometry. We observed that daily intraperitoneal administration of high dose of baicalin (10mg/kg) improved clinical appearance of uveitis compared to sham treated group. On day 10, the mean clinical score of sham treated group was 3.2±0.3 whereas that of high dose of baicalin treated groups was 2.2±0.3 ($p<0.05$). In sham treated group, IL-1a, IL-2 and IL-17 were markedly elevated and both dosages of baicalin (1mg/kg or 10mg/kg) significantly suppressed the expression of these inflammatory cytokines ($p<0.05$). There was a significant decrease of IL-17 positive T cells in spleen and peripheral mononuclear blood cells (PBMC) in mice treated with baicalin. In conclusion, intraperitoneal administration of baicalin diminished IRBP-induced experimental autoimmune uveitis in mouse by suppressing inflammatory cytokines and differentiation of Th17 cells. Our results provide laboratory evidence to support the idea that baicalin can be one of the candidate substances which can be used to treat uveitis.

IRB Status: Approved by IRB or equivalent

Disclosures:
SU AH KIM, MD: No financial relationships to disclose.

P161 (Board 61)

SUPPLEMENTING THE CORNEAL DONOR POOL USING HUMAN DECELLULARISED CORNEAS

ANDREW HOPKINSON, Laura Sidney, Siobhn Dunphy, Harminder Dua, Samantha Wilson

UNIVERSITY OF NOTTINGHAM

There is a clinical need for reliable, reproducible biomimetic corneas that are as effective, preferably superior, to cadaveric donor tissue. Decellularised tissues are advantageous compared to synthetic or semi-synthetic engineered tissues in that the native matrix ultrastructure is present and intrinsic biological cues including growth factors, cytokines and glycosaminoglycans (GAGs) may be retained. However, there is currently no reliable, standardised human corneal decellularisation protocol. The purpose of this work is to provide a systematic, direct comparison of commonly used decellularisation methods and to assess their reproducibility and reliability.

Corneal eye-bank tissue deemed unsuitable for transplantation was utilised to determine an optimal, human specific decellularisation technique. Hypertonic sodium chloride; ionic detergent sodium dodecyl sulphate; non-ionic detergent Triton-X100; and mechanical agitation, followed by nuclease treatments were investigated. Removal of detectable cellular and immune reactive material was evidenced by immunofluorescence and quantitative assays. Preservation of optical properties and light transmittance was evaluated. Retention of corneal architecture and GAGs was assessed via histological, immunofluorescence and quantitative analysis.

None of the decellularisation techniques investigated successfully removed 100% of cellular components. The techniques which had the least residual DNA were the most structurally compromised. GAG analysis demonstrated the stripping effects of the different decellularisation treatments.

The ability to utilise, reprocess and regenerate tissues deemed "unsuitable" for transplantation allows us to salvage valuable tissue. Reprocessing the tissue has the potential to have a considerable impact on addressing the problems associated with cadaveric donor shortage, which would have a significant economic benefit. Patients would directly benefit by accessing greater numbers of corneal grafts and health authorities would fulfill their responsibility for the delivery of effective corneal reconstruction to alleviate corneal blindness.

Acknowledgements: Work funded by the EPSRC Engineering, Tissue Engineering and Regenerative Medicine (E-TERM) Fellowship Scheme

IRB Status: None

Disclosures:
ANDREW HOPKINSON, PHD: No financial relationships to disclose.

P162 (Board 62)

FUNCTIONAL EFFECTS OF TRUNCATIONS IN CX46 AND CX50 IN THE HUMAN LENS

MIDUTURU SRINIVAS[1], Nefeli Slavi[1], Wang Zhen[2], Schey Kevin[2]

SUNY COLLEGE OF OPTOMETRY[1], VANDERBILT UNIVERSITY[2]

Gap junctional conductance in the lens core reduces dramatically with age (Gao et al., 2013, IOVS, 54:7174), limiting delivery of anti-oxidant molecules such as glutathione to the nucleus. Whether this reduction is due to post-translational modifications of gap junction proteins, Cx46 and Cx50, leading to alterations in their function, is not known. In this study, we identified truncation sites in Cx46 and Cx50 in the human lens using mass spectrometric analysis, and characterized their functional effects. Human lenses were dissected into outer cortex, inner cortex, and nucleus, and subjected to enzymatic digestion. Enzyme-digested peptides were analyzed by liquid chromatography (LC)-electrospray tandem mass spectrometry (ESI/MS/MS). We examined the effects of the different truncated channels on conductance and gating using in vitro electrophysiological measurements. Truncation sites were found in the C-terminus, the cytoplasmic loop and the N-terminus of Cx46 and Cx50. In the C-terminus, truncations were found at residues 238-251 in Cx46 and at residues 238-253 and 274-284 in Cx50. Levels of these C-terminal truncations were similar in the different regions of the lens. In contrast, levels of cytoplasmic loop truncations in Cx46 and Cx50 found by mass spectrometry increased dramatically from outer cortex to nucleus. For example, truncation at residue 129 in Cx46 and at residue 119 in Cx50 occurred predominantly in the nucleus. Electrophysiological studies indicated that all the C-terminal truncations were functional. The voltage gating properties and single channel conductance of channels formed by Cx46 and by Cx50 truncated at residues 251 and 253, respectively, were similar to those formed by full-length connexins. Truncations in the cytoplasmic loop of both Cx46 and Cx50, in contrast, prevented the formation of functional channels. These results might explain the large age-dependent reduction in the coupling conductance and the impediment to GSH delivery to the lens nucleus.

IRB Status: None

Disclosures:
MIDUTURU SRINIVAS: No financial relationships to disclose.

P163 (Board 63)

EFFICIENT INDUCTION OF NEURAL CREST CELLS FROM HUMAN PLURIPOTENT STEM CELLS

HIROSHI TANAKA[1], Yoshinori Nakai[1], Makoto Fukuda[2], Morio Ueno[1]; Makoto Ikeya[2]; Jyunya Tokuchida[2]; Shigeru Kinoshita[1]

DEPARTMENT OF OPHTHALMOLOGY, KYOTO PREFECTURAL UNIVERSITY OF MEDICINE[1]; CENTER FOR IPS CELL RESEARCH AND APPLICATION, KYOTO UNIVERSITY[2]

Neural crest cells (NCCs) possess multi-potency to differentiate many kinds of somatic cells. During an eye development, corneal endothelium and keratocytes are derived from NCCs. The efficient induction system of NCCs from human pluripotent stem cells (hPSCs) is a useful tool to regenerate the anterior segment of the eye. The purpose of this present study is to develop a novel method for neural crest induction from hPSCs and expand induced NCCs (iNCCs) with maintaining their characters.

hPSCs were isolated from feeder cells and then cultured on Matrigel-coated dishes in serum-free medium with chemical compounds. The proportion of p75NTR high cells was detected by use of flow cytometry. The time-period and concentration of the treatment of chemical compounds for the induction of p75NTR high cells were optimized. The expression of neural crest markers in p75NTR high cells was examined by qPCR and immunohistochemistry. The characteristics between iNCCs (PN0) and expanded iNCCs (PN10) are compared using cDNA microarray. The induction rate of p75NTR high cells peaked by 7-day treatment. The proportion of p75NTR high cells was up to 30% with a 7-day treatment of a TGF-β Inhibitor (10M). The efficiency of p75NTR high cells was increased by the 7-day treatment of a mixture of a TGF-**β** inhibitor and a GSK-3 inhibitor. The optimized concentration of a GSK-3 inhibitor (1.0 μM) increased p75NTR high cells up to 80%. p75NTR high cells co-expressed the other neural crest markers such as SOX10, TWIST, AP2-α and PAX-3 by qPCR and AP2α by immunohistochemistry. CDM with b-FGF (20ng/ml) and EGF (20ng/ml) enabled to maintain iNCCs. The global gene expression profiles of PN10 iNCCs are similar to PN0 iNCCs and different from those of original hPSCs. We developed a fundamental system to regenerate corneal endothelium and keratocytes from hPSCs through NCCs.

IRB Status: None

Disclosures:
HIROSHI TANAKA, MD: No financial relationships to disclose.

P164 (Board 64)

ADALIMUMAB AND ACUTE ACQUIRED INCOMITANT ESOTROPIA IN A PATIENT TREATED FOR UVEITIS

TAHRA ALMAHMOUD[1], Hani Sherif[2], Jean Deschenes[2]

UNITED ARAB EMIRATES UNIVERSITY, DEPARTMENT OF SURGERY[1]; MCGILL UNIVERSITY, DEPARTMENT OF OPHTHALMOLOGY[2]

Adalimumab (Humira) is a recombinant human immunoglobulin monoclonal antibody that binds specifically to Tumor Necrosis Factor alpha (TNF alpha). It is approved for the treatment of rheumatoid arthritis and Crohn's disease and recently gained wide popularity in the treatment of chronic non-infectious intraocular inflammations not responding to classical immunosuppressive therapies. There is evidence that anti-TNF alpha treatment are implicated in the development of demyelinating neurologic events.

We here report a 49-year-old male presented with severe ocular pain and double vision of 2 weeks duration while taking anti-tumor necrosis factor alpha (anti-TNF alpha) adalimumab for pars planitis refractory to standard therapies. On examination, the patient had horizontal, binocular diplopia that was worse at distance. His right esodeviation was incomitant and was 30 prism diopters (PD) for distant and 25 for near. His visual acuity was 20/50 OD and 20/25 OS. Slit lamp examination showed +2 anterior chamber cells and flare in both eyes.

The patient was diagnosed with acquired incomitant esotropia secondary to adalimumab. The symptoms resolved after discontinuation of the medication. Physician should pay close attention to the onset of neurological symptoms that wouldn't be explained by other factors in patient taking anti-TNF alpha. Discontinuation of the offending drug is the first management.

IRB Status: None

Disclosures:
TAHRA ALMAHMOUD, MD: No financial relationships to disclose.

P165 (Board 65)

A NOVEL TRUNCATION MUTATION IN GJA1 ASSOCIATED WITH PRIMARY OPEN ANGLE GLAUCOMA AND MICROCORNEA IN A LARGE CHINESE FAMILY

XUESHAN XIAO, Xiaobo Huang, NingLi Wang, Shiqiang Li, Qingjiong Zhang

STATE KEY LABORATORY OF OPHTHALMOLOGY, ZHONGSHAN OPHTHALMIC CENTER, SUN YAT-SEN UNIVERSITY

Genomic DNA was prepared from peripheral venous leukocytes of 15 individuals of a three generation Chinese family, including seven patients with POAG and microcornea, one with microcornea alone, and seven normal relatives. Whole exome sequencing was performed on genomic DNA of the proband. Candidate variants were obtained through multiple steps of bioinformatics analysis and then validated by Sanger sequencing and segregation analysis. A novel truncation mutation (c.791_792delAA, p.K264Ifs*43) in GJA1 was identified in the proband and was present in all seven patients with POAG and microcornea and the one with microcornea alone but not in the seven normal relatives in the family. It was not present in 1394 alleles from 505 unrelated controls and 192 normal controls. Extraocular signs were not observed in any patients except one who had dental enamel hypoplasia and syndactyly. This study suggests a high risk of POAG in patients with GJA1 mutation.

IRB Status: Approved by IRB or equivalent

Disclosures:
XUESHAN XIAO: No financial relationships to disclose.

P166 (Board 66)

OXIDATIVE STRESS IN NEURONS BUT NOT MÜLLER CELLS IN THE RETINAS OF DOGS WITH GLAUCOMA

KHALEEL ALYAHYA

COLLEGE OF MEDICINE KING SAUD UNIVERSITY

Objective: To test the hypothesis that oxidative stress occurs in neurons but not Müller cells in the retinas of dogs with glaucoma, and that the oxidative stress is associated with glutamate redistribution.

Animals: Sections from 12 control and 17 glaucomatous dog retinas.

Methods: For retinas embedded in plastic, serial 0.5 µm plastic sections were immunogold stained for total glutathione, taurine, and glutamate followed by image analysis for staining patterns and density. For retinas embedded in paraffin, sections were immunohistochemically stained for malondialdehyde (MDA), a marker of lipid peroxidation.

Results: Regions with different severities of damage were identified in glaucomatous retinas. In Müller cell bodies in damaged regions, immunostaining for the antioxidant taurine was not significantly changed compared to less damaged regions and control retinas ($p > 0.05\%$), while immunostaining for the antioxidant glutathione was significantly increased (x + sem, p, 0.?). In contrast to Müller cells, neurons had significantly decreased immunostaining for glutathione in damaged regions. In paraffin-embedded retinas, increased immunostaining for MDA was apparent in neurons in damaged regions of retina. A glutamate redistribution consisting of decreased levels of glutamate in neurons and increased levels of glutamate in Müller cells occurred in damaged regions.

Conclusions: Decreases in glutathione immunostaining and increases in MDA immunostaining in neurons suggest oxidative stress occurs in neurons in glaucomatous retinas. However, adjacent Müller cells in these regions maintained normal taurine immunostaining and significantly increased their levels of glutathione immunostaining, suggesting that oxidative stress was not increased in glial cells. The apparent loss of glutathione from neurons and accumulation of glutathione in Müller cells occurred in regions with glutamate redistribution and was consistent with the effects of the redistribution on the synthesis of glutathione. The results support the concept that oxidative stress and glutamate redistribution may interact to contribute to selective neuronal damage in glaucoma in dogs.

IRB Status: None

Disclosures:
KHALEEL ALYAHYA: No financial relationships to disclose.

Posterior Segment

Viewing: 10:00 – 10:30; 11:45 – 13:00

Session with Authors: 15:00 – 16:30

P263 (Board 63)

ADULT NEWT RPE CELLS ARE REPROGRAMMED INTO A UNIQUE STATE OF MULTIPOTENT CELLS TO REGENERATE MISSING NEURAL RETINA

CHIKAFUMI CHIBA[1], Rafiqul Islam[1], Ailidana Kunahong[1], Martin Miguel Casco-Robles[1], Kenta Nakamura[1], Wataru Inami[1], Fubito Toyama[2]

UNIVERSITY OF TSUKUBA[1]; UTSUNOMIYA UNIVERSITY[2]

The newt has an outstanding ability to regenerate a missing neural retina (NR) from the retinal pigment epithelium (RPE) cells. Upon a retinal injury RPE cells initially lose their epithelial structure and re-enter the cell-cycle. During this process, it has been inferred that RPE cells are reprogrammed into multipotent cells, however the nature of this RPE-derived cell remains unclear. Here, to address what state the adult newt RPE cells are reprogrammed into, we first identified Small eye Pax6 of this animal, which can be a marker of the cells which have a potency to generate both NR and RPE, and examined its expression patterns in RPE-derived cells by immunohistochemistry. As a result, we found that RPE cells which have just re-entered the cell-cycle do not exhibit Pax6-immunoreactivity, which was obviously detected in both the optic vesicle/cup of embryos and the ciliary marginal zone of larval/adult eyes. Pax6-immunoreactivity appeared in a small number of RPE-derived cells just before the RPE-derived cell population are sorted out into two rudimentary layers (pro-NR and pro-RPE), and finally localized along the pro-NR layer. Next we investigated expression of other stem-cell markers in addition to Pax6 in RPE-derived cells by single-cell qPCR. We found that the RPE-derived cells before cell division newly express pluripotency factors (c-Myc, Klf4 and Sox2; but not Oct4) and a microphthalmia factor Mitf as well as Pax6, all of which are suggested to be expressed in early optic vesicles. Taken together, these results suggest that the adult newt RPE cells are reprogrammed into multipotent cells similar to those of the early optic vesicle while preserving certain original characteristics, and then those multipotent cells are specified into two cell populations from which the pro-NR and pro-RPE layers are formed with a correct polarity.

IRB Status: Approved by IRB or equivalent

Disclosures:
CHIKAFUMI CHIBA, PHD: No financial relationships to disclose.

P264 (Board 64)

THE CONE CYCLIC NUCLEOTIDE-GATED (CNG) CHANNEL CNGB3 SUBUNIT MODULATES THE CHANNEL'S STRUCTURAL FLEXIBILITY AND CONE LIGHT RESPONSE KINETICS

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UNIVERSITY OF OKLAHOMA HEALTH SCIENCES CENTER[1]; WASHINGTON UNIVERSITY SCHOOL OF MEDICINE[2]

Cone phototransduction mediated by the cone cyclic nucleotide-gated (CNG) channel is essential for day light vision, color vision, and visual acuity. Native cone CNG channels are heterotetrameric complexes comprised of CNGA3 and CNGB3 subunits. CNGA3 is known as the ion-conducting subunit while CNGB3 is recognized as a modulator. Mutations in cone CNG channel subunits are associated with human cone diseases and mutations in CNGB3 alone account for over 50% of all known cases of achromatopsia. This work examined the structural and functional roles of CNGB3 using retinas from mice lacking CNGB3. As cones comprise only 2-3% of the total photoreceptor population in a wild-type mouse retina, we used a mouse line with CNGB3 deficiency on a cone-dominant background, i.e., Cngb3-/-/Nrl-/- mice. The channel complex assembly was analyzed by chemical cross-linking and velocity sedimentation, and the complex stability was evaluated by trypsin-TPCK digestion. Cone function was evaluated by photopic electroretinography (ERG) and transretinal ERG recordings. Although the expression level of CNGA3 was significantly reduced in Cngb3-/-/Nrl-/- retinas, CNGA3 alone was able to form homotetrameric complexes in cones lacking CNGB3. Compared with CNGA3/CNGB3 heteromeric complexes, CNGA3 homomeric complexes were more resistant to proteolytic digestion, suggesting a less flexible structure. Multiple electrophysiological recording approaches demonstrated a reduced photopic light response amplitude, decreased light response kinetics, impaired ability to adapt to background light, and reduced functional range in Cngb3-/-/Nrl-/- mice, compared with Nrl-/- controls. Thus we demonstrated that CNGB3 modulates the channel's structural flexibility and cone light response kinetics. This work provides insights into our understanding of the modulatory role of CNGB3 in cones.

IRB Status: Verified as IRB exempt

Disclosures:
XI-QIN DING, PHD: No financial relationships to disclose.

P265 (Board 65)

REPEATED WOUND STIMULATION LEADS TO A SELF-SUSTAINED ACTIVATION OF THE TGF-BETA PATHWAY IN CULTURED RPE CELLS: IMPLICATIONS FOR THE ONSET OF ADVANCED

MONTE RADEKE[1], Carolyn Radeke[1], Jane Hu[2], Dean Bok[2], Lincoln Johnson[1], Peter Coffey[1]

NEUROSCIENCE RESEARCH INSTITUTE, UNIVERSITY OF CALIFORNIA[1]; JULES STEIN EYE INSTITUTE, UNIVERSITY OF CALIFORNIA, LOS ANGELES[2]

Wound responses often contribute to degenerative disease onset and progression. In age-related macular degeneration (AMD) wet AMD is defined by vascular fibrosis. Geographic AMD is characterized by RPE cell death and failure to repair. To elucidate the critical aspects of RPE wound response we analyzed RPE gene expression in a model of protracted wound stimulation. Repeated subconfluent passage results in altered expression of over 40% of the transcriptome and loss of RPE phenotype when cultures are allowed to reach confluence and mature. In contrast, at subconfluence <5% of transcripts are differentially expressed in highly passaged RPE. Protein-protein interaction analysis reveals that the genes with differences at subconfluence can be organized into an interactome comprised of two interconnected modules enriched for functions pertaining to wound response and cell division. Among the wound response genes are activators of TGF-beta signaling. When cultured in medium containing A-83-01, an inhibitor of TGF-beta/activin receptor signaling, RPE can undergo multiple additional rounds of passaging while remaining differentiation competent in comparison to control cultures. Moreover, when highly passaged RPE are subsequently treated with A-83-01, they regain the ability to differentiate. Additionally, we found a disproportionate representation of RPE wound response genes among those genes with altered expression in wet and geographic AMD; including several genes that promote TGF-beta mediated wound responses. In conclusion, we found that extended sub-confluent culture of RPE cells leads to permanent activation of the TGF-beta pathway, which in turn results in and a state of perpetual wound response and loss of RPE phenotype. Furthermore, the preponderance of RPE-wound response genes associated with advanced AMD suggest that the transition from early to late AMD is marked by a switch to a state of chronic wound response. Accordingly, inhibition of TGF-beta signaling may serve as a potential general therapeutic approach for all forms of AMD.

IRB Status: Verified as IRB exempt

Disclosures:
MONTE RADEKE, PHD: No financial relationships to disclose.

P266 (Board 66)

GLUTAREDOXIN 2, A MITOCHONDRIAL THIOL REPAIR ENZYME, PROTECTS RPE CELLS FROM OXIDATIVE DAMAGE

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UNIVERSITY OF NORTH TEXAS HEALTH AND SCIENCE CENTER, UNT SYSTEM COLLEGE OF PHARMACY, NORTH TEXAS EYE RESEARCH INSTITUTE

The retinal pigment epithelium (RPE) is considered as a primary target for age-related macular degeneration (AMD). It is widely accepted that oxidative stress plays a major etiologic role in the development of AMD; therefore, antioxidant enzymes can be potential therapeutic targets for AMD. Glutaredoxin 2 (Grx2) is a recently identified mitochondrial thiol repair enzyme with multiple antioxidative activities. As a member of the glutathione-dependent oxidoreductase (redoxin) family, Grx2 catalyzes deglutathionylation of protein-glutathione mixed disulfides (PSSG) in mitochondria. In our previous study, we found that Grx2 protein level and enzyme activity were significantly decreased in RPE layers isolated from human AMD eyes. However, the physiological functions of Grx2 in the RPE and its possible association with AMD are completely unknown. The purpose of this study is to investigate the presence of Grx2 in RPE cells and to evaluate its potential anti-apoptotic function. Human retinal pigment epithelial (ARPE-19) cells were transfected with either a Grx2-containing plasmid or an empty vector. After transfection, cells were treated with or without 200 uM H2O2 for 24 h. Grx2 protein level and enzyme activity in Grx2 transfected cells were significantly increased as compared to non-transfected and vector transfected cells. Grx2 overexpression protected ARPE-19 cells from H2O2-induced cell viability loss. Assessment of apoptosis indicated that cells transfected with Grx2 were relatively more resistant to H2O2 with fewer cells undergoing apoptosis as compared to vector control cells. H2O2-induced PSSG accumulation in mitochondria was also attenuated by Grx2 enrichment. Furthermore, H2O2 treatment caused 50% of complex I activity loss in control cells (vector only) but only 25% in Grx2-enriched cells. Thus, the mechanism of Grx2 protection against H2O2-induced apoptosis is likely associated with its ability to preserve complex I and to prevent lethal PSSG accumulation in mitochondria.

IRB Status: None of the above

Disclosures:
HONGLI (CATHERINE) WU, PHD: No financial relationships to disclose.

P267 (Board 67)

REDUCTION OF ACUTE POST-OPERATIVE INFLAMMATION BY TOPICAL RX-10045 IN THE RABBIT PARACENTESIS MODEL

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NORTH CAROLINA STATE UNIVERSITY[1]; AUVEN THERAPEUTICS[2]

RX-10045 is an analog of Resolvin E1, a potent anti-inflammatory mediator that is endogenously produced from omega-3 fatty acid. RX-10045 may be a safer alternative to corticosteroids and NSAIDs for control of ocular inflammation. This study evaluated the therapeutic effect of RX-10045 on post-operative ocular inflammation in a rabbit model.

RX-10045 (0.03% and 0.1%), dexamethasone HCL (0.1%; DEX), or placebo was applied topically to both eyes of NZW rabbits 180, 120, 90, and 30 minutes prior to, and 15, 30, and 90 minutes after initial paracentesis (IP) performed using a 27-gauge needle to aspirate 100 uL of aqueous humor (AH). Clinical microscopic ocular inflammatory scores (OIS) (Hackett-McDonald) and IOP (TonoVet) were recorded pre-dose then at 0.5, 1, 2, 4, and 6 hours after IP. Paracentesis was repeated, rabbits euthanized, and ocular tissues collected at 0.5, 2, or 6 hours after IP. Also evaluated were AH protein concentration (Bradford assay) and inflammatory cell infiltrate on ocular histopathology.

Mean cumulative OIS, aqueous flare (AF), and cellular flare (CF) in placebo treated eyes remained elevated through 6 hours after IP. Eyes treated with RX-10045 (0.03% only) and DEX had significantly lower mean cumulative OIS, AF, and CF than placebo at 0.5 hour ($P<0.05$), and RX-10045 (0.03% and 0.1%) and DEX had significantly lower mean cumulative OIS, AF, and CF at 1 and 2 hours ($P<0.05$) after IP. IOP between groups was similar before and after IP. AH protein and inflammatory cell infiltrate were lower in 0.03% RX-10045 and DEX treated eyes compared to placebo at 0.5, 2, and 6 hours after IP.

Therefore, topical 0.03% RX-10045 was as effective as DEX in reducing paracentesis-induced ocular inflammatory scores, AH protein, and cellular infiltrate. These results strongly support further investigation of RX-10045 as a novel, effective, and safe treatment for ocular inflammation.

IRB Status: None

Disclosures:
BRIAN GILGER: Consultant/Advisor relationship with Auven Therapeutics

Anterior/Posterior Segment

Wednesday Viewing: 10:00 – 10:30; 12:00 – 13:00
Thursday Viewing: 10:00 – 10:30; 11:45 – 13:00
Session with Authors: 15:00 – 16:30

P351 (Board 51)

EXPRESSION OF G-PROTEIN GAMMA SUBUNITS IN GTγ-DEFICIENT MOUSE ROD PHOTORECEPTORS

OLEG G. KISSELEV[1,2], Alexander V. Kolesnikov[3], Elena Lobysheva[1], and Vladimir J. Kefalov[3]

DEPARTMENTS OF OPHTHALMOLOGY[1] AND BIOCHEMISTRY AND MOLECULAR BIOLOGY, SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE[2]; DEPARTMENT OF OPHTHALMOLOGY AND VISUAL SCIENCES, WASHINGTON UNIVERSITY SCHOOL OF MEDICINE[3]

Heterotrimeric G-protein transducin is central to the phototransduction cascade activation and signal amplification in retinal photoreceptors. Despite recent progress, relatively little is known about regulation of phototransduction by the transducin beta-gamma subunit complex (Gtβγ). Especially intriguing are the highly conserved expression patterns, which show rods and cones expressing unique Gβγ isoforms. The reason for the reliance on the single Gβγ isoform for phototransduction and its contribution to the unique physiological characteristics of rods and cones is unknown. To determine how other members of the G-protein gamma subunit family contribute to the specificity of receptor/G-protein interactions and the effectiveness of the downstream cascade, we have expressed transgenic constructs coding for the three known farnesylated isoforms: rod-specific Gngt1, cone-specific Gngt2, and ubiquitous Gng11, in the Gtγ-deficient mouse rod photoreceptors. Here we report the immunohistochemical and protein analysis data that show robust and uniform expression of the three individual transgenic constructs in mouse rods. The expression of transducin alpha subunit (Gtα) is restored to the wild-type levels in all cases. In the dark Gtα is targeted to the outer segments of transgenic rods, reversing the diffuse pattern found in the Gtγ-deficient rods. Exposure of the transgenic rods to bright background illumination leads to the characteristic translocation of the Gtα subunit from the outer to the inner segments, similar to the wild type phenotype. Recordings from the individual rods of the Gngt1 transgenic mice show that impaired light sensitivity observed in the Gtγ-deficient rods is fully restored. The results demonstrate that employed transgenic constructs are functionally active, which validates the overall strategy for studying other members of the G-protein gamma subunit family. Functional comparison with Gngt2 and Gngt11 transgenes is underway.

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IRB Status: None

Disclosures:
OLEG G. KISSELEV: No financial relationships to disclose.

P352 (Board 52)

BMP SIGNALLING INDUCES TRANSDIFFERENTIATION OF NR CELLS INTO RPE IN CHICK

NICOLA CORONATO, Jrg Steinfeld, Ichie Steinfeld, Heike Depner, Paul G. Layer, Astrid Vogel-Hpker

TECHNISCHE UNIVERSITT DARMSTADT, FACHBEREICH BIOLOGIE, ENTWICKLUNGSBIOLOGIE UND NEUROGENETIK

The retinal pigment epithelial (RPE) and the photoreceptors of the neural retina (NR) represent a functional unit required for vision in vertebrates. RPE defects therefore are associated with photoreceptor degeneration and blindness. During vertebrate eye development both the RPE and NR arise from neuroepithelial cells of the optic vesicle. In several species, the RPE can be induced by growth factors to transdifferentiate into NR. In contrast, growth factor-induced transdifferentation of NR into RPE in vivo has not been reported. We have previously shown, that bone morphogentic proteins (BMPs) and Wnts released from the overlying surface ectoderm induce RPE development in dorsal optic vesicle cells (Steinfeld et al., 2013). Here we show that BMPs can induce transdifferentiation of NR cells into RPE in ovo. BMP-application at optic cup stages induced a strongly hyper-pigmented RPE and led to a re-specification of neural retina progenitors within the ciliary margin zone into RPE. Remarkably, transdifferentiation of the central NR into RPE was also observed. These newly induced RPE cells expressed Mitf and Wnt2b and showed signs of differentiation, as visualised by the appearance of pigment granulae and the expression of the RPE-differentiation markers MMP115 and RPE65. Moreover, BMP exposure induced Wnt2b expression in the central and peripheral NR, which was independent of nuclear accumulation of β-catenin. The abiltiy of BMPs to induce RPE from differentiating and uncommitted NR cells will have profound impact on stem cell/medical research to find cures for ocular diseases, such as photoreceptor degeneration caused by pathological conditions of the RPE.

This work was supported by Deutsche Forschungsgemeinschaft.

IRB Status: None

Disclosures:
NICOLA CORONATO: No financial relationships to disclose.

P353 (Board 53)

RPE-FREE CHICKEN RETINAL EXPLANTS AND SPHEROIDS AS IN VITRO GLIOSIS MODELS: PEDF RESCUES DL-α-AMINOADIPATE-INDUCED GLIAL RESPONSES

PAUL LAYER, Gesine Bachmann, Gopenath Thangaraj, Alexander Greif, Jeanette Christophel

TECHNISCHE UNIVERSITT DARMSTADT, FACHBEREICH BIOLOGIE

Gliotic responses complicate human eye diseases, the causes of which often remain obscure. Retinal reaggregation models are excellent models to analyze cellular and molecular determinants leading to a three-layered retinal tissue, and thus can be instrumental to mimic and characterise pathologic states, e.g. gliosis, under defined in vitro conditions. Here, we activated MCs by the gliotoxin DL-α-aminoadipate (DL-AAA) and assayed possible protective effects by pigment epithelium-derived factor (PEDF) in explants and in histotypic retinal spheroids of the E6 chick embryonic retina. These model are suited since the avian retina i) contains only Müller cells (MCs) as glial components, ii) the RPE-free explants are devoid of a major internal PEDF source, and iii) - with reference to colour vision - can be compared to human retina. In both explants and spheres, 1mM DL-AAA treatment strongly activated Müller glial cells, as shown by GFAP, Vimentin and Glutamine synthetase IHC. ChAT and AChE IHC and enzyme histochemistry revealed that AAA treatment disrupted the cholinergic wiring in IPL areas. Moreover, stress-related catalase activity was increased under AAA treatment. Structurally, cell-free tissue gaps emerged in the middle of the INL, but TUNEL assays indicated that both in explants and spheres activated MCs were not associated with apoptosis. Remarkably, the activation of MCs was reduced upon PEDF treatment, albeit more in explants than in spheroids. These findings provide insights into molecular and cellular gliotic responses and suggest that supplementation with PEDF protects the retina against gliotic attacks. In vivo studies on mammalian retinae should establish whether PEDF supplementation could protect the human retina against gliotic attacks.

IRB Status: None

Disclosures:
PAUL LAYER: No financial relationships to disclose.

P354 (Board 54)

ROLE OF INFLAMMASOME IN DIABETIC RETINOPATHY

VELUCHAMY A BARATHI[1], Rayne R Lim[2], Bhav H Parikh[2], Yeo Sia Wey[2]; Wong Tien Yin[1]; Alessandra Mortellaro[3]; Shyam S Chaurasia[1]

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Diabetic Retinopathy (DR) is a microvascular complication of diabetes and amongst the leading causes of blindness worldwide. Early stages of DR have been extensively studied in mouse models, but not the advanced stages, proliferative DR (PDR), which has been poorly understood by the limited availability of reliable in vivo models. A double transgenic mouse model of DR named Akimba (Ins2Akita x VEGF+/-) developed recently that combines hyperglycemia and overexpression of vascular endothelial growth factor (VEGF) and displayed majority of the signs of PDR. In this study, we investigated the role of NLRP3 inflammasome components in the pathogenesis of PDR using Akimba mouse model and its parental strains, Akita (Ins2Akita) and Kimba (trVEGF029) mice. Blood glucose level measurements and retinal assessments were conducted weekly from 4 weeks to 12 weeks of age. Dark-adapted ERGs were recorded to assess the retinal function. Real-PCR and immunohistochemistry was used to study the regulation of inflammatory and NLRP3 inflammasome components in PDR. This study demonstrated the effects of the interplay between VEGF upregulation and hyperglycemia in the Akimba mice retina. Increase in macrophages was seen in all mouse models, with maximum levels in Akimba retina. This increase was highly correlated with the expression of NLRP3 inflammasome components-ASC, NLRP3, & Caspase-1 and associated with higher levels of pro-inflammatory mediators detected. Results indicated increase in activated macrophages that upregulated NLRP3 inflammasome, possibly in all the retinal layers, which released active IL-1β in the ganglion cell layer, initiating the autoinflammatory feedback loop and thereby promoting the inflammatory mediators to elicit a severe inflammatory response in DR. This study demonstrates that the Akimba mouse indicates several features of PDR and suggests an important role for NLRP3 inflammasome in the pathogenesis of DR.

IRB Status: None

Disclosures:
VELUCHAMY A BARATHI: No financial relationships to disclose.

P355 (Board 55)

NEURORETINA FATE SPECIFICATION OF SELF-ORGANIZING STEM CELLS REQUIRES REVERSIBLE WNT SUPPRESSION BY EYE FIELD GENES

TANUSHREE PANDIT[1], Deepti Abbey[1], Xin Geng[2], Alfonso Lavado[1], Yoshiki Sasai[3], Guillermo Oliver[1]

ST JUDE CHILDREN'S RESEARCH HOSPITAL[1]; OKLAHOMA MEDICAL RESEARCH FOUNDATION[2]; RIKEN CENTER FOR DEVELOPMENTAL BIOLOGY[3]

Congenital retinal abnormalities such as anopthalmia and micropthalmia and retinal degenerative diseases as retinal pigmentosa and macular degeneration cause varying degrees of irreversible vision loss in millions of people worldwide. Currently, the identity of the genes and mechanisms regulating early neuroretina (NR) specification has not been fully characterized. Recent advances using self-organizing 3D cultures of

ES and iPS cells provides us with a valuable in-vitro model in the characterization of the regulatory cascades and signaling pathways controlling normal retina development. We have previously reported that the activity of the homeobox gene Six3 is required to directly repress Wnt8b and Wnt1 during NR and forebrain development respectively. Taking advantage of available Rx knockdown ES-cell clones and recently generated Six3-/- iPS, in combination with the use of Wnt antagonists and agonists we have now evaluated the specific temporal involvement of Wnt signaling in early NR specification. Here I will describe results from this analysis showing the cross-regulation among Wnt signal and eye field genes. This work also provides additional support to the use of self-organizing 3D ES/iPS-cells as a robust in-vitro system to identify and validate candidate genes involved in eye development. (This work was supported by NIH Grant EY12162 to G.O)

IRB Status: Approved by IRB or equivalent

Disclosures:
TANUSHREE PANDIT: No financial relationships to disclose.

P356 (Board 56)

GENE THERAPY FOR A MOUSE MODEL OF ACHROMATOPSIA

SHANTA SARFARE, Seok-Hong Min, Sanford Boye, Astra Dinculescu, Shannon Boye, William Hauswirth, Christine Kay

DEPARTMENT OF OPHTHALMOLOGY, UNIVERSITY OF FLORIDA

Achromatopsia is an autosomal recessive retinal disorder characterized by cone photoreceptor dysfunction with an incidence of 1:30,000. Mutations in the cone cyclic nucleotide gated cation channel beta (CNGB3) account for 50% of the incidence of achromatopsia. Although there is a Cngb3 knockout mouse, it is difficult to investigate cone function in this model, as cones comprise a very small proportion of the mouse retina. Mice lacking the Nrl gene have no rods, increased S-cones and enhanced cone function. In this study, we investigated the results of gene replacement therapy in a Cngb3/Nrl double knockout (DKO) mouse retina to more closely approximate the cone-dominant macula of achromatopsia patients. One microliter viral vector AAV8 (Y733F)-Cngb3 (1 x1012 vector genomes/ml) where the Cngb3 gene is driven by the photoreceptor-specific IRBP/GNAT2 promoter, was injected in the subretinal space of 15 days old Cngb3/Nrl DKO mice. The treated eyes show increased photopic ERG and S-opsin mediated ERG b-wave amplitude responses compared to untreated fellow eyes up to 8 months post-injection. AAV-mediated gene delivery of CNGB3 results in long-term restoration of cone function in the Cngb3/Nrl DKO mouse. Ongoing studies include histological evaluation and immunolocalization of CNGB3 and other cone markers in the treated mice. The Cngb3/Nrl DKO mice may serve as a useful model for developing and testing therapeutic strategies for human cone photoreceptor degenerative diseases.

IRB Status: Approved by IRB or equivalent

Disclosures:
SHANTA SARFARE, PHD: No financial relationships to disclose.

P357 (Board 57)

DEFECTS IN LYN AND AIRE PATHWAYS COOPERATE TO PROMOTE AUTOIMMUNE UVEITIS

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UCSF DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY

Studies of genetic factors associated with human autoimmune disease point to a multigenic origin of autoimmune susceptibility. However, little is known about how defects in different immune tolerance checkpoints cooperate to result in full-blown disease. To address this, we chose to investigate whether genetic alterations in two pathways implicated in immune tolerance might synergize to lead to autoimmunity in mice. The intracellular protein tyrosine kinase Lyn is critical for inhibitory receptor function in both B cells and myeloid cells. Lyn-deficient mice develop lupus-like systemic autoimmune disease but not organ-specific autoimmunity. In contrast, Autoimmune Regulator (Aire) promotes the expression of tissue-specific antigens by thymic epithelial cells and T-cell negative selection. Aire-deficient mice develop multiorgan autoimmunity, including autoimmune uveitis that has been linked to absence of thymic expression of retina-specific protein, interphotoreceptor retinoid binding protein (IRBP), and expansion of IRBP-specific CD4 T cells. A knockin mouse model with a single mutant allele of Aire encoding a G228W mutation (AireGW/+) has low but detectable expression of IRBP and does not develop eye disease on a C57BL/6 genetic background. To determine whether the lack of Lyn inhibitory pathways can cooperate with a partial defect in T cell central tolerance to lead to organ-specific autoimmunity, we created a double mutant AireGW/+ Lyn-/- mouse. Remarkably, over 50% of double mutant mice developed severe uveitis that was not detected in parental genotypes and was accompanied by a rise in anti-IRBP antibodies and an expansion of IRBP-specific CD4 T cells. Interestingly, although Lyn-/- mice do not develop retinal autoimmunity, funduscopy revealed small abnormalities in the retinas of these mice. Flow cytometric analysis of Lyn-/- retinal cell populations showed an expansion of CD45hi CD11b+ myeloid cells, including increased numbers of activated F4/80+ macrophages. Furthermore, CD11b+ cells were detected in the subretinal space of Lyn-/- but not wt mice. These results suggest that Lyn-deficient retinal myeloid cells may promote uveitis induction by autoreactive T cells that have escaped negative selection due to partial loss of function of Aire.

IRB Status: No human subjects were a part of this study.

Disclosures:
IRINA PROEKT: No financial relationships to disclose.

ADDENDUM — POSTERS WITHDRAWN

Monday, 21 July 2014

BOARD 14 (P114)
INHIBITION OF LYMPHANGIOGENESIS AND HEMANGIOGENESIS IN CORNEAL INFLAMMATION SUBCONJUNCTIVAL PROX1 SIRNA INJECTION IN RATS
CHANG RAE RHO, Kyung-Sun Na, Kyung Jin Cho

BOARD 29 (P129)
EFFECTIVE AND PROSPECTIVENESS OF SELECTIVE LASER TRABECULOPLASTY IN TREATMENT OF PRIMARY OPEN ANGLE GLAUCOMA PATIENTS
GLEB KRISHTOPENKO, Nikolai Pozniak, Sergei Pozniak, Nikolai Kovshel

BOARD 30 (P130)
PRIMARY OPEN ANGLE GLAUCOMA AND PHACOEMULSIFICATION
IRINA KUDERKO, Nikolai Pozniak, Sergei Pozniak, Nikolai Kovshel, Gleb Krishtopenko, Pavel Beliakovskii

BOARD 36 (P136)
REGULATION OF THE MICROGLIAL PHAGOCYTOSIS-SENSOR TREM2 (CHR6P21) BY AN NF-KB-SENSITIVE MIRNA-34A (CHR 1P36) IN AGE-RELATED MACULAR DEGENERATION (AMD)
WALTER LUKIW, Prerna Dua, James Hill, Peter Alexandrov, Surjyadipta Bhattacharjee, Brandon Jones, Yuhai Zhao

BOARD 43 (P143)
SEQUENCE – STRUCTURE – FUNCTION OF LENS CRYSTALLINS
STÉPHANIE FINET, Fériel Skouri-Panet, Céline Férard, Elodie Duprat

BOARD 44 (P144)
CHARACTERIZATION OF THE CHILDHOOD LAMELLAR CATARACT IN TRANSGENIC MICE: IMPAIRMENT OF SECONDARY FIBER CELLS MORPHOGENESIS
RAJENDRA GANGALUM, Zhe Jing, Ankur Bhat, Yoshiko Nagaoka, Sophie Deng, Meisheng Jiang, Suraj Bhat

BOARD 48 (P148)
ALPHAA-CRYSTALLIN PREVENTS LENS EPITHELIAL CELL APOPTOSIS THROUGH NEGATIVE REGULATION OF P53-MEDIATED SIGNALING PATHWAY
DAVID LI

Board 57 (P157)
ESTABLISHING A MODEL TO INVESTIGATE ANTERIOR EPITHELIAL CELL DIVISION IN WHOLE PIG LENSES
REBECCA ZOLTOSKI

Tuesday, 22 July 2014

BOARD 1 (P201)
SIMVASTATINS INHIBIT PATHOLOGICAL RETINAL ANGIOGENESIS IN VLDLR MOUSE MODEL
SABU ABRAHAM, John Greenwood, Steven Moss, Xiaomeng Wang

BOARD 5 (P205)
CELLULAR THERAPY WITH KAINATIS OPTICONEUROPATHY
PAVEL BELIAKOUSKI, Nikolai Pozniak, Sergei Pozniak, Irina Kuderko

BOARD 17 (P217)
GENOTYPE-PHENOTYPE ANALYSIS IN PATIENTS WITH AUTOSOMAL RECESSIVE RETINITIS PIGMENTOSA DUE TO PDE6A MUTATIONS
DITTA ZOBOR, Gergely Zobor, Nicole Weisschuh, Bernd Wissinger, Susanne Kohl, Eberhart Zrenner

BOARD 23 (P223)
EXOGENOUS αβ-CRYSTALLIN PROMOTES TUBULOGENESIS IN HUMAN RETINAL ENDOTHELIAL CELLS
ROOBAN NAHOMI, Scott Howell, Ram Nagara

BOARD 49 (P249)
NOVEL PENTABLOCK COPOLYMER BASED NANOFORMULATIONS FOR SUSTAINED OCULAR DELIVERY OF PROTEIN THERAPEUTICS
ASHIM MITRA

Wednesday, 23 July and Thursday, 24 July 2014

BOARD 22 (P322)
RETINAL WHITE BLOOD CELL FLUX AND SYSTEMIC BLOOD PRESSURE IN PATIENTS WITH TYPE 1 DIABETES
GABRIELE FUCHSJÄGER-MAYRL, Stefan Palkovits, Leopold Schmetterer

BOARD 34 (P334)
FUNCTIONAL ROLES OF RAX HOMEOPROTEIN IN MOUSE RETINA DEVELOPMENT
TAKAHISA FURUKAWA