Paper

Retinal Cell Biology 1

Moderators

Akihiro Ohira
Shimane University School of Medicine, Izumo, Shimane, Japan
Yoko Ozawa
Keio University School of Medicine, Shinjuku, Tokyo, Japan

19-1 Withdrawn

Activation of TLR3 promotes the degeneration of retinal ganglion cells by up-regulating the expression of JNK3

Shravan Chintala [1] Nahrain Putris [1] Mason Geno [1]
1. Eye Research Institute, Oakland University, Rochester, MI, United States.

Purpose: To investigate whether activation of Toll-like receptor 3 (TLR3) promotes the degeneration of retinal ganglion cells (RGCs) by up-regulating the expression of JNK3, and by translocating JNK3 into the mitochondria.
Methods: TLR3-specific activator, Poly(I:C), or PBS was injected into the vitreous humor of Thy1-YFP mice. At 24 h, 48 h, and 72 h after treatments, degeneration of RGCs was assessed by using antibodies against beta-III-tubulin (Tuj1). A TLR3-specific inhibitor was injected into the vitreous humor with or without Poly(I:C). Western blot assays were performed to determine relative levels of TLR3, JNK3, pJNK3, and SARM1 expression in retinal protein extracts, and immunohistochemistry assays were performed to determine their cellular localization in the retina. Mice eyes were treated with Ploy(I:C) or PBS along with MitoTracker Red, and co-localization of MitoTracker Red and JNK3, MitoTracker Red and SARM1 was determined by immunolocalization studies by using JNK3 and SARM1 antibodies.
Results: Poly(I:C) activated TLR3, and up-regulated its downstream targets JNK3, but not SARM1 in the retina. Poly(I:C) activated TLR3 and up-regulated JNK3 specifically in RGCs and promoted a significant degeneration of RGCs over a 72 h time period. TLR3 up-regulated the expression of JNK3 in the cytoplasm of RGCs, but not in the mitochondria. TLR3-specific inhibitor down-regulated Poly(I:C)-mediated expression of JNK3, and, in turn, significantly attenuated TLR3-induced degeneration of RGCs.
Conclusions: Results presented in this study, for the first time, show that activation of TLR3 alone promotes the degeneration of RGCs by up-regulating the expression of JNK3, but not SARM1.
Commercial Relationships: Shravan Chintala, None; Nahrain Putris, None; Mason Geno, None
Support: National Eye Institute project grant EY017853-01A2

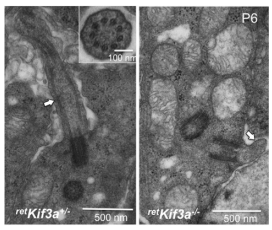
20-2

Heterotrimeric Kinesin-2 Mediates Transition Zone and Axoneme Formation of Mouse Photoreceptors

Li Jiang [1] Yuxiao Wei [1] Cecinio C. Ronquillo [1] Sen Wu [2] Robert E. Marc [1] Jeanne M. Frederick [1] Wolfgang Baehr [1, 3]
1. Department of Ophthalmology and Visual Sciences, University of Utah Health Science Center, Salt Lake City, UT, United States. 2. State Key Laboratory of Agrobiotechnology, College of Biological Sciences, China Agricultural University, Beijing, China. 3. Department of Biology, University of Utah Health Science Center, Salt Lake City, UT, United States.

Purpose: Anterograde intraflagellar transport (IFT) employing kinesin-II molecular motors has been implicated in protein trafficking of photoreceptor sensory cilia. Key players in anterograde IFT are heterotrimeric kinesin-II KIF3, consisting of KIF3A, KIF3B and KAP subunits, and IFT88, an obligatory IFT-B particle. Conditional deletions of KIF3A using Cre recombinase in post-natal retina yielded conflicting results. We therefore initiated this study to clarify role of KIF3-dependant anterograde IFT in mouse photoreceptor development and survival.
Methods: We generated embryonic retina-specific (ret) deletions of KIF3A and IFT88, before onset of photoreceptor differentiation, and tamoxifen-induced (tam) deletions of KIF3a and IFT88 in adult mice with fully developed photoreceptors. We then examined the subcellular localization of the photoreceptor outer segment (OS) proteins, including rhodopsin, cone opsins, other phototransduction and OS structure proteins in the KO mouse retinas by immunofluorescence microscopy. And the mouse photoreceptor ultrastructure and function were studied by transmission electron microscopy (TEM) and full-field electroretinography (ERG), respectively.
Results: In $^{ret}Kif3a^{-/-}$ and in $^{ret}Ift88^{-/-}$ mice, basal bodies docked to the cortex of mutant photoreceptors but failed to form transition zones (connecting cilia) and outer segments. Rhodopsin, cone pigments and other outer segment (OS) proteins were retained in $^{ret}Kif3a^{-/-}$ and $^{ret}Ift88^{-/-}$ photoreceptor inner segments as outer segments were absent. Tamoxifen-induced deletion of either KIF3a or IFT88 in the adult mouse each led to a slowly progressing photoreceptor degeneration manifested by outer segment shortening. Rhodopsin and cone pigments trafficked normally for more than 2 weeks post-induction, a time in which the OS is completely renewed. Fully mature $^{tam}Kif3a^{-/-}$ and ^{tam}Ift88$^{-/-}$ photoreceptor axonemes failed to be maintained and disintegrated slowly.
Conclusions: The results demonstrate that IFT is not required for rhodopsin transport to the OS. Rather, anterograde IFT mediated by KIF3 participates in photoreceptor transition zone (PTZ) and axoneme formation.
Commercial Relationships: Li Jiang, None; Yuxiao Wei, None; Cecinio Ronquillo, None; Sen Wu, None; Robert Marc, None; Jeanne Frederick, None; Wolfgang Baehr,

None
Support: NIH Grant EY08123-24A1



Abrogated connecting cilium formation in ^{ret}Kif3a$^{-/-}$ photoreceptors.

21-3

Molecular Basis for Retinitis Pigmentosa Associated with the Defect of SNRNP200- Pre-mRNA Splicing

Yuan Liu [1] Xue Chen [1]

1. Department of Ophthalmology, The First Affiliated Hospital of Nanjing Medical, NanJing, China.

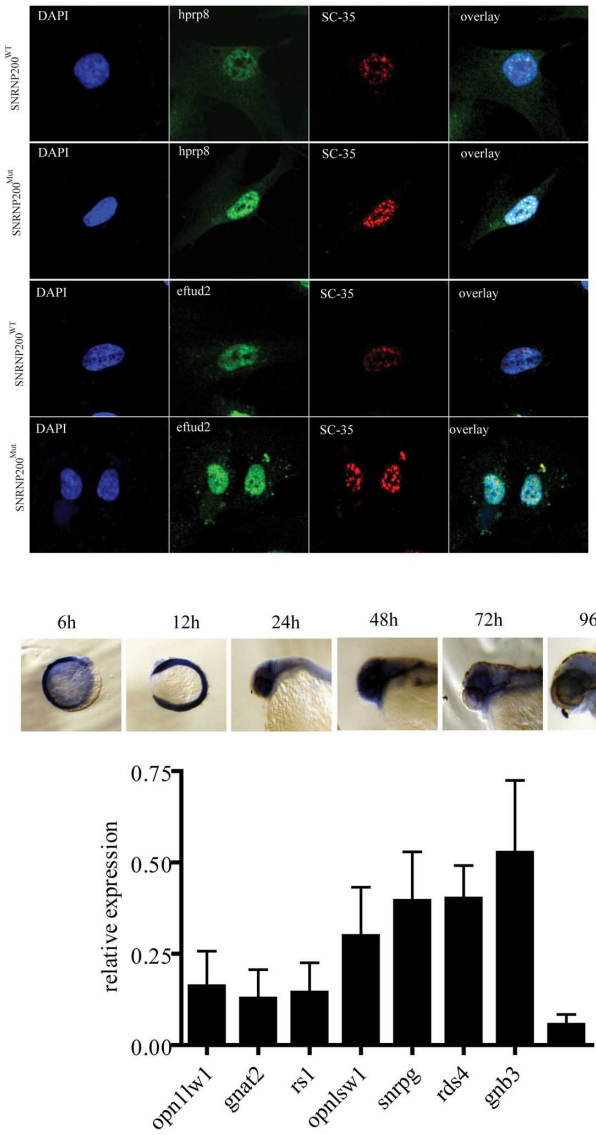
Purpose: Retinitis pigmentosa (RP) is one form of the most common inherited retinal dystrophies (IRDs) led by the progressive loss of rod/cone photoreceptors and retinal pigment epitheliums(RPE). Herein, we aim to identify how SNRNP200 defects would contribute to pre-mRNA splicing defects and retinal degenerations.

Methods: Primary fibroblasts were derived from the skin tissues of patients carrying a missense mutation, c.3260C>T (p.S1087L), in exon 25 of the *SNRNP200* gene and an unaffected member in their family. Proliferation abilities and apoptosis of the cultured fibroblasts were tested by Brdu assay and FACS, respectively.The localization of the endogenous SNRNP200 in the nucleus of the fibroblasts was determined by immnostaining. In situ analysis of snrnp200 expression in different stages of zebrafish was conducted. Morphological changes in zebrafish with snrnp200 gene knockdown (by morpholino) were also determined. Transversal cryosections of zebrafish with snrnp200 knockdown were analyzed by immunohistochemistry to visualize distinct retinal layers.

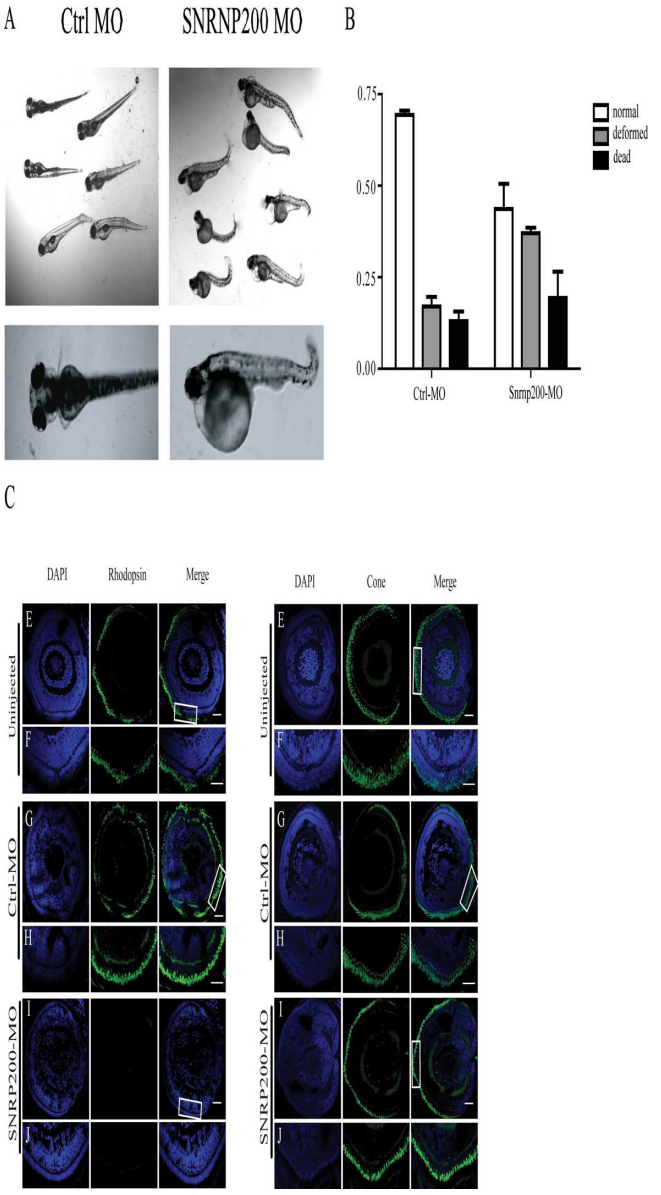
Results: Abnormal localization of trip-snRNP in the nucleus of fibroblasts carrying the mutation was revealed by immunostaining. The proliferation abilities of fibroblasts carryingthe mutation were significantly reduced when compared with those from the control, while no obvious apoptosis was detected in both wild type and mutant fibroblasts. In situ assay indicated that snrnp200was expressed extensively in all developmental stages of zebrafish, suggesting its important role in zebrafish development. Defects in photoreceptorouter segments were demonstrated by transversal cryosections of zebrafish with snrnp200 knockdown.

Conclusions: SNRNP200 defects can alter tissue-specific gene expression, generatesplicing defects, and lead to the RP phenotype.

Commercial Relationships: Yuan Liu, None; Xue Chen, None



A.Trip-snRNP expression in cells with or without mutation B.In situ analysis of snrnp200 expression in different stages of zebrafish C.the specific retinal markers expression when knockdown SNRNP200



(A,B) the phenotype and the teratogenic lethal of zebrafish model with SNRNP200-MO injection. (C)Defects in photoreceptorouter segments were demonstrated with snrnp200 knockdown.

22-4

Amelioration of age-related eye diseases in a spontaneous obese rat model by dietary intervention

Yadagiri Reddy Paduru [1] Shivalingam Pothula [1] Harishankar N [2] Uday Kumar Chekkilla [1] S. Sreenivasa Reddy [1] Shruthi Karanam [1] G Bhanuprakash Reddy [1]

1. Biochemistry, National Institute of Nutrition, Hyderabad, Telangana, India. 2. National Centre for Laboratory Animal Sciences , Hyderabad, India.

Purpose: Epidemiological studies have reported an association between metabolic syndrome (obesity, insulin resistance) and increased incidence of age-related eye diseases such asretinal degeneration and cataract, yet the underlying biochemical and molecular mechanisms remained unclear.Previously we describe retinal degeneration and cataract as a consequence of obesity in spontaneously developed obese (WNIN/Ob) rat model. In the present study we investigated the effect of diet restriction and dietary agents on obesity associated retinal degeneration and cataract using WNIN/Ob rat model

Methods: A group 40-day old WNIN/Ob rats along with age matched lean littermates were maintained on AIN-93 diet *adlibitum* for five months. Another group of WNIN/Ob rats were pair-fed with lean controls for the same period. While, a group 3-monts old WNIN/Ob rats along with age matched lean littermates were maintained on AIN-93 diet *adlibitum* for four months, two groups of WNIN/Ob rats were fed with two dietary agents, 2% ellagic acid (EA) and 0.002% procyanidin-B2 (PCB2) enriched cinnamon in the diet for the same period. Cataract and retinal degeneration were evaluated by slit-lamp microsope and electroretingram (ERG). Morphologyof retina by histology and immunoblotting of retina markers were performed in the retina. Eye lens protein profile was analyzed. Component of ER-stress and ubiquitin-proteasome system(UPS) were also analyzed in the lens and retina

Results: By morphological evaluation and ERG analysis, retinal degeneration in the WNIN/Ob rats appears to be ameliorated significantly by dietary restriction and partially by dietary agents. Immunohistochemical analysis with retinal markers further confirmed prevention of retinal degeneration in the obese rats by these dietary approaches. In addition to altered protein profile, there is a significant increase in sorbitol levels in the eye lens of WNIN/Ob compared to their lean controls. Diet restriction and feeding of dietary agents (EA and PCB2) led to decreased sorbitol levels and lower incidence of cataract in obese rats. Dietary intervention modulated ER-stress and UPS components in the lens and retina

Conclusions: These results suggest that dietary intervention could provide a viable approach to prevent obesity-associated age-related eye diseases

Commercial Relationships: Yadagiri Reddy Paduru, None; Shivalingam Pothula, None; Harishankar N, None; Uday Kumar Chekkilla, None; S. Sreenivasa Reddy, None; Shruthi Karanam, None; G Bhanuprakash Reddy, None

23-5

Reprogramming human newborn RPE cells toward retinal progenitor cells by SOX2

Razie Ezati [1] Zahra-soheila Soheili [1] Shahram Samei [2] Azadeh Etemadzadeh [1] Ehsan Ranaei pimardan [4, 1] Hamid Ahmadieh [3] Alireza Zomorodipour [1]

1. National Institute of Genetic Engineering and Biotechnology, Tehran, Iran (the Islamic Republic of). 2. Iranian Blood Transfusion Organization research Center, Tehran, Iran (the Islamic Republic of). 3. Ophthalmic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran (the Islamic Republic of). 4. Tarbiat modares university, Tehran, Iran (the Islamic Republic of).

Purpose: SOX2 is a member of the SRY-related Box family of transcription factors that play a critical role in re-programming phenomenon. This protein maintains pluripotency properties in neural progenitor cells and inhibits their differentiation. The purpose of this study was cloning of the coding sequence of SOX2 gene in

pAAV-MCS and studying its over-expression in RPE cells in culture. Adeno associated virus is a safe and non-pathogenic expression system which was used for gene transfer into RPE cells to re-program the cells.
Methods: Plex.SOX2 that contained human SOX2 gene was digested by BamH1 and Xho1 restriction enzymes and the corresponding isolated segment was inserted into pAAV-MCS vector. Green fluorescent protein (GFP) cloned in downstream of SOX2 gene as a reporter gene for determining gene transfection efficiency. Recombinant vector was sequenced and concomitantly transfected with helper vectors (pAAV-RC, pAAV-helper) to HEK293 cells. Produced viral particles collected from HEK293 culture supernatant and their titre was determined by flowcytometry. Human newborn RPE cells were infected by recombinant virus. Retinal progenitor cell markers like pax6, chx10 and neural progenitor marker,nestin, were evaluated by real time PCR and immunocytochemistry.
Results: Production of recombinant constructs was confirmed by PCR, restriction enzyme digestion and sequencing procedures. Recombinant viral production in HEK293T cells was monitored successfully. Titration result showed presence of 2×10^6 particle in 1 ml of culture supernatant. . According to time study result, human new born RPE cells were infected by recombinant virus with MOI=10 and 24h post-infection,RNA extraction and real time PCR were done. Real time data showed 80 fold over-expression of sox2 in infected cells. Among markers that were studied; nestin expression was significantly increased in real time PCR and ICC results.
Conclusions: A large number of studies have been shown important roles for SOX2 in maintaining neural progenitor/stem cell properties. Since nestin is neural progenitor cell marker, its expression in treated cells shows RPE dediffrentiation toward neural progenitor cells.
Commercial Relationships: Razie Ezati, None; Zahra-soheila Soheili, None; Shahram Samei, None; Azadeh Etemadzadeh, None; Ehsan Ranaei pimardan, None; Hamid Ahmadieh, None; Alireza Zomorodipour, None

24-6

Protective effect of B101, a mixture of nutrients, in a retinal degeneration mouse model

Tomoko Hasegawa [1] Hanako O. Ikeda [1,2] Masayuki Hata [1,2] Yuki Muraoka [1] Akira Kakizuka [3] Nagahisa Yoshimura [1]

1. Department of Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan. 2. Institute for Advancement of Clinical and Translational Science, Kyoto University Hospital, Kyoto, Japan. 3. Laboratory of Functional Biology, Kyoto University Graduate School of Biostudies, Kyoto, Japan.

Purpose: Retinitis pigmentosa is one of the leading causes of blindness, and there is no established treatment available. This study was aimed at investigating whether a mixture of nutrients, B101, has a protective effect in late-stage photoreceptor degeneration in *rd12* mice, a retinal degeneration model with an *Rpe65* gene nonsense mutation.
Methods: Thirty-four 13-month-old *rd12* mice were assigned to saline or B101 group (n = 17, each). The B101 group had *ad libitum* access to water containing 7.5 g/L B101. Morphologic changes were longitudinally examined with spectral domain optical coherence tomography from 13 to 19 months. The entire retinal thickness was measured at a point 366 μm around the optic nerve head. Photoreceptor layer thickness (sum of the outer nuclear layer, photoreceptor myoid zone, ellipsoid zone, and outer segment) was measured at points 244 μm above and below the optic nerve head center, and they were averaged. Retinal function was evaluated using photopic electroretinography b-wave amplitude (stimulation, 10.0 cds/m^2). The eyes of the 19-month-old mice were enucleated and retinas were separated from the combination of the choroid and sclera. Western blot was performed to evaluate the expression of CCAAT/-enhancer-binding protein homologous protein (CHOP, an endoplasmic reticulum stress marker) and activity of cleaved caspase-3 (a factor activated in apoptosis) in the retina and combination of the choroid and sclera in the 19-month-old mice.
Results: The total retinal thickness in the saline and B101 groups was 202.4 ± 9.0 μm and 203.0 ± 9.0 μm, respectively, at 13 months ($P = 0.79$), and the corresponding 19-month values were 185.0 ± 10.2 μm and 194.4 ± 12.6 μm ($P < 0.01$). The photoreceptor layer thickness in the saline and B101 groups was 58.5 ± 6.3 μm and 59.7 ± 4.6 μm, respectively, at 13 months ($P = 0.40$), and the corresponding 19-month values were 39.4 ± 7.4 μm and 45.3 ± 6.8 μm ($P = 0.01$).
Photopic electroretinography b-wave amplitudes in the saline and B101 groups at the 10.0 cds/m^2 stimulus were 12.2 ± 5.0 μV and 14.9 ± 6.0 μV, respectively ($P = 0.09$), at 19 months.
Western blot showed suppression of CHOP expression in the combination of the choroid and sclera and suppression of cleaved caspase-3 activity in the retina in the B101 group.
Conclusions: B101 suppressed photoreceptor degeneration in *rd12* mice even in the late stages of the disease when degeneration had progressed.
Commercial Relationships: Tomoko Hasegawa, in preparation (P); Hanako Ikeda, in preparation (P); Masayuki Hata, in preparation (P); Yuki Muraoka, in preparation (P); Akira Kakizuka, in preparation (P); Nagahisa Yoshimura, in preparation (P)

Retinal Cell Biology 2

Moderators

Peizeng Yang
The First Affiliated Hospital Chongqing Medical University/Chongqing Eye Institute, Chongqing, China
Nahoko Ogata
Nara Medical University, Kashihara, Nara, Japan

25-1

Pericyte-deficient retinopathy models, Novel animal models for progressive diabetic retinopathy

Junyeop Lee [1] Akiyoshi Uemura [3] Gou Young Koh [2] Young Hee Yoon [1]
1. Department of Ophthalmology, Asan Medical Center, University of Ulsan, College of Medicine, Seoul, Korea (the Republic of). 2. Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Korea (the Republic of). 3. Department of Retinal Vascular Biology, Nagoya City University, Graduate School of Medical Sciences, Nagoya, Japan.

Purpose: Diabetic animals do not display enough vascular alterations to represent proliferative diabetic retinopathy (PDR) during their lifespan. Instead, oxygen-induced retinopathy (OIR) models are widely used as animal models for PDR. However, OIR model omits pericyte dropout and microaneurysm formation which are the earliest and most specific structural changes of DR. The purpose of this study is to develop a progressive DR model which follows sequential vascular changes from the pericyte dropout to the vascular proliferation.
Methods: PDGF-B/PDGFR β signaling is important for pericyte-endothelial interactions, and thus disruption of this signaling axis has been suggested to play a role in pericyte apoptosis in DR. To induce pericyte dropout *in vivo*, we produced monoclonal antibody against PDGFR β from the hybridoma cell line. Pericyte-deficient retinopathy model was developed by intraperitoneal injection of this antibody into postnatal mice. In addition, we generated pericyte-deficient-OIR (PD-OIR) model by combining the conventional OIR model with intraperitoneal injection of anti-PDGFR β antibody.
Results: Intraperitoneal anti-PDGFR β antibody effectively induced pericyte dropout in the postnatal mouse retina. In these mice, retinal blood vessels were dilated and tortuous. Microaneurysms accompanied by intraretinal hemorrhage and edema appeared at the pericyte-deficient blood vessels. Intriguingly, even after the cessation of the antibody and pericyte coverage was recovered, continuous deteriorations of retinal vasculature progressed leading to capillary occlusion and intraretinal neovascularization. PD-OIR model displayed severe hemorrhage at inner retina and vitreous where mircoaneurysms and extraretinal new vessels are located, which are not found in conventional OIR model.
Conclusions: Compared to previous DR models, pericyte-deficient retinopathy and PD-OIR models are more valid animal models which follow sequential pathophysiologic cascades of human DR. One of advantages of these models is that the degree and duration of pericyte coverage can be controlled in a dose-dependent manner. Therefore, these models will be useful for studying how DR progresses, and for developing potential therapeutics targeting NPDR and diabetic macular edema.
Commercial Relationships: Junyeop Lee, None; Akiyoshi Uemura, None; Gou Young Koh, None; Young Hee Yoon, None

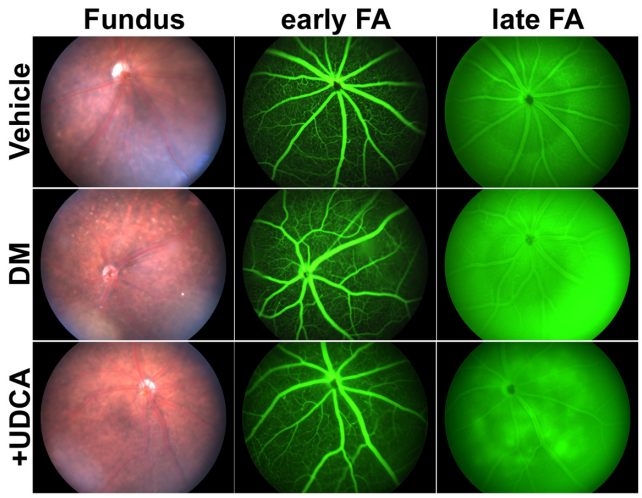
26-2

Ursodeoxycholic acid attenuates endoplasmic reticulum stress-related pericyte loss in diabetic retinopathy of streptozotocin-treated mice

Yoo-Ri Chung [1] Jeong A Choi [2] Min Ji Kang [3] Jae-Young Koh [2,4] Young Hee Yoon [1]
1. Ophthalmology, Asan Medical Center, Seoul, Korea (the Republic of). 2. Neural Injury Research Center, Asan Medical Center, Seoul, Korea (the Republic of). 3. Biomedical Science, University of Ulsan College of Medicine, Seoul, Korea (the Republic of). 4. Neurology, Asan Medical Center, Seoul, Korea (the Republic of).

Purpose: The early event of diabetic retinopathy (DR) is the loss of pericytes, which results in the breakdown of blood-retinal barrier (BRB). We investigated the role of endoplasmic reticulum (ER) stress and unfolded protein response (UPR) in this process, and verified the effect of ursodeoxycholic acid (UDCA), a chemical chaperone alleviating ER stress, in diabetic mice and retinal pericytes.
Methods: Streptozotocin (STZ)-induced diabetic mice and human retinal pericyte (HRP) cells treated with either advanced glycation end products (AGE) or modified low-density lipoprotein (mLDL), were used to verify the association of ER stress and UPR in diabetic retinopathy, with or without UDCA. Fluorescein angiography was used to evaluate the severity of BRB breakdown in mice, and the expression of UPR markers was verified by western blot analysis.
Results: Among the UPR markers, the expressions of GRP78 and those involved in PERK pathway were increased in STZ-induced diabetic mice. The treatment of UDCA attenuated this increased expression of UPR markers, and the diffuse leakage of fluorescein dye in angiography was improved in UDCA-treated diabetic mice compared to non-treated diabetic group. The expression of UPR markers involved in PERK pathway was also increased in AGE- or mLDL-exposed HRP, which was attenuated by UDCA.
Conclusions: We verified that ER stress is involved in DR of STZ-induced diabetic mice, and that UDCA attenuated the expression of UPR and showed protective effect against pericyte loss. This suggests that UDCA, as a chemical chaperone that alleviates ER stress, may have protective effect and needs further evaluation for its efficacy in diabetic retinopathy.

Commercial Relationships: Yoo-Ri Chung, None; Jeong A Choi, None; Min Ji Kang, None; Jae-Young Koh, None; Young Hee Yoon, Allergan (C), Bayer (C), Alcon (C)
Support: NRF-2013R1A2A2A01068457



Fundus photographs and fluorescein angiography (FA) in STZ-induced diabetic mice with or without UDCA treatment. Diffuse leakage of fluorescein dye was noted in non-treated diabetic group (DM) compared to control group (Vehicle), which was attenuated in UDCA-treated diabetic group (+UDCA).

27-3

Enriched CD146+ adipose stromal cells display increased migration and adhesion, Ameliorate retinal ischemia-reperfusion injury

Yogesh Jonna [3] Ahmed Ramadan [3, 1] Josy Augustine [3] Gangaraju Rajashekhar [2, 1]

1. Opthamology, Eugene and Marilyn Glick Eye Institute, Indianapolis, IN, United States. 2. Opthamology, Cellular and Integrative Physiology, Indiana University School of Medicine, Indianapolis, IN, United States. 3. Opthamology, Indiana University School of Medicine, Indianapolis, IN, United States.

Purpose: We have demonstrated previously that intravitreal injection of adipose stromal cells (ASC) in the eyes of diabetic rats improved the retinal function. Well defined ASC population with highest safety and efficacy are needed to plan for future human clinical trials. Recently, CD146+ population of vascular pericytes have been recognized as potential utility in particular therapeutic areas. In this study, we aim to identify if CD146+ population of pericyte ASC have better ability to migrate and adhere to the areas of injury in retinal blood vessels and improve the retinal function in I/R model of retinopathy.
Methods: Unilateral retinal I/R were done in adult Lewis rats by transiently elevating the intraocular pressure for 1h. After day 7 of reperfusion, the animals were randomized to receive intravitreal CD146+ ASC, CD146- ASC (10,000 cells/eye) or saline injections. After further 6-7 days, retinal function was assessed by Electroretinogram (ERG), retinal apoptosis and gliosis by confocal microscopy and retinal whole mounts after 2 months for ASC localization to retinal vasculature. *In vitro*, CD146 was knockdown by CD146 siRNA with Lipofectamine RNAiMAX transient transfection. A scratch wound-healing assay was used to test ASC migration and an adhesion assay that measures the adherence of ASC to Laminin quantified via crystal violet uptake.
Results: Retinal I/R resulted in a significant reduction in "b" wave amplitude, which was significantly improved by CD146+ ASC compared with CD146- ASC at day-6 post injection and remained high at one month. Confocal microscopy performed on retinal whole mounts from injured eyes that received CD146+ ASC demonstrated increased localization and homing to the retinal vasculature in comparison to CD146- ASC. Immunocytochemistry confirmed decreased apoptosis and gliosis with CD146+ASC ($P<0.05$). *In vitro*, CD146-ASC demonstrated reduced ability to close wound as compared to CD146+ ASC at 12-hour post injury ($P<0.05$). Knocking down CD146 also significantly reduced adhesion to Laminin ($P<0.05$).
Conclusions: Our results show that CD146+ ASC, via improved adhesion and migration, improves the function of retina and rescues I/R injury induced retinal degeneration. The data suggest that CD146 plays a vital role in ASC to be more effective at being incorporated into the retinal perivasculature that is damaged in ischemic retinopathy.
Commercial Relationships: Yogesh Jonna, None; Ahmed Ramadan, None; Josy Augustine, None; Gangaraju Rajashekhar, Cell Care Therapeutics Inc (C)
Support: SRPinAM, NIH-NHLBI T35 Training Grant, NEI R01 (EY023427-01), CMMF and Knights Templar of Wisconsin

28-4

Altered ER-stress and ubiquitin-proteasome system leads to photoreceptor death in obese rat model

G Bhanuprakash Reddy [1] S. Sreenivasa Reddy [1] Karanam Shruthi [1] Yadagiri Reddy Paduru [1] Radha Ayyagari [2]

1. Biochemistry, National Institute of Nutrition, Hyderabad, Telangana, India. 2. Ophthalmology, University of California, San Diego, CA, United States.

Purpose: Obesity is associated with various progressive age-related diseases, including retinal degenration. Previously we reported photoreceptor death in spontaneous obese WNIN/Ob rat model. However, underlying molecular mechanism for retinal degeneration in this obese rat model is not known. The objective of this study is to investigate the role of ER-stress and ubiquitin-proteasome system (UPS) in retina of WNIN/Ob rat model
Methods: Morphology and ultrastructure of retina of 12-month old obese rats and age matched lean littermate controls rats by histology and ultrastructure analysis. qRT PCR and immunoblotting of retina markers, components of UPS, markers of ER stress and apoptotis were performed in the retina. Proteasome activity was assayed by fluorometric method. Immunohistochemistry was performed for mediators of apoptosis, which was further confirmed by TUNEL assay
Results: Significant retinal degeneration was observed in 12-month obese rats, particularly photoreceptor

loss, Expression of retinal marker genes by immunohistochemistry and qRT-PCR confirmed these abnormalities. Results showed altered UPS, existence of ER stress, up-regulation of apoptotic markers and apoptosis in the cerebral cortex of obese rats. It appears that altered UPS and increased ER-stress might play a role in photoreceptor death in obese rat

Conclusions: Dysregulated UPS could be one of the underlying mechanisms for the retinal degenerations observed in the spontaneous obese rat model.

Commercial Relationships: G Bhanuprakash Reddy, None; S. Sreenivasa Reddy, None; Karanam Shruthi, None; Yadagiri Reddy Paduru, None; Radha Ayyagari, None

29-5

Transplantation of rod progenitors after ex vivo gene correction improves vision in a model of autologous cell replacement for retinitis pigmentosa

Alona O. Cramer Barnea [1] Mandeep S. Singh [1] M. Dominik Fischer [1] Samantha De Silva [1] Michelle E. McClements [1] Alun R. Barnard [1] Robert E. MacLaren [1]

1. Clinical Neurosciences, University of Oxford, Oxford, United Kingdom.

Study Group: Nuffield Laboratory of Ophthalmology

Purpose: Photoreceptor degeneration due to retinitis pigmentosa is a primary cause of inherited retinal blindness. Patient-specific induced pluripotent stem cells (iPSc) could provide a source of cells to replace lost tissue. However, the use of patient-derived cells would require that the damaging gene mutation be corrected prior to transplantation. Ex-vivo gene therapy of rod progenitors and subretinal transplantation of treated cells are here studied in Rhodopsin knockout ($Rho^{-/-}$) mice. Delivery of the rhodopsin gene via adeno-associated virus (AAV) is compared with gene delivery via non-viral minicircle (MC) DNA

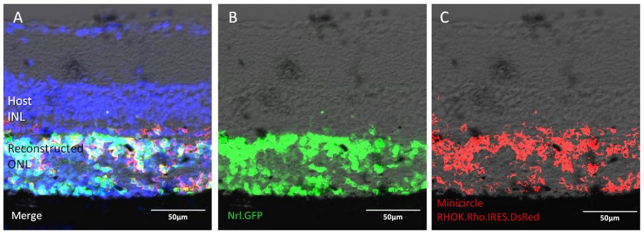
Methods: Rod progenitors were obtained from *Rho*$^{-/-}$, Tg(Nrl-EGFP) mice, where green fluorescent protein (GFP) is expressed specifically in rods. Cells were treated ex vivo using either serotype 2 AAV (Y444F) or MC carrying the *Rhodopsin* gene and a DsRed reporter. Magnetic assisted cell sorting against CD73 was performed to enrich the rod population in treated cultures and sorted cells were transplanted under the retinae of adult $Rho^{-/-}$ mice. Assays of visual function were performed prior to transplantation and at three weeks and two months post transplantation, including behavioural light avoidance, optomotor response, pupil constriction and electroretinography. In vivo scanning laser ophthalmoscopy and histology were used to assess transplanted cells

Results: Ex vivo expression of DsRed was achieved by both MC and AAV prior to transplantation. Post transplantation histology revealed co-labelled GFP and DsRed cells, expressing *Rhodopsin* as well as other rod markers, and interacting with the host retina. Significant improvements in measures of visual function were observed three weeks post transplantation of treated cells, compared to sham transplantation. Significantly greater improvement was achieved after two months in animals treated with MC compared to AAV therapy

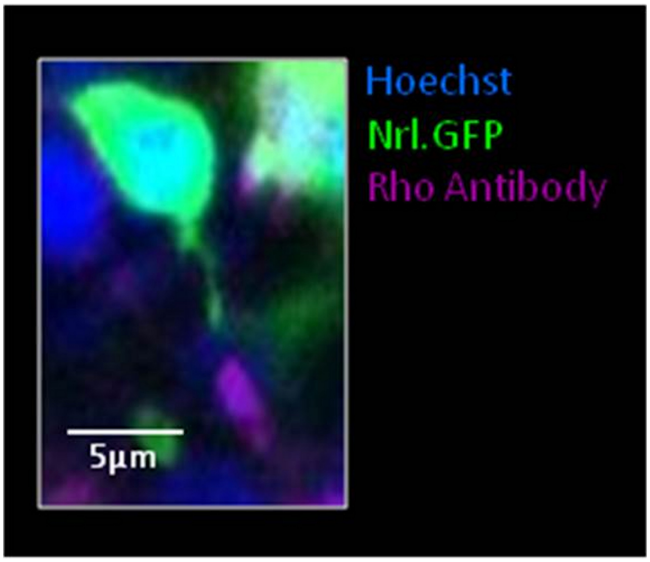
Conclusions: These results show successful transplantation of genetically corrected rod progenitors in a model of retinal disease and afford a foundation for the development of ex vivo gene therapy in human photoreceptor progenitors. Establishing an efficient non-viral method for gene delivery provides a novel system for cell therapy and may offer a platform of genetic treatment for a wide range of diseases in which the gene in focus exceeds the size limit for packaging in AAV

Commercial Relationships: Alona Cramer Barnea, None; Mandeep Singh, None; M. Dominik Fischer, None; Samantha De Silva, None; Michelle McClements, None; Alun Barnard, None; Robert MacLaren, None

Reconstituted outer nuclear layer (ONL) in an adult *Rho*$^{-/-}$ mouse after transplantation of rod progenitors (green) treated with minicircle ex vivo gene therapy (red)



Transplanted *Rho*$^{-/-}$, *Tg(Nrl-EGFP)* cell expressing rhodopsin after ex vivo gene therapy



30-6

Investigating the role of bone marrow derived CD34+ve cells in NMDA injured mouse retina upon transplantation

Akshay Anand [1] Neeru Jindal [1]

1. Neuroscience Research Lab, Department of Neurology, Postgraduate Institute of Medical Education and Research, Chandigarh, India.

Purpose: Retinal degenerative diseases result in the loss of ganglion cells are one of the major causes of blindness. NMDA activity resembles to glutamate neurotransmitter, high concentration of which results in excitotoxicity of

neurons through NMDA receptor. Stem cell therapy has emerged as a potential therapy to slow down the apoptotic death of RGCs and to replace the lost RGCs. Our approach is to investigate the incorporation and survival of bone marrow derived CD34+ve stem cells in NMDA injured retina at different time point.

Methods: All experiments were conducted with IAEC approval. Initially NMDA with dose of 50mM and 100mM was injected into vitreous cavity of C57/BL6J mice. Morphometric analysis showed significant decrease in ganglion cell number two days after NMDA injection. Immunohistochemistry analysis showed decreased expression of Brn3b and upregulation of CNTF, doublecortin and Nestin expression after 100mM NMDA injection. Real Time PCR analysis showed upregulation of GFAP and nestin expression. CD34+ve cells were sorted from mouse bone marrow cells by FACS sorter. At the time of acquisition, 10-11% cells were CD34+ve. These cells were sorted and after sorting, around 97% cells were CD34+ve. For tracking, the donor cells were labelled with CFDA dye. 100,000 (2μl) cells were transplanted intraviterously into one eye of (100mM dose) NMDA mouse model. To the contralateral eye, equal volume of PBS was injected which served as injury control. Stem cell migration and incorporation was studied at three different time points i.e. 7, 14 and 21 day after transplantation.

Results: CD34+ve cells were found in the vitreous cavity at 7day of transplantation. At 14 day, CD34+ve cells were scattered within retinal layers. Brn3b expression was found to be increased after CD34+ve stem cell transplantation at 21 day. At 21 day injury, CNTF, GDNF and expression got upregulated and this expression was found to be downregulated 21 day after transplantation of CD34+ve stem cell.

Conclusions: CD34+ve cells have potential to translate into clinical benefits offering future insights for exploring as well as examining the preclinical effects.

Commercial Relationships: Akshay Anand, None; Neeru Jindal, None

Retinal Cell Biology 3

Moderators

Chi-Chao Chan
National Eye Institute, National Institutes of Health, Bethesda, MD, United States
Atsushi Hayashi
University of Toyama, Toyama, Tomaya, Japan

52-1

Enhanced proliferation of retinal pigment epithelial cells by low-level laser irradiation

Yalong Dang [1,2] Chun Zhang [2]
1. Department of Ophthalmology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China. 2. Department of Ophthalmology, Peking University Third Hospital, Beijing, China.

Purpose: AMD is one of the major causes of irreversible blindness in the world. RPE degeneration is the initial and characteristic feature of AMD. Aged RPE cells have limited abilities of proliferation and phagocytosis. Therefore, methods for rejuvenating the aged RPE cells are promising therapies for such patients.The aim of thi study is to analyze whether low-level laser irradiation exerts regulatory effects on proliferation and differentiation in human ARPE19 cells.
Methods: Human ARPE-19 cells were seeded in a 100mm TC-treated dish after receiving low level laser irradiation (8J) daily. Controls were sham irradiated. After 2 days of treatments, cells were harvested, collected for further analysis. Fluorescence-activated cell sorter (FACS) was used to reveal the changes in the cell cycle after LLLI. RT-PCR and Western blotting were utilized to quantify the different markers related to RPE proliferation and maturation. In addition, the potential mechanisms were investigated.
Results: After 8J of LLLI, a significant decrease of RPE 65, CRALBP, VEGF-A, and cytokeratin 18 in ARPE-19 cells was noticed. Additionally, an increase of cyclin D1, CDK4, CDK6 expression was equally observed after 8J of LLLI treatment. Cell-cycle studies revealed an increase of ARPE cells in S-G2/M phase after LLLI treatment. CKIs, such as p21, p15, p16, p57 was also down-regulated.
Conclusions: Our results demonstrated the low level laser irradiation could boost the proliferation of RPE cells. The results indicate young or rejuvenated cells could be obtained from LLLI so that AMD patients might recover vision again.
Commercial Relationships: Yalong Dang, None; Chun Zhang, None
Support: National Science Foundation, Research Grants, ID, 81371017. International Cooperation Foundation of Henan Province, Regular Grants, 2014, 2013

53-2

PEDF Improves Mitochondrial Function in RPE cells During Oxidative Stress

Yuan He [1]
1. Ophthalmology, The Second Affiliated Hospital of Xi'an College of Medicine, Xi'an, China, Xi'an, Shaanxi, China.

Purpose: Oxidative stress plays an important role in health and aging. We have shown that oxidative stress impairs mitochondrial function and promotes RPE cell death in an age-dependent manner. This study investigates the role of PEDF in limiting oxidative stress-induced damage to RPE cells through mitochondrial pathways.
Methods: Three groups of early-passaged RPE cells from donors aged 50-55, 60-65, and 70-75 year yo were either preconditioned with PEDF followed by exposure to sublethal doses of H_2O_2 or post-treated with PEDF after H_2O_2 treatment. Effects of PEDF on mitochondrial function and cell viability were examined.
Results: Oxidative stress induced an age-dependent increase in LDH release, ROS levels, and cell death and a decrease in ATP production and $\Delta \Psi m$ in human RPE cells. Preconditioning or post-stressed treatment with PEDF resulted in increased cell viability, inhibition of cytochrome c release and caspase 3 cleavage, and improved mitochondria function denoted by a decrease in **ROS generation and increases in** ATP production and $\Delta \Psi m$. Oxidative stress also disrupted the reticular network, trafficking, and distribution of the mitochondria and blocked activation of PI3K, Akt, and Erk signaling in the cells. PEDF mitigated negative effects of oxidative stress on mitochondrial remodeling and cellular distribution and unblocked its control of PI3K/Akt and MAPK signaling. Although PEDF potentiated both PI3K/Akt and MAPK signaling in the cells, stabilization of mitochondrial networks and function was dependent on its activation of PI3K/Akt. Specificity of PEDF' s activity was confirmed using the pharmacological inhibitors LY294002, SH6, and U0126. We also show that in the absence of oxidative stress, pharmacological inhibition of the PI3K/Akt pathway alone was sufficient to disrupt mitochondrial structure and function. In addition, PEDF blocked effects of oxidative stress on expression of cyclophilin D and UCP2, genes controlling mitochondrial function, and the apoptotic genes. Control of ROS levels by PEDF was specifically linked to UCP2 regulation since PEDF induced expression of this gene in UCP2 deficient cells was associated with a decrease in ROS production.
Conclusions: We provide evidence that PEDF promotes resilience of aging RPE cells to oxidative stress by stabilizing mitochondrial networks and function and that mitochondrial dynamics in human RPE cells are controlled, in part, through the PI3K/Akt pathway.
Commercial Relationships: Yuan He, None
Support: grants from the Ben Franklin Foundation, PA, USA, The Juvenile Diabetes Research Foundation, USA, the National Natural Science Foundation of China (No.81100665), the Departments of Health (No.2010D56), Education (No. 2013JK0798) and Science and Technology (No.2011K14-02-07 and 2013KJXX-31) of Shaanxi Province,

China, grants from Xi' an Medical University (10FC01) and The Second Affiliated Hospital of Xi' an Medical University (10EFYFC01).

54-3

Cell Death in Age-Related Macular Degeneration, an Immunopathological and Ultrastructural Model

Chi-Chao Chan [1] Nicholas A. Popp [1] Christopher Ardeljan [2] Shida Chen [1,3] Mones Abu-Asab [2]

1. Laboratory of Immunology, Immunopathology Section, National Eye Institute, National Institutes of Health, Bethesda, MD, United States. 2. Histology Core, National Eye Institute, National Institutes of Health, Bethesda, MD, United States. 3. Zhongshan Ophthalmic Center, Guangzhou, Guangdong, China.

Purpose: The etiology of age-related macular degeneration (AMD) remains elusive despite the characterization of many factors contributing to the disease in its late-stage phenotypes. AMD features an inflammatory involvement and allostatic overload, which result in inflammatory-mediated cell death. This study evaluates the prevalent types of cell death in AMD.

Methods: AMD lesions in paraffin-fixed, archived slides of human eyes were microdissected and examined for the expression of various inflammatory cytokines and cell death markers. Their ultrastructure was examined by transmission electron microscopy.

Human ARPE-19 cells and mouse retinal stem cells were subjected to inflammatory stimuli and/or under oxidative stress to examine their effects on relevant inflammatory cytokines, cell death markers, and ultrastructure.

Results: The inflammatory cytokines IL-1 β , IL-18, and IL-6 were elevated in the AMD lesions and stimulated *in vitro* cell cultures. Both molecular studies as well as ultrastructural pathology suggest that pyroptosis and perhaps necroptosis, which are characterized by mitochondrial degeneration, autophagosome formation, cytoplasmic organelle degradation, and nuclear fragmentation, are the predominant mechanisms of cell death at play, with only minimal evidence for apoptosis.

Conclusions: Our study suggests that inflammation, pyroptosis and necroptosis play a significant role in the pathogenesis of neuronal cell death in AMD.

Commercial Relationships: Chi-Chao Chan, None; Nicholas Popp, None; Christopher Ardeljan, None; Shida Chen, None; Mones Abu-Asab, None

Support: NEI intramural research program

55-4

MicroRNA-150 regulates pathologic ocular neovascularization by modulating endothelial cell function

Chi-Hsiu Liu [1] Ye Sun [1] Jie Li [1] Lucy P. Evans [1] Katherine T. Tian [1] Jing Chen [1]

1. Department of Ophthalmology, Boston Children's Hospital/Harvard Medical School, Boston, MA, United States.

Purpose: Pathologic ocular neovascularization (NV) commonly causes blindness in several vascular eye diseases. It is critical to define the factors dysregulated in pathologic NV in order to develop effective targeted therapeutics. MicroRNAs are small non-coding RNAs regulating gene expression at the post-transcriptional level, and may mediate developmental and pathologic angiogenesis. Here we investigated the potential role of a specific microRNA, *miR-150*, in regulating ocular NV.

Methods: Two mouse ocular angiogenesis models were used, an oxygen-induced retinopathy (OIR) and a laser-induced choroidal neovascularization (CNV) models. In OIR, neonatal mice were exposed to 75% oxygen from postnatal day 7 to 12. MicroRNA array was performed with OIR retinas compared with room air controls, followed by confirmation with RT-PCR and localization with laser capture microdissection (LCM) isolated retinal neural and vascular layers. Pathologic retinal NV in OIR was quantified in *miR-150* knockout (*miR-150*$^{-/-}$) and wild type (WT) mice, and WT mice injected intravitreally with *miR-150* mimic and negative control. For CNV, adult *miR-150*$^{-/-}$ and WT mice were treated with laser and CNV lesions were analyzed at 1 week post laser. Effects of *miR-150* on endothelial cell function were analyzed in human retinal vascular endothelial cell (HRMEC) culture. *MiR-150* putative targets were validated in both retinas and in HRMECs.

Results: Expression of *miR-150* was significantly suppressed in OIR retinas. *MiR-150* was found highly expressed in LCM isolated normal retinal blood vessels, and significantly suppressed in pathologic NV isolated from OIR retinas. *MiR-150*$^{-/-}$ retinas showed increased retinal NV in OIR. Intravitreal injection of *miR-150* mimic significantly decreased NV in OIR retinas. In the laser-induced CNV model, *miR150*$^{-/-}$ mice showed significantly larger lesions compared with WT. HRMECs treated with *miR-150* mimic revealed substantially decreased levels of proliferation, migration, and tubular formation. Loss of *miR-150* leads to strong upregulation of predicted target angiogenic genes (*Cxcr4, Dll4, Fzd4, Plxnd1, and Kdr*) in retinas *in vivo* and in HRMECs *in vitro*.

Conclusions: Our findings indicate that vascular enriched *miR-150* is an endogenous inhibitor of pathologic ocular NV. *MiR-150* may be a valuable target to develop potential treatments for neovascular eye diseases.

Commercial Relationships: Chi-Hsiu Liu, None; Ye Sun, None; Jie Li, None; Lucy Evans, None; Katherine Tian, None; Jing Chen, None

56-5

Conditional knockout of Wnt co-receptors, Lrp5 and Lrp6, manifests persistent hyaloid vasculatures

Yoshinobu Odaka [1] Gowri Nayak-Sarangdhar [1] Maya Dassanayake [2] Shruti Vemaraju [1] Richard A. Lang [1]

1. Ophthalmology, Vision Systems Group, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States. 2. Wayne State University School of Medicine, Detroit, MI, United States.

Purpose: Developing retina and lens acquire oxygen and nutrients from the hyaloid vascular system (HVS) in the

vitreous humor. In human embryos the VHS completely regresses during the third trimester; however, 95% of pre-termed babies present an impaired VHS involution, which could lead to a permanent vision loss. Various loss-of-function mutations in Wnt signaling participants in humans and mice associate with abnormal hyaloid vascular development and remodeling. Previously, our laboratory identified macrophages in the vitreous provide Wnt7b ligand and trigger endothelial cell apoptosis (Labov et al, Nature 2005; Rao et al, Development 2007). Yet, roles of Wnt receptors such as Lrp5 and Lrp6 in endothelial cells have not been explored in VHS regression.

Methods: Lrp5 and Lrp6 conditional knockout mice were generated by crossing $Lrp5^{flox/flox}$; $Lrp6^{flox/flox}$ and $Lrp5^{flox/flox}$; $Lrp6^{flox/+}$; $Pdgfb\text{-}iCreERT^{+/-}$ (endothelial cell specific tamoxifen inducible Cre recombinase). Forty five μg of tamoxifen was introduced by intragastric injection at postnatal day 3. At end point (P8), eye balls were enucleated from pups after transcardiac perfusion with PBS followed by 4% paraformaldehyde fixation. The vasa hyaloidea propria (VHP), a posterior part of VHS, was carefully excised from the intraocular space without perturbing blood vessels, mounted on a slide, and immunostained for confocal microscopic analyses.

Results: Endothelial cell specific Lrp5 and Lrp6 double homozygous knockout mice showed significantly higher number of VHP vessels at postnatal day 8 as compared to littermate control group. Furthermore, in knockout animals, caspase 3 activity was downregulated.

Conclusions: Blocking Wnt signaling in endothelial cells at postnatal day 3 and onward sufficiently presents a phenotype of suppressed cell death in VHS, suggesting Wnt signaling cascades promote apoptosis in the hyaloid. Our results may imply Wnt agonists have therapeutic potential for treating pediatric patients with congenital abnormalities in HVS.

Commercial Relationships: Yoshinobu Odaka, None; Gowri Nayak-Sarangdhar, None; Maya Dassanayake, None; Shruti Vemaraju, None; Richard Lang, None

Support: Cincinnati Children's Research Foundation, NEI (EY021636)

57-6

HIF-1alpha and HIF-2alpha in the neuron are essential for the retinal vascular development

Toshihide Kurihara [1, 2] Yoshihiko Usui [2] Edith Aguilar [2] Peter D. Westenskow [2] Martin Friedlander [2]

1. Ophthalmology, Keio University School of Medicine, Tokyo, Japan. 2. The Scripps Research Institute, La Jolla, CA, United States.

Purpose: Hypoxia-inducible factor (HIF) plays important roles in cellular hypoxia and stress response. Previously, we have reported contributions of HIF and its negative regulator VHL in neurons, astrocyte, myeloid cells, and pigment epithelium cells in the developing and mature retina (Development 2010, J Cell Biol. 2011, J Clin Invest. 2012). Recently, we found that conditional VHL deletion in the retinal neuron induced an arrest of retinal vascular development and persistent fetal vasculature (PFV) (ARVO 2014). In this study, we explored functions of individual HIF isoforms (HIF-1alpha and HIF-2alpha) in the VHL-deleted retina.

Methods: Retinal neuronal specific conditional VHL knockout mice were obtained by crossing CRX-Cre mice with $VHL^{floxed/floxed}$ mice (VHL;CRX-Cre mice). These mice were further crossed with HIF-$1^{floxed/floxed}$ mice or HIF-$2^{floxed/floxed}$ mice (VHL/HIF-1;CRX-Cre VHL/HIF-2;CRX-Cre, or VHL/HIF-1/HIF-2;CRX-Cre mice). Fundus photography, immunohistochemistry, histology, and electrophysiology were examined to explore the phenotype.

Results: As we previously reported, VHL:CRX-Cre mice showed an arrest of retinal vascular development and proliferative hyaloidal vascular endothelial cells on the surface of the retina and photoreceptor degeneration resembling human PFV. VHL/HIF-1;CRX-Cre showed similar phenotypes to VHL:CRX-Cre mice. On the other hands, VHL/HIF-2;CRX-Cre had vascular development arrest, but not the PFV phenotype. VHL/HIF-1/HIF-2;CRX-Cre mice showed complete lack of retinal vasculature more severe than VHL/HIF-2;CRX-Cre mice.

Conclusions: These data suggested that individual HIF-alpha isoforms had differential roles in retinal vascular development. HIF activation may be targeted to prevent or treat retinal angiogenic diseases or developmental anomaly such as PFV.

Commercial Relationships: Toshihide Kurihara, None; Yoshihiko Usui, None; Edith Aguilar, None; Peter Westenskow, None; Martin Friedlander, None

Support: Takeda Science Foundation and the Uehara Memorial Foundation

Lens - Paper

Moderators

Wei Han
The First Affiliated Hospital, Zhejiang University, Hangzhou, Zhejiang, China
Daijiro Kurosaka
Iwate Medical University School of Medicine, Morioka, Iwate, Japan

58-1

A new histological evaluation to detect residual OVDs in cataract surgery

Hidetsugu Mori [1] Haruhiko Yamada [1] Kanji Takahashi [1]
1. Opthalmology, Kansai Medical University, Hirakata Hospital, Hirakata, Japan.

Purpose: To establish a new hitstological evaluation to detect residual ophthalmic viscosurgical devices(OVDs) and its adhesion to the corneal endothelium in phacoemulsification and aspiration(PEA) surgery.
Methods: In this study, we conducted research by using adult postmortem porcine eyes. Viscoat®, HealonV®, Healon® and DiscoVisc® were used as OVDs. Each OVD was mixed with fluorescent conjugated dextrans to make it visible. All surgery were performed by one surgeon(H. M). Surgical procedures were as follows, After making a corneal side port, a continuous curvilinear capsulorhexis was conducted under the water irrigation. Then, corneal tunnel incision was made and the anterior capsule was removed. Each single OVD was injectioned by 0.4cc through a corneal side port. The lens was removed using single-hand phacomulsification technique. After the lens was completely removed, ultrasound oscillation was done for ten seconds in anterior chamber to simulate human eye with hard lens nucleus. After operation, the anterior segment was isolated by equatorial incision and the tissue was frozen by shimmering liquid nitrogen. Twenty micrometers thin-sliced sections were made by Cryostat from limbus to limbus by sagital manner. Each one out of ten slides from all sectioned slides(700 sections in each eye) were picked up and dried naturally at room temperature and were photographed immediately with a fluorescent microscopy with cool CCD camera(Olympus, Tokyo, Japan) . Using WinRoof® software(Mitani shoji, Tokyo, Japan) , we measured the percentage of OVDs which cover corneal endothelium and the volume of OVD clots remaining in the anterior chamber.
Results: In regard to endothelial coating ability, Viscoat® showed statistically higher coverage ratio compared to Healon®and DicoVisc® (mean value, Viscoat®99.0%, HealonV®82.6%, Healon®60.1%, DicoVisc®65.6%, $p<0.05$, Fisher PLSD). The volume of residual OVD clots in the HealonV® was statistically larger than that of Healon® and DicoVisc®(mean value, Viscoat®0.56ml, HealonV®0.86ml, Healon®0.18ml, DicoVisc ®0.21ml ,$p<0.05$, Fisher PLSD).
Conclusions: In this study, we could clearly observe the distribution of OVDs and how they coat corneal endothelium. Moreover, we could perform quantitative evaluation of residual OVDs. This results showed that Viscoat® and HealonV® had high potential to the corneal endothelium protection during the PEA surgery.

Commercial Relationships: Hidetsugu Mori, None; Haruhiko Yamada, None; Kanji Takahashi, None

59-2

Intraoperative floppy-iris syndrome associated with antipsychotic

Masato Matsuo [1] Ichiya Sano [1] Yoshifumi Ikeda [1] Etsuko Fujihara [1] Masaki Tanito [1]
1. Matsue Red Cross Hospital, Matsue, Japan.

Study Group: Division of Ophthalmology, Matsue Red Cross Hospital, Matsue, Shimane, Japan

Purpose: Association between intraoperative floppy-iris syndrome (IFIS) during cataract surgery and use of urologic selective a1-adrenergic receptor antagonists (a1-blocker) has been well established. We report 3 cases of IFIS without a history of selective a1-blocker use while with a long-term history of antipsychotics use.
Methods: Report of 3 Japanese cases who showed typical features of IFIS during the phacoemulsification procedure in both eyes.
Results: Case 1. A 39-year-old man with chronic angle closure glaucoma showed IFIS in both eyes. For treatment of schizophrenia, he had been used several classes of antipsychotics including 1stgeneration antipsychotics (1stG) haloperidol for 5 months and chlorpromazine as needed; dopamine system stabilizer (DSS) aripiprazole for 2 months; dopamine serotonin antagonist (DSA) olanzapine for 7 years and quetiapine for 1 month; and serotonin dopamine antagonist (SDA) risperidon for 3 years and blonanserin for 3 weeks. Case 2. A 63-year-old woman with schizophrenia had a history of antipsychotics use including DSS aripiprazole for 8 months, DSA quetiapine for 6 months, and SDA risperidon for more than 10 years. Case 3. A 65-year-old woman with organic mental disorder had history of antipsychotics use including 1stG haloperidol for more than 10 years.
Conclusions: The IFIS induced by antipsychotics can be explained by their a1-blocker effect during long-term use, since every classes of antipsychotics identified in our cases had a1-blocker effect to some degree. Although possible association between some antipsychotics and IFIS had been reported in clinical cases, several drugs we identified from our cases were novel in the literature. Surgeons need to keep in mind the possibility of IFIS in patients with current and past use of this commonly prescribed group of drugs.
Commercial Relationships: Masato Matsuo, None; Ichiya Sano, None; Yoshifumi Ikeda, None; Etsuko Fujihara, None; Masaki Tanito, None

60-3

Spectral-Domain Optical Coherence Tomography Analysis of Corneal Epithelial Thickness Changes after Cataract Surgery

Tianyu Zheng [1] Yi Lu [1] Wenwen He [1]

1. EYE and ENT Hosp of Fudan Univ, Shanghai, China.

Study Group: cataract study group of Shanghai EENT hospital

Purpose: To observe the corneal epithelial thickness and cellular changes after cataract surgery.

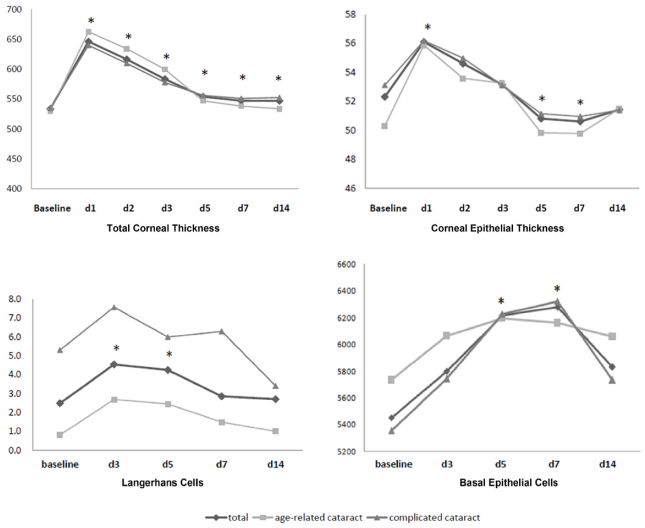
Methods: Prospective, observational study. 72 eyes of 72 patients undergoing cataract surgery were reviewed on baseline and d1, d2, d3, d5, d7, d14 after surgery. Ophthalmic examination, spectral-domain optical coherence tomography, pentacam and corneal laser-scanning confocal microscopy were performed. Corneal epithelial thickness (ET), total corneal thickness (CT), corneal basal epithelial cell density and Langerhans cell (LC) density, best-corrected visual acuity (BCVA), simulated keratoscope (SimK) and DeltaK were recorded.

Results: At baseline, central cornea had thinner CT (533.4 ± 31.4 μ m) but thicker ET (52.3 ± 4.1 μ m) than the peripheral. On d1 after surgery presented a sharp increase both in central CT (646.1 ± 184.0 μ m) and ET (56.1 ± 8.6 μ m). Then CT gradually decreased but still greater than baseline level on d14, while ET decreased immediately to baseline on d2 and d3, and was even thinner on d5 and d7 (thinnest at 50.6 ± 5.7 μ m, $p<0.05$ compared to baseline), then increased and returned to baseline on d14. Significant LC infiltration presented in corneal epithelium on d3 and d5, before the period of ET thinning. As the LC infiltration declined, corneal basal epithelial cell density increased significantly at d5 and d7 to restore the baseline ET. BCVA, SimK, and DeltaK were found more related to ET than CT early after cataract surgery by Pearson correlation coefficient analysis.

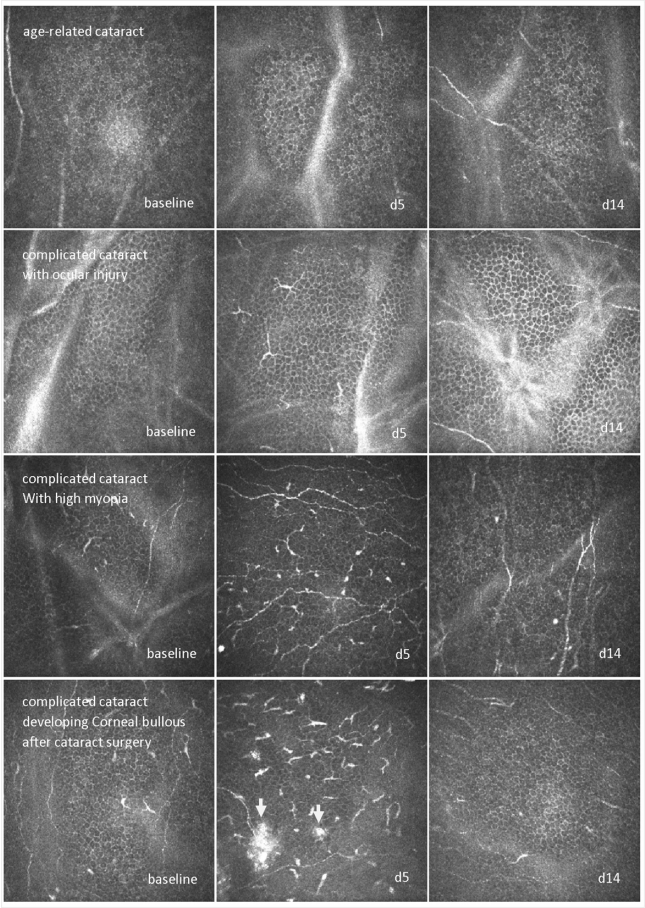
Conclusions: After immediate decrease of edema, corneal epithelium experienced a period of thinning on d5 and d7 after cataract surgery following significant LC infiltration, then epithelial proliferation was enhanced and ET was successfully restored. This process could be one of the reasons influencing BCVA and corneal refraction early after cataract surgery.

Commercial Relationships: Tianyu Zheng, None; Yi Lu, None; Wenwen He, None

Support: Supported by the National Natural Science Foundation of China (NSFC) No. 81270989 and No. 81300747, Specialized Research Fund for the Doctoral Program of Higher Education (SRFDP) No. 20130071120096, Training Program for 100 excellent academic leaders by Shanghai health bureau No. XBR2011056.



Central corneal thickness (CT), epithelial thickness (ET), Langerhans Cell (LC) density (cells/field in maximum) and basal epithelial cell density (cells/mm^2) after cataract surgery (*significantly different compared with baseline among the total subjects, by paired-sample t test).



Changes of corneal epithelium after cataract surgery on in vivo laser-scanning confocal microscopy. Langerhans cell infiltration declined on the last visit.

61-4

A lens model for the in vivo expression and self assembly of human proteins

John I. Clark [1]

1. Biological Structure, Univ of Washington, Seattle, WA, United States.

Purpose: The transparent lens is excellent for studies of the progressive expressionof proteins temporally and spatially and their self-assemble in vivo. While there is a long history of studying protein aggregation in ageing cataracts, little is known about aggregation of amyloid proteins in vivo. The purpose of the current study was to characterize variations in the the expression yellow fluorescent proteins (YFP) - amyloid fusion proteins in the transparent lens of the eye. The hypothesis was that spatial and temporal differences result from the dynamics of the cellular distribution and progressive aggregation with age.

Methods: Human cDNAs for alphaB crystallin (α B), betaB crystallin (β B) and amyloid beta (A β) (generously provided by J.Sullivan) fusion proteins were cloned into Invitrogen Gateway Tol2 destination vectors downstream of the α A crystallin promoter (generously provided by B.Link), in frame with the yellow fluorescent protein (YFP). The α A crystallin promoter drives vector constructs of human YFP fusion proteins specifically in zebrafish lens cells (Kurita et al. (2003) Dev Biol 255:113). Control and experimental vectors were injected into fertilized wild-type ABWIK zebrafish embryos at the one- or two-cell stage. At four days to six days post-fertilization (dpf), the lenses of developing zebrafish were imaged in 3D using fluorescence or multiphoton microscopy.

Results: Alternating patterns of expression were observed in layers of lens fiber cells in vivo. Similarities in expression of YFP, αB-YFP and βB-YFP suggest the regulation of normal proteins produced similar patterns of fluorescnece which was observed using multphoton microscopy. Expression of A β -YFP was observed cortical cells that contained bright condensed fluorescent aggregates. A β-YFP was not observed in every cell and localizations were asymmetric. Peripheral cells appeared to express A β -YFP at higher levels than central cells where protective mechanisms may reduce expression or aggregation. Patterns of fluorescence were altered with age.

Conclusions: Zebrafish were used to identify timing, spatial distributions, and aggregation of human amyloid proteins in vivo. Cells were identified that were protective against expression and aggregation of amyloid proteins.

Commercial Relationships: John Clark, None

Support: Grant EY 04542 from the N.E.I.

62-5

Normal Expression of AQP0 Protein is Required for Maintaining the Integrity of Interlocking Domains and Transparency of the Lens

Woo-Kuen Lo [1] Sondip K. Biswas [1] Lawrence Brako [1] Mary Gadalla [1]

1. Neurobiology, Morehouse School of Medicine, Atlanta, GA, United States.

Purpose: We have recently discovered that aquaporin-0 (AQP0) targets interlocking protrusions to control the integrity and transparency of the lens. We have found that the complete loss of AQP0 specifically causes severe disruption of interlocking protrusions which leads to fiber cell separation and cataract formation in the AQP0-/- homozygous lenses at age 1 month and older. Here, we use the AQP0+/- heterozygous lenses to compare the changes of membrane structures and the N-cadherin-catenin complexes during a slower cataract formation process.

Methods: In the AQP0+/- lenses of C57B6-J mice (1 to 12 weeks old), the presence of AQP0 in protrusions was detected by immunolabeling using whole-mount samples. Changes in membrane structures and the N-cadherin-catenin complexes were examined with confocal microscopy, SEM and TEM. The age-matched wild-type and AQP0-/- lenses were used for control and comparison.

Results: The nuclear cataracts were first found in the AQP0+/- lenses at age 2 months in which interlocking protrusions in the nuclear fibers were significantly disrupted. In contrast, protrusions in the cortical fibers (~240 μ m deep) exhibited only a minor elongation and deformation which did not cause visible fiber-cell separation and cortical opacification. Furthermore, the enlarged extracellular spaces between cortical fiber cells were rarely observed in the cataractous AQP0+/- lenses (2-3 months old), but they were found extensively in the same cortical regions of the cataractous AQP0-/- lenses (1 month old). TEM revealed that many elongated and deformed protrusions were dispersed within the enlarged extracellular spaces in the AQP0-/- lenses. Confocal microscopy analysis also showed that a significant decrease of the N-cadherin-catenin complexes in the cortical fibers were associated with the AQP0-/- lens, but not with the AQP0+/- lens.

Conclusions: Although the reduced amount of AQP0 is able to delay disruption of protrusions and formation of enlarged extracellular spaces with opacities in the cortical fibers in the AQP0+/- mice, the normal expression of AQP0 is required for maintaining the integrity of protrusions and transparency of the entire wild-type lens. The N-cadherin-catenin complexes may play a cooperative adhesion role in maintaining the integrity of cortical fibers during the delayed cataractogenesis in the AQP0+/- lenses.

Commercial Relationships: Woo-Kuen Lo, None; Sondip Biswas, None; Lawrence Brako, None; Mary Gadalla, None

Support: NIH Grant EY05314

63-6

Non-essential role for cilia in coordinating precise alignment of lens fibre

Yuki Sugiyama [1] Elizabeth Shelley [1] Li Wen [1] Frank J. Lovicu [1] John McAvoy [1]

1. Save Sight Institute, University of Sydney, Sydney, NSW, Australia.

Purpose: The primary cilium, a microtubule-based organelle found in most cells, is a centre for mechano-sensing fluid movement and cellular signalling, notably the Hedgehog pathway. Recently we found that each lens fibre cell has an apically situated primary cilium that is polarized to the side of the cell facing the anterior pole. The direction of polarity is similar in neighbouring cells so that in the global view, lens fibres exhibit planar cell polarity (PCP) along the equatorial-anterior polar axis. Ciliogenesis has been associated with the establishment of PCP, although the exact relationship between PCP and the role of cilia is still controversial. Here we assessed the role of the primary cilia in coordinating the precise alignment/orientation of the fibre cells.

Methods: A conditional allele of IFT88, a key component of the intraflagellar transport (IFT) complex, was removed from the lens using MLR10- and LR-Cre lines to disrupt primary cilia formation. We also examined the lens phenotype in knockouts of Bardet–Biedl Syndrome (BBS) proteins 4 and 8, the components of the BBSome complex which mediates cilium assembly.

Results: We found IFT88 is expressed in lens epithelial and fibre cells colocalising with the cilium marker, acetylated-tubulin. When IFT88 was conditionally removed from the lens after E12.5 with the MLR10 Cre line the IFT88 protein was absent from most lens cells. In IFT88-depleted cells the rod-like structure labelled by acetylated-tubulin was shortened, proving the disruption of primary cilia formation. However no obvious histological defects were detected in IFT88;MLR10 conditional knockout (CKO) lenses. Consistent with this, in primary lens epithelial explants prepared from IFT88;MLR10 CKO lenses, the centrosomes/basal bodies still showed polarised localisation at the apical surface of elongating cells upon FGF-induced fibre differentiation. Earlier depletion of IFT88 from the lens placode at E9.5 with the LR-Cre line also did not cause abnormal lens formation. In BBS4 and 8 knockout lenses the pattern of the anterior sutures formed by the apical tips of elongating/migrating fibres were comparable to the control lenses.

Conclusions: These results indicate that primary cilia do not play an essential role in the precise cellular alignment of fibre cells. Thus, it appears that in the lens cilia are not required to establish PCP.

Commercial Relationships: Yuki Sugiyama, None; Elizabeth Shelley, None; Li Wen, None; Frank Lovicu, None; John McAvoy, None

Support: NHMRC (Australia), NIH (USA EY03177)

Retina 1

Moderators

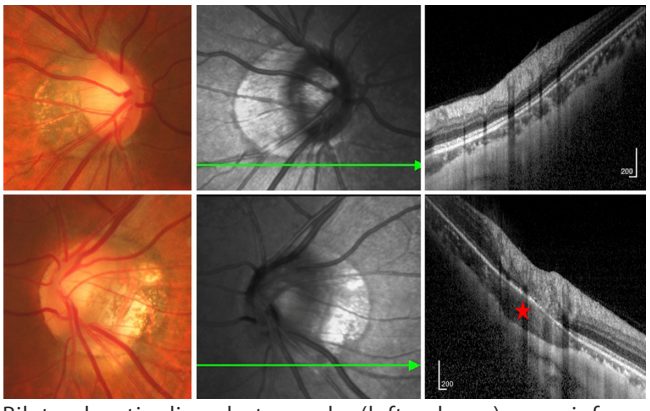
Sunil Srivastava
Cleveland Clinic, Cleveland, OH, United States
Yoshinori Mitamura
Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Tokushima, Japan

98-1

Unilateral Peripapillary Intrachoroidal Cavitation and Optic Disc Rotation

Yi Dai [1] Jost B. Jonas [2] Xinghuai Sun [1]
1. Ophthalmology, Eye & ENT Hospital, Fudan University, Shanghai, China. 2. Ophthalmology, Medical Faculty Mannheim of the Ruprecht-Karls-University, Heidelberg, Germany.

Purpose: To examine the morphology of the optic nerve head in patients with unilateral peripapillary intrachoroidal cavitations (PICCs).
Methods: The hospital-based observational study included patients with unilateral PICCs. Tomographic images of the parapapillary fundus were taken by enhanced depth imaging mode of optical coherent tomography. The ocular biometric parameters were compared between the affected eyes and the contralateral unaffected eyes.
Results: The study population consisted of 30 patients with a mean age of 42.7 ± 13.8 years (range:22-72 years), mean axial length of 26.7 ± 2.4 mm (range, 22.00-32.30 mm) and mean refractive error of -8.71 ± 5.21 diopters (range, -20.50 diopters to +0.50 diopters). In the eyes affected by PICC as compared with the contralateral eyes, the vertical optic disc diameter (P=0.001) and the minimal disc diameter (P=0.03) were significantly shorter, the ratio of minimal to maximal disc diameter was significantly lower (P=0.02), and the angle of disc rotation was significantly higher (P<0.001).
Conclusions: In patients with unilateral PICCS, the eyes with PICCs as compared with the contralateral eyes have optic discs which are more spindle-like configured due to a disc rotation around the vertical axis and around the sagittal axis.
Commercial Relationships: Yi Dai, None; Jost Jonas, None; Xinghuai Sun, None
Support: National Science Foundation of China (81170838)



Bilateral optic disc photographs (left column), near-infrared reflectance optical cohenrence tomographic (OCT) images (medium column), and cross sectional enhanced depth imaging -OCT images (right column) from a 43 year old patient. The axial length of the right and left eyes is 27.8mm and 27.4mm. The left eye showed a PICC (red star).

99-2

Retinal Vessels Analysis, In Vivo Visualization of Systemic Microvasculature in Pre eclampsia and Hypertension in Pregnancy

Mohammadreza Peyman [1] Azam Bakhtiari [2] Mimiwati Zahari [1] Siti Zawiah Omar [2]
1. Ophthalmology, University of Malaya, Kuala Lumpur, Malaysia. 2. University Malaya, Kuala Lumpur, Malaysia.

Purpose: The main objective of this study is to correlate the retinal vessels caliber with severity of hypertension in pregnancy
Methods: A total of 37 patients including normal pregnancy (14 cases), 11 patients with diagnosis of pre-eclampsia and 12 patients with PIH were recruited in this study. Blood samples were drawn to measure complete blood count, uric acid. The proteinuria status of participants was assessed with 24 hour urine analysis. Fundus photography was carried out at the time of diagnosis. Using image analysis software, measurements summarized as the central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE).
Results: The average age of women was 27.6 ± 7.8 years. Arteriolar diameter in patients with pre-eclampsia (136.44 ± 14.24μm) was significantly less than arteriolar diameter in patients with PIH (155.37 ± 20.11μm) [Mann-Whitney Test, p=0.03]. Compared to healthy pregnant controls (174.25 ± 15.31μm), women with pre-eclampsia and PIH had a lower arteriolar diameter (p<0.05). No significant difference was found between venular diameters among three groups (Kruskal-Wallis Test, p>0.05). No significant difference was found between mean arterial blood pressure in patients with pre-eclampsia (108.95 ± 15.24 mmHg) and PIH (106.46 ± 13.48 mmHg) [Mann-Whitney Test p=0.5]. A significant reverse correlation was noted between serum levels of uric acid and retinal arteriolar diameter in patients with high blood pressure [Spearman's rho=-0.8, p=0.001].
Conclusions: Significant retinal arteriolar vasoconstriction in patients with pre-eclampsia compared with patients with PIH in absence of significant difference in mean arterial blood pressure might be due to endothelial cell dysfunction and subsequent substance alteration that may induce vasoconstriction in retinal and systemic arterioles. Presence of significant reverse correlation between uric acid levels with arteriolar diameter may provide a non-invasive tool to monitor the disease progress or other organs status and may predict consequent morbidity and mortality.
Retinal vessels analysis can be a non-Invasive tool for in vivo visualization of systemic microvasculature in Pre eclampsia and Hypertension in Pregnancy.
Commercial Relationships: Mohammadreza Peyman,

None; Azam Bakhtiari, None; Mimiwati Zahari, None; Siti Zawiah Omar, None

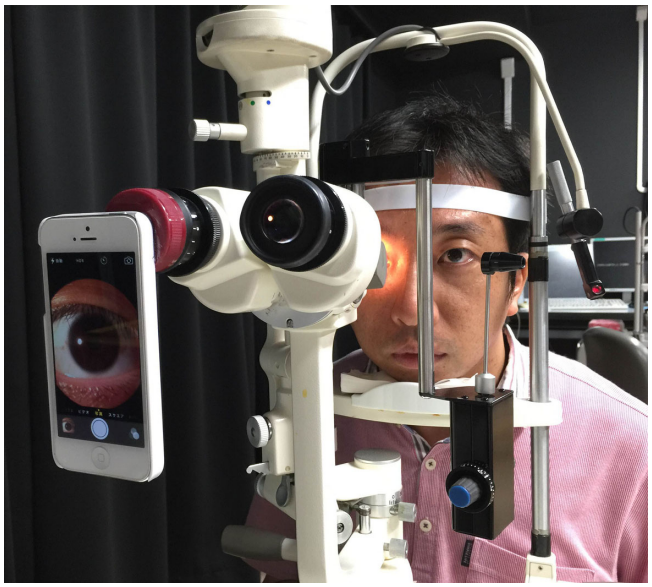
100-3

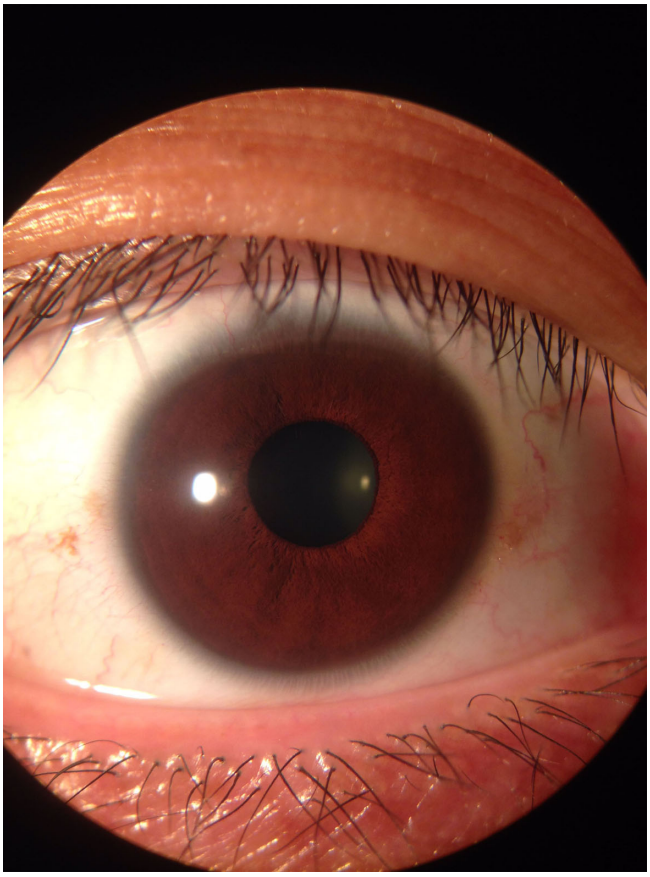
Slit-lamp adapter for smartphone

Sumihiro Kawano [1, 2] Hiroki Kawano [1] Hiroto Terasaki [1] Toyoko Yanagita [2] Taiji Sakamoto [1]

1. Ophthalmology, Kagoshima Univ Faculty of Med, Kagoshima, Kagoshima, Japan. 2. Ophthalmology, Tarumizu Chuo Hospital , Tarumizu, Kagoshima, Japan.

Purpose: To produce a slit-lamp adapter for smartphone and to evaluate its utility.
Methods: A small pinhole was formed in the center of a PS 8K bottle cap (Daiichi Glass Corp, Tokyo, Japan) and a smartphone case was attached on it. After this device was attached to slit-lamp (SM70 and SM70N, TAKAGI Corp, Japan), photographs were taken using a built-in camera with this smart phone for exterior of the eye , anterior eye and ocular fundus of the eyes with various pathologies .
Results: A clear image was obtained from ocular surface, anterior segment, cornea, iris, lens but also fundus using this device. Fundus images were obtained by contact lens.
Conclusions: Our device could be easily made by hand with little cost. High quality images were easily obtained from anterior and posterior eyes. The present device would be very useful not only for physicians but also medical staffs in monitoring the diseases of patients.
Commercial Relationships: Sumihiro Kawano, None; Hiroki Kawano, None; Hiroto Terasaki, None; Toyoko Yanagita, None; Taiji Sakamoto, None


PS-8K bottle cap attachment with iPhone5


slit-lamp photography taken by PS-8K bottle cap attachment

101-4

Intraoperative OCT (iOCT) utilizing a microscope integrated system with heads-up surgeon display, The DISCOVER Study

Sunil Srivastava [1] Peter Kaiser [1] Rishi Singh [1] Justis Ehlers [1]

1. Cole Eye Institute, Cleveland Clinic, Cleveland, OH, United States.

Purpose: To evaluate the feasibility and the utility of a real time prototype microscope integrated *i*OCT system with heads up surgeon display feedback system in both anterior segment and posterior segment surgery.
Methods: A prospective IRB approved study was initiated for *i*OCT utilizing the RESCAN 700 (Carl Zeiss Meditec, Germany). Real-time *i*OCT imaging was analyzed and surgeon feedback forms were obtained and reviewed.
Results: Interim 6 months results reveal 175 eyes enrolled. 70% of eyes were enrolled in the posterior segment surgery arm. 99% of cases had successful imaging performed. No adverse events were identified attributable to *i*OCT. Surgeon feedback revealed in 14% of membrane peeling cases, surgeons identified residual membranes with *i*OCT which required additional peeling. Additionally, in 10% of membrane peeling cases, *i*OCT confirmed complete peeling. Furthermore, in over 70% of cases, *i*OCT provided valuable feedback to the surgeon to assist with surgical decision making. In over 35% of cases, information provided by *i*OCT altered the surgical procedure.
Conclusions: MIcroscope integrated intraoperative OCT is feasible with a heads-up display system. *i*OCT

provides valuable feedback to the surgeon which can alter intraoperaive decision making. Additional software development, instrument refinement and tracking is needed to further advance the use of *i*, OCT in the ophthalmic surgery theater.

Commercial Relationships: Sunil Srivastava, Zeiss (C), Bioptigen (P), Synergetics (P), Santen (C), Clearside (F), Novartis (F), Sanofi (F), Regeneron (C), Bausch and Lomb (C); Peter Kaiser, Zeiss (C), Optovue (C); Rishi Singh, Zeiss (C); Justis Ehlers, Zeiss (C), Bioptigen (P), Synergetics (P)

102-5

Quantitative Analysis of Diabetic Choroidopathy using En Face Swept Source Optical Coherence Tomogrpahy

Tarek Alasil [1, 2] Daniela Ferrara [2] Caroline R. Baumal [2] James G. Fujimoto [3] Jay S. Duker [2] Nadia K. Waheed [2]

1. Ophthalmology, Yale Eye Center, New Haven, CT, United States. 2. Ophthalmology, New England Eye Center, Boston, MA, United States. 3. Department of Engineering and Computer Science and Research Laboratories of Electronics, Massachusetts Institute of Technology, Cambridge, MA, United States.

Purpose: To define the choroidal layers in diabetic and normal subjects using en face images from swept-source optical coherence tomography (SS-OCT).

Methods: Diabetic subjects and age matched normal subjects were enrolled in a prospective cross-sectional study. All subjects were prospectively scanned with a prototype SS-OCT system obtaining 100,000 A-lines/sec at a wavelength of 1050nm and axial resolution of 6 µm. A motion correction algorithm was applied to correct and merge two orthogonal 12x12 mm scans into a single volumetric dataset. En face images were generated at intervals of 4.13 µm (1 pixel) relative to the retinal pigment epithelium (RPE)/Bruch's membrane complex. Systemic analysis of en face SS-OCT images was completed by two independent observers to define the choriocapillaris (CC) and choroidal vascular (CV) layers.

Results: The p values for the differences between the two independent observers in identifying the RPE, CC, transition, and sclera were 0.99, 0.79, 0.44, and 0.15, respectively. In the diabetic group, the mean thickness ± standard deviation (SD) of each layer was, CC (43.7 ± 10.5 µm), and CV (264.5 ± 61.2 µm). In the normal group, the mean thickness ± SD of each layer was, CC (65.0 ± 24.7 µm), and CV (275.4 ± 59.3 µm). CC was significantly thinner in the diabetic group when compared to the normal group ($p < 0.001$). Multivariable linear regression analysis demonstrated significant thinning of CC with increasing age ($p < 0.001$) and being diabetic ($p < 0.001$) and significant thinning of total choroidal thickness with increasing age ($p = 0.001$) and presence of diabetic retinopathy ($p = 0.015$).

Conclusions: Diabetic choroidopathy may precede clinically apparent diabetic retinopathy. In general, diabetic patients have thinner choroids when compared to normal eyes. There is significant thinning of CC in diabetic eyes, regardless of the presence of diabetic retinopathy. En face SS-OCT might provide a useful tool to better understand the pathophysiology of diabetic choroidopathy.

Commercial Relationships: Tarek Alasil, None; Daniela Ferrara, None; Caroline Baumal, None; James Fujimoto, Carl Zeiss Meditech, Inc. (F), Optovue, Inc. (F); Jay Duker, Carl Zeiss Meditech, Inc (F); Nadia Waheed, None

103-6

First Result of the Hamburg Eye Vessel Study, Novel technique for SD-OCT based analysis of arteries and veins

Robert Kromer [1] Shafin Rahman [2] Maren Klemm [1]

1. University Eye Clinic Hamburg-Eppendorf, Hamburg, Germany. 2. University of Manitoba, Manitoba, MB, Canada.

Purpose: The Hamburg Eye Vessel Study facilitates the analysis of vessels of the eye using novel techniques using SD-OCT. The morphology of retinal vessels is closely linked to cardiovascular risk factors and diseases. We describe an innovative SD-OCT-based automated technique analyzing retinal vessels and correlating the results with the age of the patients.

Methods: This proof of concept study included 100 eyes of 100 healthy patients (age ranging from 18 to 72 years). Peripapillar nerve fiber layer (circular scan around optic nerve head) were taken with SD-OCT (Spectralis(R), Heidelberg Engineering, Heidelberg, Germany) with an ART of at least 30 thus reducing noise. All measurements are undertaken by the same investigator. The retinal vessels were automatically segmented in the infrared reflectance image. In the SD-OCT image the retinal nerve fiber layers were detected. The average signal reduction distal of each vessel was calculated for every single layer in comparison to its direct surrounding area. All algorithms were written in commercially available software package Matlab (MathWorks Inc., USA). The resulting data was analyzed using Pearson correlation and ANOVA analysis.

Results: It resulted an average signal deduction of 69,36 ± 7,101 [%] distal of each vessel. Signal reduction was significantly higher for arteries compared to veins. There was a significant correlation for the vessel index of all vessels with the age of the patients ($r=0.47$ ($p<0.001$)) and for arteries with the age of the patients.

Conclusions: Our results show that there is an age-dependent signal reduction distal of vessels, especially for arteries. This indicates that it might be possible to detect the age-dependent arteriosclerosis using our novel SD-OCT analysis technique. Further studies especially with patients with known cardiovascular changes are planned in order to provide more evidence.

Commercial Relationships: Robert Kromer, - (P); Shafin Rahman, None; Maren Klemm, None

Cornea 1

Moderators

Friedrich E. Kruse
University of Erlangen-Nuremberg, Erlangen, Germany
Noriko Koizumi
Faculty of Life and Medical Sciences, Doshisha University, Kyoto, Kyoto, Japan

104-1

Changes in corneal haze five years after endothelial keratoplasty for Fuchs endothelial dystrophy

Sanjay V. Patel [1] Keith H. Baratz [1] Jay W. McLaren [1]
1. Ophthalmology, Mayo Clinic, Rochester, MN, United States.

Purpose: To determine changes in anterior corneal and interface haze through 5 years after Descemet-stripping endothelial keratoplasty (DSEK) for Fuchs endothelial dystrophy.
Methods: In a prospective, observational study, eyes with Fuchs endothelial dystrophy were examined before and at intervals through 5 years after DSEK. All eyes had confluent central guttae with subtle or clinically definite corneal edema prior to DSEK, but without subepithelial fibrosis or vascularization by slit-lamp examination. Corneal haze (backscatter), expressed in scatter units (SU), was quantified by using *in vivo* confocal microscopy (ConfoScan 4, Nidek Technologies) in the anterior cornea (subepithelial region) and at the lamellar graft-host interface. All backscatter measurements were referenced to that of a standard to account for any fluctuations in the intensity of the confocal microscope light source or the sensitivity of the detection system over the course of the study. Comparisons between measurements over time were made by using paired t-tests.
Results: Forty-four eyes of 44 subjects underwent DSEK for Fuchs dystrophy. Anterior corneal backscatter decreased from 2183 ± 607 SU before DSEK to 1741 ± 490 SU at 6 months after DSEK (p=0.009), with continued improvement between through 5 years after DSEK (1452 ± 459 SU, p=0.002 vs. 1 year). Interface backscatter decreased from 1449 ± 608 SU at 1 month after DSEK to 1169 ± 243 SU at 2 years after DSEK (p=0.008), and thereafter remained unchanged through 5 years (1034 ± 215 SU).
Conclusions: After endothelial keratoplasty for Fuchs dystrophy, corneal haze is higher from the anterior (subepithelial) region of the cornea than from the lamellar interface. The early improvment in anterior corneal haze after restoration of endothelial function can be explained in part by resolution of corneal edema. The later progressive improvement in anterior haze through 5 years suggests that there is gradual slow repair of corneal stromal ultrastructural changes.
Commercial Relationships: Sanjay Patel, None; Keith Baratz, None; Jay McLaren, None
Support: Research to Prevent Blindness; Mayo Foundation

105-2

Factors Associated with the Endothelial Cell Loss after Descemet's Stripping Automated Endothelial Keratoplasty

Takefumi Yamaguchi [1, 2] Nobuhito Ishii [1] Seika Shimazaki-Den [1] Yoshiyuki Satake [1] Jun Shimazaki [1, 2]
1. Department of Ophthalmology, Ichikawa General Hospital, Tokyo Dental College, Chiba, Japan. 2. Department of Ophthalmology, Keio University School of Medicine, Tokyo , Japan.

Purpose: To evaluate the influencial factors for the postoperative decrease of endothelial cell density (ECD) after Descemet's stripping automated endothelial keratoplasty (DSAEK).

Methods: Two-hundred twenty six eyes of 207 patients who underwent DSAEK at Tokyo Dental College from June 2007 and Apr 2011 were included. Patients were examined in a routine fashion after DSAEK. Donor age, graft ECD, recipient factors, including corneal sensation, tear secretion, duration of bullous keratopathy (BK), iris defect, iris damage, with/without laser iridotomy, irreversible mydriasis, and causative disease for BK were analyzed as the influencial factors for the postoperative ECD loss. The iris damage and iris defect were scored based on its severity.
Results: Visual acuity (LogMAR) was significantly improved from 1.18 ± 0.57 to 0.48 ± 0.54 at 3 months, 0.34 ± 0.35 at 6 months, 0.31 ± 0.35 at 12 months and 0.26 ± 0.32 at 24 months (all $P < 0.0001$). ECD was decreased from 2600 ± 304 cells/mm^2 to 1332 ± 550 at 3 months, 1244 ± 520 at 6 months, 1104 ± 545 at 12 months and 949 ± 499 at 24 months. The Graft ECD was positively correlated with postoperative ECDs at 3, 6, 12 and 24 months (all, $P < 0.05$). Iris damage and iris defect scores were inversely correlated with postoperative ECDs at 3, 6, 12 and 24 months (all, $P < 0.05$), whereas the other parameters were not correlated with postoperative ECDs ($P > 0.05$).
Conclusions: The graft ECD and iris factors of the recipients were identified as the influential factors for the endothelial cell survival after DSAEK.
Commercial Relationships: Takefumi Yamaguchi, None; Nobuhito Ishii, None; Seika Shimazaki-Den, None; Yoshiyuki Satake, None; Jun Shimazaki, None

106-3

Investigation of the efficacy of Descemet's membrane removal during cultivated corneal endothelial cell injection in a rabbit model

Shinichiro Nakano [1] Naoki Okumura [1, 2] Junji Kitano [1] Shigeru Kinoshita [2] Noriko Koizumi [1]
1. Department of Biomedical Engineering, Doshisha University, Kyoto, Japan. 2. Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Kyoto, Japan.

Purpose: We previously reported our method of cultivated corneal endothelial cell (CEC) transplantation for the treatment of corneal endothelial dysfunction. As removal of pathologic Descemet' s membrane (DM) is thought to provide clinical benefits in cases of Fuchs' endothelial corneal dystrophy, we conducted this present study to investigate the efficacy of DM removal during cultivated CEC-injection therapy in a rabbit corneal endothelial dysfunction model.
Methods: Corneal endothelium was intensively scraped off up to the peripheral area in 12 rabbit eyes to create a corneal endothelial dysfunction model. Of those eyes, a 5.0 x 10^5 amount of cultivated rabbit CECs with rho-associated protein kinase (ROCK) inhibitor Y-27632 was injected into the anterior chamber of 4 eyes following DM removal (removal group) and in 4 eyes without DM removal (non-removal group), while 4 eyes without CECs injection were used as a control. In all eyes, slit-lamp examinations and corneal thickness- and intraocular pressure (IOP) measurements were performed for 14 days, followed by immunohistochemical analysis.
Results: All control eyes exhibited severe corneal edema and failed to recover corneal clarity. All eyes in the removal and non-removal groups recovered corneal clarity at 7-days postoperative, however, that recovery was slower in the removal group than in the non-removal group. Corneal thickness was significantly greater in the removal group than in the non-removal group at 2-days post-therapy (1163.0 ± 32.0 μ m and 794.0 ± 29.6 μ m, respectively; $p<0.01$), however, at 7-days post-therapy it had recovered to a similar thickness in both groups (501.3 ± 11.0 μ m and 615.3 ± 54.5 μ m, respectively). None of the eyes exhibited IOP elevation. Immunohistochemical analysis revealed the reconstruction of a homogeneous monolayer of polygonal cells expressing ZO-1, N-cadherin, and Na^+/K^+-ATPase in both the removal and non-removal groups.
Conclusions: The findings of this study indicate that corneal endothelium is effectively reconstructed by CEC-injection when DM is removed in a rabbit corneal endothelial dysfunction model. However, the DM removal might cause slow recovery of corneal thickness and endothelial reconstruction, thus necessitating an optimization of the cell injection protocol.
Commercial Relationships: Shinichiro Nakano, None; Naoki Okumura, Doshisha University (P), Senju Pharmaceutical Co. (P), JCR Pharmaceuticals Co. (P); Junji Kitano, None; Shigeru Kinoshita, Kyoto Prefectural University of Medicine (P), Senju Pharmaceutical Co. (P), JCR Pharmaceuticals Co. (P); Noriko Koizumi, Doshisha University (P), Senju Pharmaceutical Co. (P), JCR Pharmaceuticals Co. (P)
Support: Research Center Network for Realization of Regenerative Medicine from JST; Program for the Strategic Research Foundation at Private Universities from MEXT

107-4

Intraoperative Optical Coherence Tomography for Anterior Segment Surgeries

Hiroto Mitamura [1, 2] Takefumi Yamaguchi [1, 2] Daisuke Tomida [1] Nobuhito Ishii [1] Seika Den Shimazaki [1] Yoshiyuki Satake [1] Jun Shimazaki [1, 2]

1. Department of Ophthalmology, Ichikawa General Hospital, Tokyo Dental College, Ichikawa, Chiba, Japan. 2. Department of Ophthalmology, Keio University School of Medicine, Shinjyuku, Tokyo, Japan.

Purpose: To present the efficacy of intraoperative optical coherence tomography (iOCT, RESCAN700, Carl Zeiss Meditec) for the anterior segment surgeries.
Methods: iOCT is a microscope integrated intraoperative optical coherence tomography system, which enables the real-time imaging of the ocular tissues during surgeries. iOCT was applied to perform Descemet' s stripping automated endothelial keratoplasty (DSAEK) in 2 eyes with bullous keratopathy, complicated cataract surgery with posterior capsule rupture in 1 eye, intraocular lens (IOL) exchange in 1 eye.
Results: In DSAEK surgery, Descemet' s membrane (DM) was clearly visualized in high-contrast OCT images, which made it easy to remove DM. And after the air injection during DSAEK, the interface fluid between the graft and the recipient cornea was visualized by iOCT, and complete attachment of the DSAEK graft was confirmed at the end of surgeries. In an eye with posterior capsule rupture during cataract surgery, the deformed wound structure due to the prolapse of vitreous was observed using iOCT, which was improved by removing the vitreous from the wound. In IOL exchange, the intraoperative visualization of the posterior capsule using iOCT was useful to keep posterior capsule from intraocular manipulation during surgery and to avoid posterior capsule rupture .
Conclusions: iOCT enabled the real-time visualization of ocular tissue and its structure, thereby improving the safety of surgical steps in DSAEK, IOL exchange and complicated cataract surgery.
Commercial Relationships: Hiroto Mitamura, None; Takefumi Yamaguchi, None; Daisuke Tomida, None; Nobuhito Ishii, None; Seika Den Shimazaki, None; Yoshiyuki Satake, None; Jun Shimazaki, None

108-5

Hyaluronan protects corneal endothelial cells against the noxious effect of extracellular histones after phacoemulsification

Hiroki Kawano [1] Taiji Sakamoto [1]

1. Department of Ophthalmology, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Kagoshima, Japan.

Purpose: Histones are DNA-binding proteins and are involved in chromatin remodeling and regulation of gene expression. Histones can be released after tissue injuries or surgery. The extracellular histones cause cellular damage and organ dysfunction. Regardless of their clinical significance, the role and relevance of histones in ocular

tissues are unknown. This study purpose is to determine whether histones are released after cataract surgery and they can be toxic to corneal endothelial cells.

Methods: The presence of histone after phacoemulsification in a swine eye was examined by immunohistochemistry. Cultured human corneal endothelial cells (HCECs) were exposed to histones for 18 hours. The cell viability was determined by WST-8 assay, and the secretion of interleukin 6 (IL-6) was evaluated by enzyme-linked immunosorbent assay. The effect of specific inhibitors of ERK, JNK, and p38 mitogen-activated protein kinase (MAPK) were evaluated. The protective effect of hyaluronan on this phenomenon was also studied.

Results: Cellular debris containing histones was observed in the anterior chamber of swine eyes after phacoemulsification. Exposure of HCECs to ≥50 μg/ml of histones led to cytotoxic effects. The secretion of IL-6 was significantly increased after exposure of HCECs to histones ($P < 0.01$), and was decreased by ERK1/2 and p-38 MAPK inhibitors ($P < 0.01$). Co-incubation of hyaluronan and histones led to the formation of histone aggregates, and decreased the cytotoxic effects and IL-6 productions ($P < 0.01$).

Conclusions: Cell debris containing histones are released extracellularly during phacoemulsification. Exposure of HCECs to histones causes secretion of IL-6 and cytotoxicity. The intraoperative use of hyaluronan may decrease the cytotoxic effects of histones released during cataract surgery.

Commercial Relationships: Hiroki Kawano, None; Taiji Sakamoto, None

109-6

Mutation in Collagen, type XVII, alpha 1 (COL17A) causes Epithelial Recurrent Erosion Dystrophy (ERED) in northern Sweden

Frida Jonsson [1] Berit Byström [2] Ludvig Backman [3] Therese Kellgren [4] Patrik Ryden [4] Patrik Danielson [3] Ola Sandgren [2] Irina Golovleva [1]

1. Medical Biosciences, Umeå University, Umeå, Sweden. 2. Clinical Sciences, Umeå University, Umeå, Sweden. 3. Integrative Medical Biology, Umeå University, Umeå, Sweden. 4. Mathematics and Mathematical Statistics, Umeå University, Umeå, Sweden.

Purpose: Corneal dystrophies are a clinically and genetically heterogeneous group of inherited disorders that bilaterally affect corneal transparency. The diseases are defined according to the affected corneal layer and by molecular genetics. Autosomal dominant corneal dystrophies affecting the epithelial basement membrane and Bowman's layer are usually caused by mutations in *TGFBI*. In this study we aimed to identify genetic cause of a dominantly inherited Epithelial Recurrent Erosion Dystrophy (ERED)-like disease that is common in northern Sweden.

Methods: 20 patients from the same geographic area in northern Sweden were examined. Four unrelated families were subject of genealogical studies**.** Known mutations in the genes causative of corneal dystrophies were excluded by array-based allele specific primer extension (APEX). Haplotype assessment was done by genotyping using OmniExpressBeadChip (Illumina) and identification of potentially causative genomic variants was done by whole exome sequencing (WES).

Results: DNA from 4 patients all residing in northern Sweden, were tested by APEX for 339 previously reported sequence variants in 13 genes associated with corneal dystrophies (*COL8A*, *TGFBI*, *VSX1*, *CHST6*, *KRT3*, *KRT12*, *GSN*, *TACSTD2*, *CYP4V2*, *SOD1*, *TCF8/ZEB1*, *SLC4A11* and *UBIAD1*); however, no mutations were detected. In addition, no potential disease-associated variants were identified upon sequencing of the entire coding region and all intron-exon boundaries of *TGFBI* gene. By genealogical studies a common ancestor was identified in four nuclear families. Six affected individuals shared 26 Mb region on chromosome 10q23-q26 and WES resulted in the identification of a novel mutation, c.2816C>T, p.T939I, in the *COL17A1* gene coding for collagen type XVII alpha 1. By bioinformatics tools p.T939I was predicted to be possibly damaging or tolerant however was not previously reported (1000 genomes data set and ESP) and was absent in 115 matched controls. It appeared to be a founder mutation that segregated with disease in a genealogically expanded pedigree dating back 200 years. Furthermore, *COL17A1* expression in cornea was demonstrated by RT-PCR and immunohistochemistry.

Conclusions: Our finding highlight the roll of *COL17A1* in dominant corneal dystrophies and improves understanding of the pathogenesis of Bowman's layer dystrophy and ERED.

Commercial Relationships: Frida Jonsson, None; Berit Byström, None; Ludvig Backman, None; Therese Kellgren, None; Patrik Ryden, None; Patrik Danielson, None; Ola Sandgren, None; Irina Golovleva, None

Cornea 2

Moderators

Roger W. Bauerman
Singapore Eye Research Institute, Duke-NUS, Singapore, Singapore
Yuichi Hori
Toho University Omori Medical Center, Ota, Tokyo, Japan

218-1

A Significant Role of Endoplasmic Reticulum Stress in Ocular Chronic Graft-Versus-Host Disease

Shin Mukai [1] Yoko Ogawa [1] Kazuo Tsubota [1]
1. Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan.

Purpose: Chronic graft-versus-host disease (cGVHD) is a disabling complication after allogeneic hematopoietic stem cell transplantation (HSCT) and has a highly negative impact on patients' quality of life. However, no effective treatments for cGVHD have been created. The main focus of this study is on ocular cGVHD, and we plan on working toward establishing useful therapies for cGVHD. According to a previous report, cellular senescence is involved in ocular cGVHD, and cGVHD can be regarded as an age-related disease. (Kawai, M. et al. *Sci Rep.* 2013) In addition, literature precedent suggests that endoplasmic reticulum (ER) stress plays an important role in aging and age-related diseases (Brown et al. *Front Physiol.* 2012). Based on these findings, we envisage (1) that ER stress is increased in ocular cGVHD and (2) that mitigation of ER stress can be effective treatment for ocular cGVHD.
Methods: 1. Whole bone marrow transplantation (BMT) was conducted to obtain a mouse model of cGVHD. In the case where donors are B10.D2 mice and recipients are BALB/c mice, it is allogeneic transplantation and produces a mouse model of cGVHD. In contrast, BMT from BALB/c to BALB/c mice is syngeneic transplantation and produces control samples. (Zhang, Y. et al *J Immunol.* 2002)
2. We analyzed cGVHD-affected and control mice Day 21 and Day 28 after BMT. To investigate whether ER stress is increased in organs affected by cGVHD, we focused on the ER stress marker 78 kDa glucose-regulated protein (GRP78). We compared test and control samples to see the difference in the amount of GRP78 using real-time PCR and immunohistochemistry.
Results: Histopathologic findings showed inflammatory cell infiltration and excessive fibrosis, which are the characteristic features of lacrimal gland cGVHD.
Immunofluorescence staining revealed that ER stress was increased in the murine lacrimal glands affected by cGVHD. GRP78 was expressed in the cytoplasm surrounding nuclei in the endothelia (Figure 1). Results of qPCR also suggest that ER stress is elevated in the GVHD-affected lacrimal glands.
Conclusions: This study has suggested that ER stress is elevated in the lacrimal glands affected by cGVHD and that ER stress is linked with ocular cGVHD. Hence, mitigation of ER stress can be useful for treatment of ocular cGVHD.
Commercial Relationships: Shin Mukai, None; Yoko Ogawa, None; Kazuo Tsubota, None

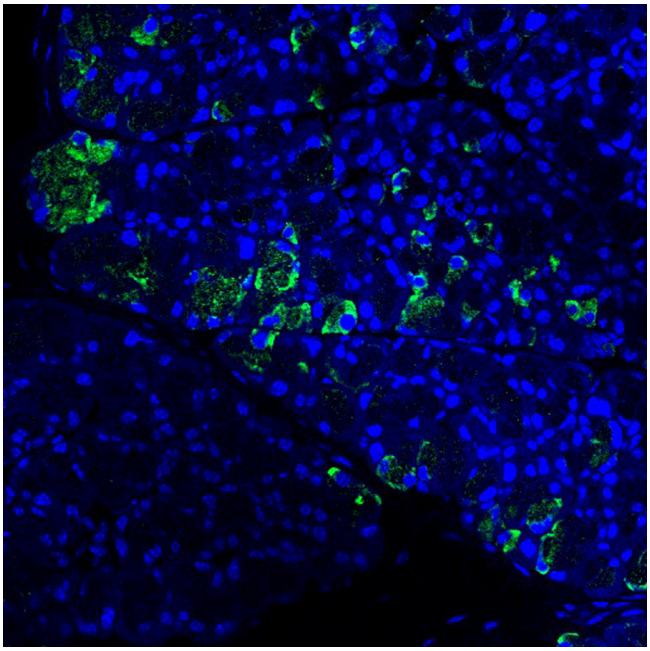


Figure 1. Fluorescence image of the cGVHD-affected lacrimal gland. GRP78 is stained green and cell nuclei are blue.

219-2

Oxidative stress and heavy metalions in severe dry eye syndrome patients in comparison with non-dry eye syndrome individuals

Ying Liu [1] Masatoshi Hirayama [1] Tetsuya Kawakita [1] Shigeto Shimmura [1] Kazuo Tsubota [1]
1. Ophthalmology, School of Medicine, Keio University , Tokyo, Japan.

Study Group: Dry eye

Purpose: Heavy metal ions have been reported to be associated with production of reactive oxygen species, which may lead to an increase in oxidative stress to cause aging and disorders in various organs including eyes. Aging is one of the most important factors in the pathogenesis of dry eye syndrome (DES). We conducted a case-control study to explore the relationship between oxidative stress and DES, comparing oxidative stress levels in blood and urine, as well as mineral levels accumulated in hairs between patients with severe DES and volunteer patients with non-DES as controls.
Methods: Seventeen patients (34 eyes) with the severe DES were selected from the clinic of Ophthalmology, Keio University Hospital, Tokyo, Japan as cases. The control group consisted of 14 volunteers (26 eyes) who were admitted to the same hospital and had no history of DES. All cases and controls underwent the Schirmer test, tear break-up time test (BUT), vital staining test and subjective symptom examination. The blood, urine, hair and tear samples were collected at the clinic. Tear osmotic

pressure (Tearlab), minerals in the hairs, and oxidative stress markers in blood and urine were measured on the same day.
Results: All study participants were females. The mean age at enrollment was 65.9 ± 11.6 years for cases, and 63.9 ± 15.2 years for controls. The mean osmolarity of 10 cases was 320.1 ± 26.3 mOsms/L (normal mean, 309.9 ± 11.0 mOsms/L). It was shown that the level of Mercury (Hg) was significantly higher accumulation in cases than in controls (p-value=0.0117). Comparing with the controls the elevated level of sodium (Na) (mineral that essential to biological activity) in patients indicated the tendency of increasing accumulation of the mineral(not significant). The level of serum albumin, blood Hb and blood RBC were significantly lower in patients than controls (p-value = 0.0138, 0.0025 and 0.0010, respectively). There was a higher level of 8-OHdG in tears in patients. The mean 8-OHdG/CRE (8-hydroxy-2'-deoxyguanosine creatinine reduced level) in urine and superoxide dismutase (SOD) activity in blood showed no significant difference between the cases and controls.
Conclusions: The high level of Hg concentration in the hairs was found in cases with severe DES, which suggested that Hg may be associated with DES.
Commercial Relationships: Ying Liu, None; Masatoshi Hirayama, None; Tetsuya Kawakita, None; Shigeto Shimmura, None; Kazuo Tsubota, None

220-3

Efficacy and Safety of Topical Diquafosol Tetrasodium Treatment for Chronic Graft-versus-Host Disease-Related Dry Eye Disease, A Randomized Comparative Clinical Study

Yoko Ogawa [1] Naoyuki Kozuki [1] Mio Yamane [1] Mizuka Kamoi [1] Yumiko Saijo-Ban [1] Tetsuya Kawakita [1] Dogru Murat [1] Kazuno Negishi [1] Shigeto Shimmura [1] Kazuo Tsubota [1]
1. Department of Ophthalmology, Keio University School of Medicine, 35 Shinanomachi Shinjuku-ku , Japan.

Purpose: To investigate the safety and efficacy of topical diquafosol tetrasodium (DIQ), a $P2Y_2$ agonist that stimulates tear fluid and mucin secretion on the ocular surface and improves tear functions, as a novel treatment for chronic graft-versus-host disease (cGVHD)-related dry eye disease.
Methods: A randomized controlled trial was conducted. Twenty-two cGVHD patients with mild to moderate dry eye disease were alternatively assigned to a topical DIQ treatment group (11 patients) or a non-DIQ control group (11 patients). In addition to baseline treatment, the DIQ group received 3% topical DIQ, six times per eye per day, while the non-DIQ group did not. The patients' eyes were examined 2 weeks and 1 month after the start of the trial, and the results of each patient' s eyes were averaged.
Visual analog scale symptom scores, corneal sensitivity, Schirmer I test value, tear clearance value, tear film break-up time (TBUT), and ocular surface vital staining scores were recorded at baseline, and 2 and 4 weeks after the start of the trial in both groups. Conjunctival brush cytology was performed before and 4 weeks after the start of treatment in 3 patients in the DIQ group. The MUC5AC, MUC1, MUC4, and MUC16 gene expressions were examined by real time PCR.
Results: After DIQ treatment, significant improvements were found in symptoms, the tear clearance value, TBUT, vital staining scores, and MUC4 gene expression at the end of the trial ($P<0.05$). TBUT, fluorescein and rose bengal scores were significantly improved in the DIQ group compared to the control group at 2 and 4 weeks after the start of the trial ($p< 0.01$, 0.01, and 0.05 respectively). The MUC5AC, MUC1, and MUC16 gene expressions tended to increase in the DIQ group at 4 weeks compared to the start of the trial. There were no significant DIQ-related events, except for irritation in 2 of the 11 cases.
Conclusions: The results suggest that adding 3% topical DIQ to conventional treatment is effective for cGVHD patients with mild to moderate dry eye, by improving mucin gene expression, ocular surface findings, and tear clearance.
Commercial Relationships: Yoko Ogawa, None; Naoyuki Kozuki, None; Mio Yamane, None; Mizuka Kamoi, None; Yumiko Saijo-Ban, None; Tetsuya Kawakita, None; Dogru Murat, None; Kazuno Negishi, None; Shigeto Shimmura, None; Kazuo Tsubota, None
Support: the Japanese Ministry of Education, Science, Sports and Culture, #23592590 and #26462668
Clinical Trail: UMIN000006862

221-4 Withdrawn

Activation of HIF-1a (hypoxia inducible factor-1a) prevents dry eye-induced acinar cell death in the lacrimal gland

Yuri Seo [1] Yong Woo Ji [1] Hyemi Noh [1] Areum Yeo [1] EungKweon Kim [1] HyungKeun Lee [1]
1. Institute of Vision Research, Department of Ophthalmology, Yonsei University College of Medicine , Seoul, Korea (the Republic of).

Purpose: To determine and investigate the protective mechanisms against dry eye (DE) stress in mice.
Methods: Six to 8-weeks-old(C57BL/6) mice (Charles River Laboratory, Wilmington MA) and MMTV-Cre-dependent hypoxia-inducible factor (HIF)-1a conditional knock out(CKO) mice were placed in a low humidity controlled environment chamber(<15% of humidity) and supplemented with subcutaneous injections of 0.1mL of scopolamine hydrobromide, 5mg/mL(Sigma-Adlrich Chemical Co., St. Louis, MO), 3 times a day for the duration of the experiment. After 2 weeks of DE induction, mice were sacrificed and their lids, eyeballs, and lacrimal glands (LGs) were collected. Sizes of lacrimal glands were measured grossly. Lacrimal gland acinar cell organelle structures were observed with TEM (transmission electron microscope). ER (Endoplasmic Reticulum) stress and autophagy level, COX-2, MMP-9 and HIF1a level in LGs were quantified using immunoblot. Glycolytic activities were measured using LC-MS method. Infiltration of $CD3^+$ and $CD11b^+$ cells were measured with FACS analysis.
Results: DE induced prominent blood vessel loss without apoptosis or necrosis in the LG. Autophagic vacuoles, distressed mitochondria, and stressed ER were observed

via TEM. Immunoblotting confirmed the increase in autophagic markers. Glycolytic activities were enhanced with increasing levels of succinate and malate that, in turn, activated HIF-1a. The areas of stable HIF-1a expression overlapped with COX-2 and MMP-9 up-regulation in LGs of DE-induced mice. In HIF-1a CKO mice in which HIF-1a expression was lost in the LG, normal LG polarities and morphologies were completely lost with DE induction, and tremendous acinar cell apoptosis was observed. Similar to Sjogren' s syndrome, $CD3^+$ and $CD11b^+$ cells infiltrated HIF-1a CKO LGs.

Conclusions: DE induced the expression of HIF-1a that activated autophagy signals to prevent further acinar cell damage and to maintain normal LG function.

Commercial Relationships: Yuri Seo, None; Yong Woo Ji, None; Hyemi Noh, None; Areum Yeo, None; EungKweon Kim, None; HyungKeun Lee, None

222-5

Prospective Placebo-Controlled Trial of Intense Pulsed Light (IPL) Therapy for Meibomian Gland Dysfunction (MGD)

Jennifer P. Craig [1] Yen-Heng Chen [1] Philip Turnbull [1]

1. Ophthalmology, The University of Auckland, Auckland, New Zealand.

Study Group: Ocular Surface Laboratory

Purpose: To determine the effect of intense pulsed light (IPL) therapy on tear film quality in meibomian gland dysfunction (MGD).

Methods: This prospective, double-masked, randomised, placebo-controlled trial compared ocular surface and tear film parameters before and after 3 consecutive IPL treatments with multiple homogenously sequenced light pulses (E>Eye, E-Swin, France) at T1, T2 (T1+2 weeks) and T3 (T1+6 weeks) on one eye (randomised) of 28 participants with MGD (19F, 9M, aged 45 ± 16 years). Symptoms (SPEED questionnaire) as well as lipid layer grade (LLG), noninvasive break up time (NIBUT), tear evaporation (TER) and tear meniscus height (TMH) were evaluated relative to baseline and control values.

Results: LLG and NIBUT improved significantly from baseline to T3 in the treated eyes ($p<0.0001$) but not in the control eyes ($p>0.05$). SPEED symptom score reduced in both the treated ($p<0.0001$) and control ($p=0.002$) eyes with 86% recording reduced symptoms in the treated eye by T3. Improvements in TER approached statistical significance ($p=0.08$) in the treated eye but not in the control eye ($p=0.165$). IPL had no significant effect on TMH in either eye ($p>0.05$).

Conclusions: Improvements in tear quality and reduced dry eye symptoms suggest that the application of IPL has potential therapeutic benefit for patients with MGD.

Commercial Relationships: Jennifer Craig, E-Swin (F); Yen-Heng Chen, E-Swin (F); Philip Turnbull, E-Swin (F)

Support: NZAO Summer Studentship

Clinical Trail: 365741

223-6

A Simplified Xeroscope for the Noninvasive Measurement of Tear Break-up Time

Darien B. Gaw [1] Bernard Gil O. Tinio [1]

1. Department of Ophthalmology, Makati Medical Center, Makati City, NCR, Philippines.

Purpose: To develop and validate a noninvasive method for measuring tear break-up time (NIBUT) using readily available materials by comparing it to clinical standard fluorescein tear break-up time (TBUT) assessment.

Methods: A prototype xeroscope was made from a recycled round plastic lid measuring 16 cm. in diameter. At its inner lining, a fluorescent green stick-on paper printed with black concentric grid pattern was fixed. A 30-blue LED strip was fixed on the inner rim of the lid using adhesive tape and was powered by an AC-DC power adapter set at 6V at 350mA (2W). A central cut-out measuring 2 cm. x 1 cm. was made on the lid serving as its viewfinder through which the precorneal grid reflection was observed.

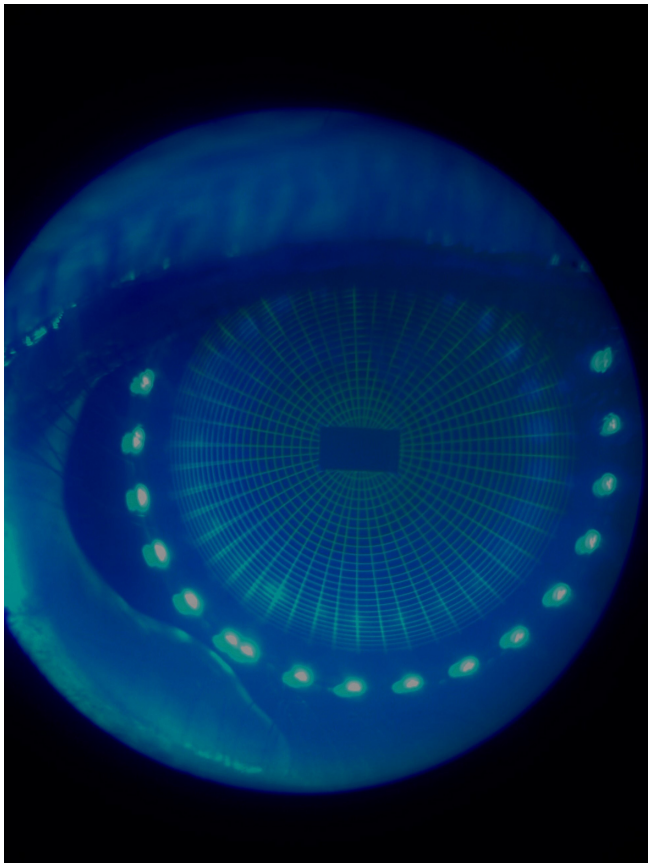
The device was tested on 50 patients, aged 21 to 65 years old, with no apparent ocular surface disorders and eyelid abnormalities. Measurement of NIBUT was done first on both eyes using the prototype xeroscope followed by standard fluorescein TBUT measurement. A 10-minute break in between test procedures was given to all participants to ascertain no residual tearing was carried over on the following procedure.

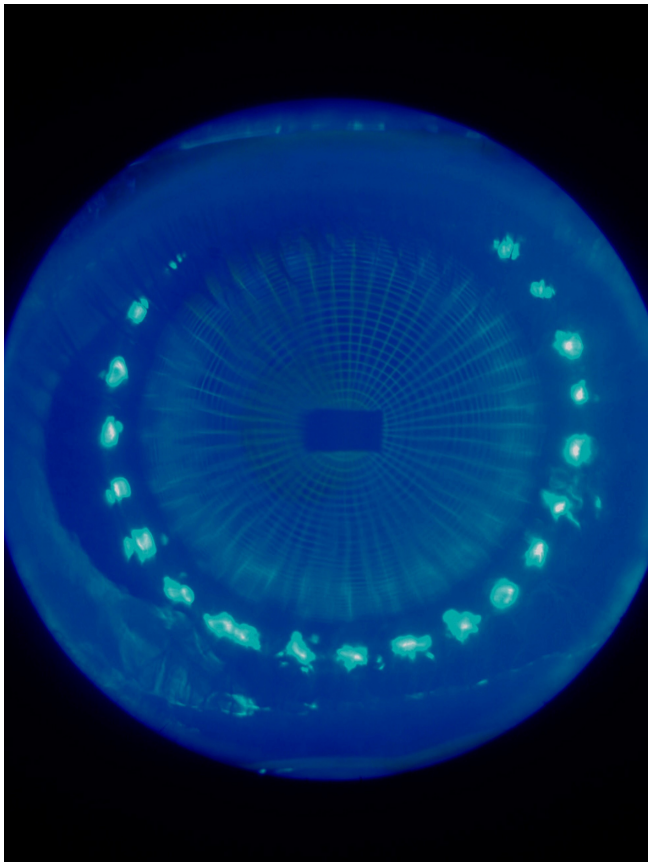
Viewed through a slit-lamp biomicroscope, the interval between a complete blink and the appearance of randomly distributed progressive distortion on the precorneal grid reflection was measured in seconds and was designated as noninvasive tear break-up time (NIBUT). The clinical standard using sterile single-use 1 mg Fluorescein sodium ophthalmic strip for TBUT evaluation was performed for comparison. The two values, mean NIBUT and TBUT from each eye were then consigned to linear-regression analysis to determine their correlation.

Results: Fifty patients were enrolled in the study. A total of 100 eyes were examined for both test procedures. The mean age of the participants was 48.52 years old (range 21-62). The mean NIBUT for both eyes was 20.10 seconds (range 11.56-36.17) while mean TBUT was 18.70 seconds (range 10.20-30.40). The mean difference between NIBUT and TBUT was 1.40 second. Regression correlation analysis revealed a strong positive linear correlation between the NIBUT and TBUT of both eyes with a computed R-squared value of 0.9697.

Conclusions: The noninvasive technique using the prototype device provides a good alternative approach for measuring tear film stability without disturbing normal tear physiology and dynamics at a fraction of the cost of commercially-available xeroscopes and tearscopes.

Commercial Relationships: Darien Gaw, None; Bernard Gil Tinio, None





Cornea 3

Moderators

Kong Yong Then
Hospital University Kebangsaan, Malaysia, Malaysia
Motokazu Tsujikawa
Osaka University Graduate School of Medicine, Suita, Osaka, Japan

224-1

Patient experience of cultured limbal epithelial transplantation - development of a Quality of Life (QOL) based outcome assessment questionnaire

Derek Ho [1] Carol Porteous [2, 3] Richard Cable [2, 3] Catey Bunce [2, 3] Alex Shortt [2, 3]
1. Ophthalmology Department, William Harvey Hospital, Ashford, United Kingdom. 2. Moorfields Eye Hospital, NIHR Biomedical Research Centre, London, United Kingdom. 3. UCL Institute of Ophthalmology, London, United Kingdom.

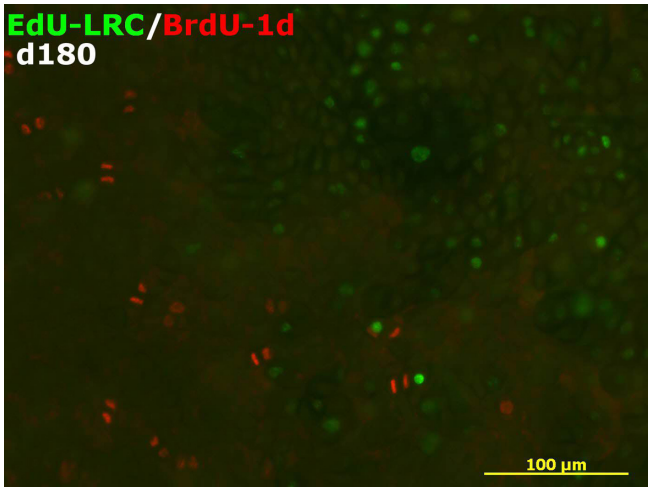
Purpose: In corneal limbal stem cell deficiency (LSCD), loss of vision occurs because a healthy epithelium cannot be maintained. Over the past 10 years a new laboratory-based technique of cultured limbal epithelial transplantation has been introduced as part of the surgical treatment of LSCD. To date, outcome measures reported for this treatment have largely been based upon subjective assessment by the treating physician. This study aims to bring clinicians and patients together to develop a questionnaire tool for the evaluation of LSCD severity and the impact of treatment on the patients QOL and vision.
Methods: Using a Delphi approach, ophthalmologists with corneal and external disease experience were independently interviewed to determine which components of the NEI-VFQ and ADVS questionnaires they considered relevant to LSCD patients. Institutional ethics approval was obtained and patients with established LSCD were invited to participate in a focus group. A structured meeting was held where patients' experiences with LSCD were discussed and the impact on their quality of life explored. Thematic analysis was performed on the focus group transcripts.
Results: The Delphi exercise with LSCD experts resulted in the elimination of several irrelevant questionnaire items. A long-list of QOL questions was created, which served as a framework in the focus group discussion. Thematic analysis of the transcripts revealed the key QOL indicators and enabled the construction of a provisional LSCD-QOL questionnaire. These key themes included, co-morbidities and symptoms; depth perception; daily activities; public transportation; independence, psycho-social and emotional impact and future concerns. This LSCD-QOL questionnaire is currently being validated in a cohort of 30 patients with LSCD.
Conclusions: The use of Patient Reported Outcome Measures (PROM) is rapidly gaining popularity with patients, doctors and healthcare providers. They encourage patient involvement in care, facilitate better clinical decisions by doctors, and allow evidence-based resource allocation by healthcare authorities. Once validated, the LSCD-QOL tool will provide qualitative measurement of the impact of LSCD on patients' lives, as well as an unbiased gauge of disease progression and intervention effectiveness, with particular importance in clinical trials of novel treatments for LSCD.
Commercial Relationships: Derek Ho, None; Carol Porteous, None; Richard Cable, None; Catey Bunce, None; Alex Shortt, None

225-2

Localization of label retaining cells and rapidly proliferating cells in 6-month cultured primary human limbal epithelial cell sheets

Hideyuki Miyashita [1] Kazuo Tsubota [1] Shigeto Shimmura [1]
1. Ophthalmology, Keio University School of Medicine, Tokyo, Japan.

Purpose: Stem cells *in vivo* undergo cell cycle slowly, whereas their progeny rapidly proliferate to produce differentiated cells. In this study, we confirmed whether the proliferation heterogeneity is maintained in primary cultured human limbal epithelial cell sheets, which are used for a stem cell transplantation therapy.
Methods: Human limbal epithelial cells isolated from US eyebank eyes were primary cultured on plastic cell culture inserts, with a feeder layer of human mesenchymal stem cells prepared in the bottom of a paired well. Cells were fed with modified supplementary hormonal epithelial medium containing KGF and Y-27632, but not EGF. Semi-confluent cells (day 6) were serially labeled with EdU (1 μ M) for 3 days, and one of triplicated inserts was fixed immediately after serial labeling. Remained inserts were fed daily with medium to washout EdU label. On day 179, cells were fed with BrdU (10 μ M) containing medium for 1day to label rapidly proliferating cells. Cryosections or PFA-fixed whole mount cell culture inserts were stained with EdU detection kit, followed by anti- BrdU immunostaining.
Results: Immediately after serial labeling, almost all epithelial cells were labeled with EdU. Whole mount staining showed that 6-month cultured sheets contained label retaining cells (LRCs), which were heterogeneously distributed in the cell sheets. BrdU positive cells, which underwent S phase from day 179 to day 180, were mainly observed in LRC-sparse areas.
Conclusions: Primary cultured human limbal epithelial cell sheets are clustered into LRCs-rich areas and rapidly proliferating areas, indicating the proliferative heterogeneity in *in vitro* sheets.
Commercial Relationships: Hideyuki Miyashita, None; Kazuo Tsubota, None; Shigeto Shimmura, None
Support: JSPS KAKENHI Grant Number 25861650



EdU (green)-labeled LRCs and BrdU (red)-labeled rapidly prolifeating cells in the 6-month cultured primary human limbal epithelial cell sheet.

226-3

Development of an Autologous Treatment Protocol for Limbal Epithelial Stem Cell Deficiency using Optimally Transparent, Mechanically Suitable RAFT Tissue Equivalents

Isobel Massie [1] Alvena K. Kureshi [1] Julie T. Daniels [1]

1. Department of Ocular Biology & Therapeutics, UCL Institute of Ophthalmology, London, United Kingdom.

Purpose: Limbal epithelial stem cell (LESC) deficiency can cause blindness. LESC may be cultured from donor rims and expanded on human amniotic membrane (HAM), which may be used as a carrier for transplantation. However, clinical graft manufacture using HAM can be unreliable and the use of donor cells requires immunosuppression post-transplantation. We have developed RAFT tissue equivalents (TE) (produced by wicking water away from type I collagen hydrogels) and shown that human limbal epithelial cells (hLE) isolated from donor rims can be expanded on RAFT TE. The aims of this study were to optimize the optical and mechanical properties of RAFT TEs for clinical application and to develop a protocol for hLE culture from limbal biopsies so that treatment could be autologous.
Methods: Hydrogels were produced using 1, 2 or 3mg/ml type I collagen in 0.6, 1.2 or 2.4ml volumes. Water was wicked away to produce RAFT TEs that were compared to HAM substrate for transparency, thickness and mechanical strength. Limbal biopsies (4mm^2) were taken from fresh, whole globes and collagenase-digested overnight at 37 ° C. The resulting mixed population cell suspension was split between 2 RAFT TEs and cell growth observed. Cell phenotype was assessed using light microscopy and putative stem cell marker, p63 α , expression was assessed using confocal microscopy.
Results: The total amount of collagen used to produce RAFT TEs correlated with RAFT TE thickness (range, 53-411 μ m), and break force (range, 0.105–0.732 Newtons), and inversely with RAFT TE transparency (range 13–81%). RAFT TEs made using 0.6ml of 3mg/ml collagen were of comparable thickness, transparency and strength to HAM and were used for biopsy studies. hLE colonies were observed from all biopsies taken (4 globes). Confluent hLE on RAFT TEs were small, tightly-packed with scant cytoplasm and had cobblestone morphology. Confocal imaging revealed high levels of p63 α expression.
Conclusions: RAFT TEs can be produced with similar optical and mechanical properties to HAM, but have further advantages such as increased reliability and reproducibility, no requirement for screening and increased availability. hLE can be isolated from limbal biopsies, enabling autologous treatment. Future work will determine the optimal location for biopsy harvesting.
Commercial Relationships: Isobel Massie, None; Alvena Kureshi, None; Julie Daniels, None
Support: Technology Strategy Board Grant 101109

227-4

Limbal reconstruction using bio-engineered limbal plate in rabbit eye of limbal deficiency experimental model

Hong Kyun Kim [1]

1. Department of Ophthalmology, Kyungpook Nat'l University School of Medicine, Daeu, Korea (the Republic of).

Purpose: Although bio-engineered limal epithelial cell sheets have showed a good results in some patients with limbal deficiency patients, long term results have been variable depend on the degree of limbal destruction. Full reconstruction of limbal niche and supply of limbal stem cell is essential for the complete physiologic recovery in the patients. This study aimed to develop bio-engineered limbal tissue for the reconstruction of stem cell microenvironment.
Methods: Human corneal limbal tissues were decellularized by 0.25% trypsin-EDTA in hypotonic Tris buffer (10mM, pH 7.2) and human corneal limbal epithelial cells were re-seeded on the lenticule. Limbal deficient experimental model was established by alkali burn with 1N NaOH and decellularized and bio-engineered lenticule was transplantated in the burned site after 1 week. The operated corneas were collected at 1 week and 4 weeks. Limbal deficiency and invasion of conjunctival cells were examined by impression cytology and routine histology, and harvested corneas were analyzed by immuonohistochemistry.
Results: Immonologic staining results showed that human cornea lenticules were successfully decellularized and limbal epithelial cells reseeded expressing Keratin 3, 5, 12, and ABCG2. Impression cytology after alkali burn showed numerous goblet cells at inside of limbus, and several neovascularization were observed. Transplantation of the limbal plate showed that limbal epithelial cells inhibited conjunctival cell migration into center cornea compare to lenticule only, and had more transparent than the other at 4 weeks after operation.
Conclusions: Data in this study showed that bio-engineered plate was suitable for limbal reconstruction and corneal epithelial cells were remained and kept its phenotype without any infiltration of inflammatory cells. Collectively, our data suggest that bio-engineered human cornea lenticule has great potential for the replacement of allogenic cornea transplantation to treat severe corneal

diseases.
Commercial Relationships: Hong Kyun Kim, None
Support: This study was supported by Korean National Research Fund (NRF-2012R1A1A1010163)

228-5

Low oxygen increases CD44 expression in lacrimal gland derived mesenchymal stem cells

Mathias Roth [1] Kristina Spaniol [1] Claus Kordes [2] Dieter Häussinger [2] Silke Schwarz [3] Nicole Rotter [3] Gerd Geerling [1] Stefan Schrader [1]

1. Department of Ophthalmology, University Düsseldorf, Düsseldorf, Germany. 2. Department of Gastroenterology, Hepatology and Infectious Diseases, University Düsseldorf, Düsseldorf, Germany. 3. Department of Otorhinolaryngology, University Ulm, Ulm, Germany.

Purpose: Quantitative tear deficiency due to lacrimal gland insufficiency is one of the major causes for the development of severe dry eye syndrome. So far, there exists no causative treatment. The application of lacrimal gland-derived mesenchymal stem cells (LG-MSC) for the regeneration of lacrimal gland tissue could result in a novel therapy in severe cases. In order to optimize the culture conditions, the purpose of the present study was to evaluate the influence of reduced oxygen concentration on the phenotype, the proliferative capacity and the differentiation potential of murine LG-MSC.
Methods: Murine (C57BL/6) LG-MSC were cultured in 21% oxygen (high oxygen) and in 5% oxygen (low oxygen). Cells from both culture conditions were characterized by flow cytometry. Proliferation was evaluated by colony forming unit assays (CFU) and a cellsorter-assisted proliferation assay. Differentiation potential into adipocytes and osteocytes was evaluated by Oil-Red-O- / Alizarin-Red staining and quantitative Real Time polymerase chain reaction (qRT-PCR). Reactive oxygen species (ROS) levels were measured with a with ROS Assay Stain kit in flow cytometry.
Results: Cells from both culture conditions revealed adipogenic and osteogenic differentiation potential and presented a MSC-specific flow cytometric phenotype. In low oxygen, cells yielded less ROS, showed a more homogenous and stable morphology without stress fibres, a higher colony forming potential and an increased proliferation capacity. Low oxygen significantly increased the expression of CD44+ LG-MSC (CD44 median fluorescence intensity, 5% O^2, 3562.76 ± 1359.89; 21% O^2, 846.00 ± 251,73, p=0.015)
Conclusions: For the first time, the effect of low oxygen concentration on LG-MSC has been evaluated. Our findings indicate, that low oxygen preserves the morphology, as well as the proliferative capacity and significantly increases the number of LG-MSC expressing CD44, which might be a promising marker to identify a potent MSC subpopulation.
Commercial Relationships: Mathias Roth, None; Kristina Spaniol, None; Claus Kordes, None; Dieter Häussinger, None; Silke Schwarz, None; Nicole Rotter, None; Gerd Geerling, None; Stefan Schrader, None

229-6

Meibomian Gland Dysfunction Related Dry Eye in Ectodysplasin A Mutant Mice

Yenchiao Wang [1] Xiaoxiao Chen [1] Baikai Ma [1] Sanming Li [1] Hui He [1] Tingting Liu [1] Liyin Zhang [1] Zuguo Liu [1] Wei Li [1]

1. Eye Institute of Xiamen University, Xiamen, China.

Study Group: Eye Institute of Xiamen University, Xiamen, Fujian, China

Purpose: Meibomian glands play an important role in the integrity of tear film and ocular surface. Meibomian gland dysfunction (MGD) has been the most frequent cause of evaporative dry eye, however, the pathophysiological process of MGD induced dry eye is largely unknown. This study was designed to investigate the tear film and ocular surface change in meibomian gland deficient ectodysplasin A mutant (Tabby) mice.
Methods: Slit lamp microscope observation, tear film break-up time (BUT) test, fluorescein staining, and Schirmer test were performed to evaluate the ocular surface and tear status of Tabby mice. Scanning electron microscope (SEM), H&E staining, PAS staining, immunofluorescent staining of K12, K10, and Sprr1b was performed on corneal and conjunctival tissues from Tabby mice and wild type mice at different time points from 3 weeks to 32 weeks. K12, K10, and Sprr1b gene expression in the corneal epithelium from Tabby and wild type mice at 4 weeks was determined by Real-time PCR.
Results: Tabby mice demonstrated scabrous corneal surface from 8 weeks, then sequentially developed central corneal stromal edema, new blood vessel ingrowth, and granuloma tissue formation. Aqueous tear secretion maintained relatively stable, while there was a tendency of shortened BUT and increased fluorescein score from 4 to 16 weeks. H&E staining showed rugged corneal epithelium in Tabby mice from 4 weeks. The granuloma tissue loci at 32 weeks showed dramatically thickened stroma with prominent cell infiltration, as well as significant stratification and keratinization of the epithelium. SEM showed dramatic reducing of microvilli on the apical surface of the corneal epithelium in Tabby mice. Conjunctival goblet cell density did not show obvious decrease in Tabby mice. Keratin K12 and K10 expression in Tabby mice from 4 to 8 weeks did not show apparent change compared with that of wild type mice, however, Sprr1b emerged in the superficial layer of the central corneal epithelial cells at 4 weeks and increased gradually to peripheral cornea and full thickness of the epithelium at 8 weeks in Tabby mice, while kept negative in the wild type mice throughout the observation period.
Conclusions: Tabby mice exhibit typical tear film and ocular surface pathological changes of evaporative dry eye. These mice may be valuable model of MGD related dry eye, thus could be applied in the future study of dry eye mechanism and treatment.
Commercial Relationships: Yenchiao Wang, None; Xiaoxiao Chen, None; Baikai Ma, None; Sanming Li, None; Hui He, None; Tingting Liu, None; Liyin Zhang , None; Zuguo Liu, None; Wei Li, None

Retina 2

Moderators

Gislin Dagnelie
Johns Hopkins University School of Medicine, Baltimore, MD, United States
Akihiro Kakehashi
Jichi Medical University, Saitama Medical Center, Saitama, Saitama, Japan

279-1

Efficacy of ranibizumab in branch and central retinal vein occlusion, Outcomes from the BRIGHTER and CRYSTAL studies

Sanjeewa Wickremasinghe [1]
1. University of Melbourne, The Royal Victorian Eye and Ear Hospital, Melbourne, East Melbourne, VIC, Australia.

Purpose: BRIGHTER (NCT01599650) and CRYSTAL (NCT01535261) are two ongoing 24-month (M) studies assessing the long-term efficacy of pro re nata (PRN) dosing regimen of ranibizumab (RBZ) 0.5 mg, in patients with branch and central retinal vein occlusion (BRVO and CRVO)
Methods: In BRIGHTER, patients with BRVO (N=455) were randomized (2:2:1) to RBZ (n=183), RBZ+laser (n=180) or laser (n=92) alone. In CRYSTAL, all patients with CRVO (N=357) were assigned to receive RBZ treatment. In both studies patients received three loading dose injections followed by monthly RBZ until best corrected visual acuity (BCVA) stabilization; thereafter on a PRN basis until M12, when monitoring frequency could also be reduced. The primary endpoint was mean change in BCVA from baseline at M6 (with superiority assessment vs laser, BRIGHTER) and M12 (CRYSTAL)
Results: In both the studies, almost 20% had disease duration of >12M, and approximately a quarter had ischemia. In BRIGHTER, at M6, mean BCVA letter gain was 14.8/14.4/6.0 in RBZ/RBZ+laser/laser, with an average of 4.8 (RBZ) and 4.5 (RBZ+laser) injections. Mean BCVA letter gains by baseline VA subgroups (≤39/40-59/≥60) were, 20.9/19.5/11.6 (RBZ); 19.5/16.8/11.1 (RBZ+laser); 18.7/11.4/-1.4 (laser). Gains by prior duration of BRVO (≤12/>12 months) were, 16.4/8.4 (RBZ); 15.0/11.5 (RBZ+laser); 5.9/7.1 (laser), and gains by baseline ischemic/non-ischemic status were, 14.3/11.9 (RBZ); 14.4/11.8 (RBZ+laser); 9.2/2.7 (laser; Fig. 1).
In CRYSTAL, at M12, mean BCVA improved by 12.3 letters with a mean of 8.1 injections; mean BCVA gains by baseline VA subgroups (≤39/40-59/≥60) were, 18.0/12.7/8.9 letters (Fig. 2). Gains by prior duration of CRVO (<3/ ≥3-<6/≥6-<9/≥9 -<12/ ≥12 months) were, 13.4/12.1 /9.1/13.8/10.0 letters, and gains by ischemic/non ischemic status were, 11.6 /12.1 letters
Conclusions: These findings support that patients with BRVO and CRVO can be effectively managed by individualized RBZ dosing regimen. In both studies, patients with lower baseline VA had higher BCVA gains. Patients with shorter duration of disease (<12M) benefited more from RBZ treatment than those with longer duration of BRVO and CRVO; RBZ treatment provided similar VA gains in both ischemic and non-ischemic eyes
Commercial Relationships: Sanjeewa Wickremasinghe, Novartis (F), Novartis (R), Bayer Healthcare (C)
Support: Travel grants - Novartis; Speaker fees- Novartis, Bayer Healthcare
Clinical Trail: BRIGHTER NCT01599650; CRYSTAL NCT01535261

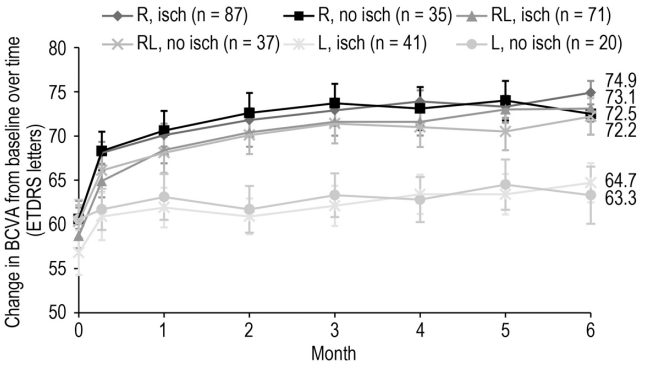


Figure 1, BRIGHTER, Absolute change in BCVA from baseline based on ischemic status

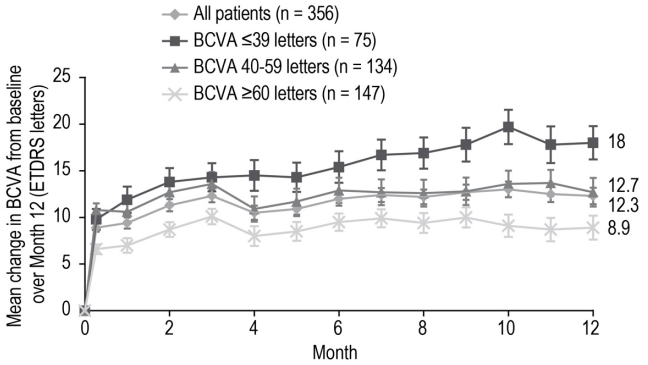


Figure 2, CRYSTAL, Mean change in BCVA from baseline over 12 months

280-2

Effects of Ranibizumab in Zonel and Zone II Retinopathy of Prematurity patients

Yi Chen [1] Jing Feng [1] Xiaoxin Li [1] Jianhong Liang [1] Hong Yin [1]
1. Eye Department, Peking Universtiy People's Hospital, Beijing, China.

Purpose: To evaluate the effectiveness and complications associated with the use of ranibizumab in the treatment of zone I and zone II retinopathy of prematurity (ROP).
Methods: Data from patients who had received intravitreal ranibizumab (IVR) injections in Peking University Peoples Hospital for the treatment of ROP from July 2012 to December 2013 were collected. The main outcome measures were the regression of ROP and the complications that were associated with the IVR injections
Results: In total, 151 eyes from 85 patients (56 male and 29 female) were analyzed. The mean birth weight was 1438.6 ± 334.5 g, mean gestational age was 30.1 ± 2 weeks, mean age at the time of intervention was 37.0 ± 6.2 gestational weeks (range, 32-45 weeks), mean follow-up was 4.9 ±

3.3 months (range, 1.4-20.8 months), After receiving IVR injections, 120 eyes (79.5%) exhibited ROP regression after single injection. 26 eyes (17.2%) required additional laser treatment for ROP regression after the absence of a positive response to the IVR injections. 5 eyes (3%) progressed to stage 4 ROP and required vitrectomies to reattach the retinas. 50 of 120 eyes which were regressed after single IVR had recurrence of ROP and need additional laser or additional IVR. All of the eyes (100%) had attached retinas after the various treatments that they received. No notable systemic complications related to the IVR injections were observed.
Conclusions: IVR injection seems to be an effective and well-tolerated method of treating zone I and zone II ROP. Recurrence of ROP is common and long-term follow up may need. Long-term effects and side effects may be assessed in future prospective randomized trial.
Commercial Relationships: Yi Chen, None; Jing Feng, None; Xiaoxin Li, None; Jianhong Liang, None; Hong Yin, None

281-3

Predictors for the Development of Referral-Warranted Retinopathy of Prematurity in the Telemedicine Approaches to Evaluating of Acute-Phase ROP (e-ROP) Study

Graham Quinn [1]
1. The Children's Hospital of Philadelphia, philadelphia, PA, United States.

Study Group: e-ROP Cooperative Group

Purpose: To determine predictive factors for the development of referral-warranted ROP (RW-ROP)
Methods: Secondary analysis was performed of the results of 979 infants with birth weight (BW) of <1251g who did not have RW-ROP in either eye at the first e-ROP examination and who had at least one subsequent ROP examination performed by a study-certified ophthalmologist. The infants were participants in the NEI sponsored "Telemedicine Approaches to Evaluating Acute-Phase ROP (e-ROP)" study, a prospective cohort observational study conducted in 13 clinical centers in the US and Canada. The main outcome measure is the incidence of RW-ROP (defined as presence of plus disease, Zone I ROP, or ROP stage 3 or greater in either eye and based on the clinician's diagnostic examination) and associations with predictive factors.
Results: Among 979 infants without RW-ROP at first study-related eye exam (median post-menstrual age, 33 weeks, range, 29 to 40 weeks) who underwent at least two eye exams, 149 (15.2%) developed RW-ROP. In a multivariate model, significant predictors [odds ratio (95% confidence interval)] for RW-ROP were, male sex [1.80 (1.13 – 2.86) vs. female], nonblack race [2.76 (1.50 – 5.08) for White vs. Black and 4.81 (2.19-10.6) for other vs. Black], low BW [5.16 (1.12 – 7.20) for ≤500g vs. >1100g], low gestational age [9.79 (3.49 – 27.5) for ≤24 weeks vs. ≥28 weeks], number of quadrants with preplus disease [7.12 (2.53 – 20.1) for 1-2 quadrants and 18.4 (4.28 – 79.4) for 3-4 quadrants vs. no preplus], stage 2 ROP [4.13 (2.13 – 8.00) vs. no ROP], presence of retinal hemorrhage [4.36 (1.57 – 12.1) vs. absence], need for respiratory support [4.99 (1.89 – 13.2) for need of controlled mechanical ventilator, 11.0 (2.26 – 53.8) for need of high frequency oscillatory ventilation vs. no respiratory support], and slow weight gain [2.44 (1.22 – 4.89) for weight gain ≤12g/day vs. >18g/day]. These characteristics predicted development of RW-ROP significantly better than BW and gestational age (area under ROC curve=0.88 vs. 0.78, p<0.0001).
Conclusions: When controlling for very low BW and prematurity, presence of preplus disease, stage 2 ROP, retinal hemorrhage, and need for ventilation at time of first study-related eye exam were strong independent predictors for RW-ROP. These predictors may help identify infants in need of timely eye examinations.
Commercial Relationships: Graham Quinn, None
Support: NEI Cooperative Agreement EY017014

282-4

Prosthetic vision rehabilitation, What we have learned in a few short years, and how much more can be done

Gislin Dagnelie [1] Pamela Jeter [1] Duane Geruschat [1] Robert Massof [1] Michael Barry [2] Judith Goldstein [1] James Deremeik [1] Olukemi Adeyemo [1]
1. Ophthalmology, Lions Vision Ctr, Johns Hopkins University, Baltimore, MD, United States. 2. Biomedical Engineering, Johns Hopkins University, Baltimore, MD, United States.

Purpose: To present approaches to rehabilitation for retinal implant recipients; lessons learned through self-report and assessment by rehabilitation experts; and implications for a Prosthetic Low Vision Rehabilitation (PLoVR) curriculum.
Methods: We combined three elements as the basis of our curriculum, A self-report questionnaire for individuals with ultra-low vision (ULV) through rating of items requiring minimal functional vision; an expansion of classic blindness and low vision rehabilitation approaches towards activities with minimal visual requirements; and a Functional Low Vision Observer Rated Assesssment (FLORA) to arrive at a semi-formal rating of Argus II wearer's visual performance. Argus II clinical trial participants played a critical role in each of these 3 developments.
Results: The psychometric properties of the ULV questionnaire were established as highly reliable in a population of 85 ultra-low vision individuals. A single vision dimension was sufficient to explain 87% of the variance in the data, regardless of the origin of vision loss (congenital vs. acquired) or the nature of ULV (prosthetic vs. native). Visual skills training in Argus II recipients was found to be most successful in individuals with good prior blindness skills, high motivation levels, and multiple goals.
Conclusions: Thus far we have only established elements of the PLoVR curriculum, and have limited our study population with those who have native ULV and Argus II clinical trial participants. The results obtained in Argus II patients give us confidence that the methodology can be expanded to other forms of prosthetic/restored visual ability.
Commercial Relationships: Gislin Dagnelie, Second Sight Medical Products (F), Second Sight Medical Products (C), Second Sight Medical Products (P), QLT Inc (F), QLT Inc

(C); Pamela Jeter, None; Duane Geruschat, Geruschat Low Vision Consulting, LLC (I), Second Sight Medical Products (C), Spark, Inc (C); Robert Massof, Emerald Events (I); Michael Barry, QLT Inc (C); Judith Goldstein, None; James Deremeik, None; Olukemi Adeyemo, None
Support: NIH grant R01 EY021220

283-5

Prototype suprachoroidal retinal prostheses, a preliminary clinical trial of three patients

Penelope J. Allen [1, 2] Jonathan Yeoh [1, 2] Robert Briggs [2, 3] Mark McCombe [1, 2] Wilson Heriot [1, 2] Lauren Ayton [1] Chi Luu [1] Anthony Burkitt [2] Robert Shepherd [4, 2] Robyn Guymer [1, 2]

1. Macular Research Unit, CERA, Melbourne, VIC, Australia. 2. Surgical, Bionic Vision Australia, Melbourne, VIC, Australia. 3. ENT, Royal Victorian Eye and Ear Hospital, Melbourne, VIC, Australia. 4. Bionics Institute, Melbourne, VIC, Australia.

Study Group: Bionic Vision Australia Consortium

Purpose: To determine whether implantation and electrical stimulation of a retinal prosthesis in the suprachoroidal space in patients with retinitis pigmentosa is surgically safe and efficacious.
Methods: Three patients with retinitis pigmentosa were recruited from a database of such patients in the Cente for Eye Research Australia, to enter a clinical trial of a prototype retinal prosthesis to be placed in the suprachoroidal space and then electrically stimulated for a period of eighteen months.
Results: The surgical implantation of the devices was uncomplicated in all three patients. However postoperatively all three patients developed suprachoroidal haemorrhage and in one case, vitreous haemorrhage. This cleared spontaneously in all three patients.
Over the eighteen months of the trial no ocular complications developed and the trial was extended. The only complication noted was infection around the percutaneous connector in two of three patients.
The devices have remained stable with 100% of the electrodes functioning in all three patents with no lead wire breakages or loss of electrode function.
Stimulation of the devices enabled reliable phosphenes to be generated for all three patients within safe charge limits and phosphene maps have been generated. Functional vision has also been assessed on activities of daily living, orientation and mobility tasks.
Conclusions: Implantation of a retinal prosthesis in the suprachoroidal space is surgically safe and the device remains stable over at least an eighteen month period. No ocular complications developed in our three patients and no further ocular surgery was required during this period. Stimulation resulted in reliable phosphenes within safe charge limits.
Assessment of functional vision resulted in better results for the device switched "ON" and showed that the device is useful for navigational vision.
Commercial Relationships: Penelope Allen, Bionic Vision Australia (P); Jonathan Yeoh, None, Bioinic Vision Australia (P); Robert Briggs, None; Mark McCombe, Bionic Vision Australia (P); Wilson Heriot, None; Lauren Ayton, None; Chi Luu, None; Anthony Burkitt, Bionic Vision Australia (P); Robert Shepherd, Bionic Vision Australia (P); Robyn Guymer, None
Support: ARC Special Research Initiative Grant
Clinical Trail: NCT01603576

284-6

Bionic eye research, retinal ganglion cell responses to electrical stimulation with temporal patterns resembling light-evoked spike trains

Michael R. Ibbotson [1, 2] Raymond C. Wong [1] David J. Garrett [3] David B. Grayden [4] Shaun L. Cloherty [1]

1. National Vision Research Institute, Australian College of Optometry, Melbourne, VIC, Australia. 2. Optometry and Vision Science, University of Melbourne, Melbourne, VIC, Australia. 3. Department of Physics, University of Melbourne, Melbourne, VIC, Australia. 4. Department of Electrical and Electronic Engineering, University of Melbourne, Melbourne, VIC, Australia.

Purpose: People with degenerative retinal diseases such as retinitis pigmentosa lose most of their photoreceptors but retain ~30% of their retinal ganglion cells (RGCs). Retinal prostheses aim to bypass the lost photoreceptors and restore vision by directly stimulating the surviving RGCs. In most studies, electrical stimuli have consisted of electrical pulse trains delivered at fixed frequencies. These stimuli do not have the statistics of natural vision, potentially compromising their ability to convey meaningful information. Here we test whether electrical stimulation of alpha and beta RGCs can evoke neural spike trains with statistics resembling normal light responses.
Methods: Whole-cell patch clamp recordings were made from individual cat RGCs *in vitro*. We recorded the responses of cells to short sequences of visual stimulation. These responses were converted to trains of electrical stimulation that were presented to the same cell via an epiretinal stimulating electrode. We quantified the efficacy of the electrical stimuli and the latency of the responses.
Results: Spikes were evoked with sub-millisecond latency. Median response latencies were 0.55 ms and 0.75 ms for ON (n = 8) and OFF (n = 6) alpha cells, respectively, and 0.5 ms and 0.6 ms for ON (n = 7) and OFF (n = 4) beta cells. The mean stimulus efficacy was 0.79 for alpha-ON and 0.97 for alpha-OFF cells. Median stimulus efficacy for beta cells was 0.13 for ON cells and 0.31 for OFF cells. For beta cells evoked spikes were followed by periods of inhibition, preventing responses to subsequent electrical pulses.
Conclusions: These data demonstrate that meaningful spike trains, resembling normal responses of RGCs to visual stimulation, can be reliably evoked by epiretinal prostheses in alpha RGCs. This suggests that the reduced efficacy of electrical stimulation at high frequency, as previously reported, is not a significant challenge to retinal implants. However, this is not the case for beta RGCs.
Commercial Relationships: Michael Ibbotson, None; Raymond Wong, None; David Garrett, None; David Grayden, None; Shaun Cloherty, None
Support: Australian Research Council

Cornea 4

Moderators

Shizuya Saika
Wakayama Medical University School of Medicine, Wakayama, Wakayama, Japan
Naoyuki Maeda
Osaka University Graduate School of Medicine, Suita, Osaka, Japan

285-1

Can Biomarkers predict the treatment outcomes of Crosslinking in Keratoconus?

Natasha Pahuja [1] Dr Rohit Shetty [1] Arkasubhra Ghosh [2] Vishal Arora [4] Abhijit Roy [2] Harsha Rao [3]
1. CORNEA REFRACTIVE, NARAYANA NETHRALAYA, bangalore, India. 2. Grow lab, NARAYANA NETHRALAYA, Bangalore, India. 3. L V Prasad eye institute, Hyderabad, India. 4. NARAYANA NETHRALAYA, Bangalore, India.

Purpose: To investigate the biomarkers using gene expression analysis in Keratoconus patient corneal epithelium in order to understand the deregulated pathways that may drive pathophysiology of the disease and correlate it to the clinical outcome after crosslinking
Methods: Gene expression analysis for Matrix Metalloproteinase9 (MMP9), interleukin 6 (IL6), Lysyl Oxidase (LOX) and collagen (COLIVA1 and COlIA1) was performed in corneal epithelium from 30 KC subjects undergoing corneal crosslinking using quantitative PCR. These patients were followed up to document the change in mean keratometry (Km), spherical equivalent and corrected distant visual acuity (CDVA) at 1, 3 and 6 month duration. The levels of these biomarkers were correlated with clinical parameters and treatment outcome to evaluate their prognostic potential
Results: Collagen related genes and collagen crosslinker LOX mRNA levels were significantly reduced in KC patients, which was also confirmed by IHC (Immunohistochemical analysis of Lox and ColIVA1). Clinical outcome at 6 months showed statistically significant improvement in CDVA in patients that correlated with statistically significant, higher levels of LOX and COlIA1. Moreover patients with higher levels of pre-operative COLIVA1 showed significant flattening as reflected by postoperative Km compared to those with lower levels of this basement membrane protein. These data were analysed using a multivariate linear regression model (R^2=0.8)
Conclusions: The improved CDVA is possibly related to remodeling of the cornea post surgery causing change in corneal aberrations. Biomarkers can be studied to predict the clinical outcome post surgery. Patients with higher levels LOX and COlIA1 have a significant better CDVA post crosslinking. Additionally COLIVA1, secreted by the basement membrane of corneal epithelium may have a role in predicting the clinical outcome of crosslinking thus of significant prognostic value.
Commercial Relationships: Natasha Pahuja, None; Dr Rohit Shetty, None; Arkasubhra Ghosh, None; Vishal Arora , None; Abhijit Roy, None; Harsha Rao, None
Clinical Trail: NCT01746823

286-2

Quantitative Analysis of Collagen Lamellae in the Normal and Keratoconic Human Cornea by Second Harmonic Generation Imaging Microscopy

Naoyuki Morishige [1] Ryu-taro Shin-gyou-uchi [1] Hiroya Azumi [1] Hiroaki Ohta [1] Koh-hei Sonoda [1]
1. Ophthalmology, Yamaguchi University, Ube, Japan.

Purpose: To characterize the structural properties of collagen lamellae in the normal and keratoconic human corneal stroma, we measured their width and angle relative to Bowman's layer (BL).
Methods: Thirteen normal and four keratoconic corneas were examined. Collagen lamellae in tissue blocks from the central cornea were visualized by second harmonic generation imaging microscopy. Images obtained in 1-μm steps from BL to Descemet's membrane (DM) were subjected to three-dimensional reconstruction. The reconstructed data sets were divided into 10 layers of equal depth (L1–L10) for analysis. The width of lamellae adherent to BL (L0) was also determined.
Results: For the normal cornea, the width (mean ± SD) of collagen lamellae was 6.5 ± 1.7 μm at L0, decreased to 4.3 ± 1.3 μm at L1, and then increased gradually with progression toward DM to 122.2 ± 34.5 μm at L10, whereas the angle of lamellae was 20.9 ± 5.4 degrees at L1 and decreased initially to 10.6 ± 3.2 degrees at L2 before declining gradually to 2.7 ± 2.2 degrees at L10. The width and angle of collagen lamellae in the keratoconic cornea were significantly larger at deep stroma and smaller at anterior stroma, respectively, relative to those in the normal cornea.
Conclusions: In the normal human cornea, collagen lamellae adjacent to BL are narrow and form a steep angle with BL, whereas they increase in width and their angle relative to BL flattens with progression toward DM. These properties of collagen lamellae are altered in keratoconus and are likely related to abnormalities of corneal shape.
Commercial Relationships: Naoyuki Morishige, None; Ryu-taro Shin-gyou-uchi, None; Hiroya Azumi, None; Hiroaki Ohta, None; Koh-hei Sonoda, None

287-3

Predicting post Photorefractive Keratectomy Corneal Haze through Analysis of Corneal Gene Expression Patterns

Vishal Vohra [1] Dr Rohit Shetty [1] Arkasubhra Ghosh [2] Natasha Pahuja [1]
1. Cornea and External eye disease , Narayana Nethralaya, Bangalore, India. 2. GROW Research Laboratory, Narayana Nethralaya Foundation, Bangalore, India.

Study Group: Narayana Nethralaya Study group

Purpose: Subepithelial corneal haze is a complication of Photorefractive keratectomy (PRK). Our aim is to study biomarkers involved in development of corneal haze by

gene expression analysis of corneal epithelium of patients undergoing PRK.
Methods: Corneal epithelium of patients undergoing PRK was collected intraoperatively. Epithelium of 4 eyes of 2 patients who developed haze postoperatively and that of 6 eyes of 3 age matched controls was analysed. Quantitative real-time PCR was performed for inflammatory markers, corneal structure genes, fibrosis associated genes and regulators of signaling cascade. Gene expression microarrays were performed for the mRNA samples.
Results: Mean age of cases and controls was 26 and 28 years respectively. Mean spherical equivalent of cases and controls was -3.37D &- 2.38D respectively. Gene expression analysis showed that Collagen I and Collagen IV were reduced in haze patients. TIMP1 showed a reducing trend along with MMP2 & 14. Inflammatory factor TNF α was elevated in haze patients, but IL6 and IL1 did not show appreciable changes. Regulators of signaling cascades EGFR and Wnt3a were reduced in haze patients. Global expression patterns in corneal epithelium were further analyzed by microarrays.
Conclusions: Development of haze is a multifactorial process that depends on molecular status of corneal epithelium prior to surgery. These initial data demonstrate that pre-screening of patients based on their corneal biomarker status may help identify patients prone to develop haze. The molecular processes underlying these deregulated biomarkers may be targets for management of post-PRK corneal haze.
Commercial Relationships: Vishal Vohra, None; Dr Rohit Shetty, None; Arkasubhra Ghosh, None; Natasha Pahuja, None

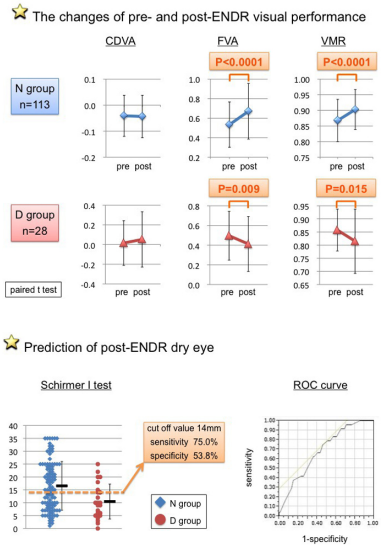
288-4

The evaluation of functional visual acuity after nasolacrimal duct recanalization

Tomoyuki Kamao [1] Atsushi Shiraishi [1] Naomi Takahashi [2] Yuichi Ohashi [1]

1. Ophthalmology, Ehime University Graduate School of Medicine, Toon, Ehime, Japan. 2. Ophthalmology, Uwajima City Hospital, Uwajima, Ehime, Japan.

Purpose: Dry eye can develop after nasolacrimal duct recanalization and is often severe enough to require treatment using lacrimal endoscope. We investigated the effect on visual performance using a functional visual acuity measurement system in patients performed endoscopic nasolacrimal duct recanalization (ENDR). And we determined whether development of dry eye could be predicted before ENDR.
Methods: One hundred forty-one ENDR were performed in 110 patients (male 34 in 27 patients, female 107 in 83 patients) presenting with nasolacrimal duct obstruction at an Ehime University Graduate School of Medicine from December 2010 to December 2013. The mean age at surgery was 70.6 years (range, 29-94 years). We measured corrected distance visual acuity (CDVA), functional visual acuity (FVA), visual maintenance ratio (VMR), tear meniscus height (TMH) with anterior segment optical coherence tomography and Schirmer I test values. The values were measured at pre-ENDR and 6 months after removing bicanalicular lacrimal pathway tubes (post-ENDR). Dry eye was diagnosed based on the results of post-ENDR examinations. Subjects were divided into the post-ENDR non-dry eye (N) and dry eye (D) groups, and each examination value was evaluated.
Results: Twenty eight eyes (19.8%) in 23 patients diagnosed dry eye after ENDR. No significant difference was detected between two groups in pre or post-ENDR in CDVA. FVA and VMR were significantly increased after ENDR in the N group ($p<0.0001$), but decreased in the D group ($p=0.009$, 0.015). TMH (N, $p<0.0001$, D, $p=0.007$) and Schirmer I test values (N, $p<0.0001$, D, $p=0.0002$) were significantly lower in post-ENDR than pre-ENDR. When pre-ENDR results in the two groups were compared, no significant differences were seen in CDVA, FVA, VMR or TMH, but Schirmer I test values were significantly lower in the D group than the N group ($p=0.003$). The sensitivity and specificity for dry eye diagnosis before ENDR were calculated to be 88.9 and 65%, respectively when a cut-off value was determined as ≦ 14 mm in Schirmer I test.
Conclusions: Our results suggest that ENDR can provide better vision related quality of life as well as relief from epiphora. However, ENDR can also disturbed visual performance if the patients developed dry eye after surgery. It should be carefully considered a potential dry eye before nasolacrimal duct recanalization.
Commercial Relationships: Tomoyuki Kamao, None; Atsushi Shiraishi, None; Naomi Takahashi, None; Yuichi Ohashi, None



289-5

Engineering a Light Attenuating Contact Lens

Farah Shareef [1] David Szlachta [1] Genesis Contreras [1] Andrew Chen [1] Dimitri Azar [2,1] Michael Cho [1]

1. Bioengineering, University of Illinois at Chicago, Chicago, IL, United States. 2. Ophthalmology and Visual Sciences, University of Illinois at Chicago, Chicago, IL, United States.

Purpose: Diminished iris functionality from trauma induced damage or congenital iris defects causes a loss of controlled light exposure to the eye. Patients with permanent iris damage experience poor vision caused by photophobia, glare and haloes. Commercial artificial irises have severe post-implant complications and lack a dynamic response to light. A novel light attenuating contact lens

that mimics the functionality of the natural iris was developed using DEA, a photo-responsive material, within a biocompatible polymer matrix. In response to blue light, DEA is activated to change opacity and decrease light transmission.

Methods: DEA powder dissolved in acetone was combined with polydimethylsiloxane (PDMS, Sylgard 184, Sigma) to form composite films subjected to a 4-day wash cycle to create the photo-responsive contact lens. Optical properties such as activation and reversal times, and percent light transmission were measured using a UV/Vis Spectrophotometer for wavelengths 320-720nm. In vitro cell culture experiments with indirect exposure were conducted for up to 1 week. Cells stained with live/dead viability assay were imaged using a confocal microscope at days 1, 3, and 7. Comparison of wash cycle water to water with known DEA concentrations approximated potential leaching.

Results: Our photo-responsive contact lens contained 0.025 to 0.4 weight percent DEA uniformly distributed in PDMS. Optical properties indicated activation of DEA by blue light, and response and reversal times within 1 second. Wavelength scans showed up to 40% attenuation of UV and visible light. A 92% cell viability rate was calculated for cells exposed to our contact lens. Over 1 week, normal morphology and proliferation was observed with no significant difference in survival of control and experimental cultures. Leaching studies determined less than 0.5% DEA leached out of our product.

Conclusions: The combination of DEA within a PDMS polymer matrix provides a new design for our light attenuating contact lens. Quick reversible activation to decrease light transmission based on incident light allows our contact lens to alleviate the adverse symptoms of decreased iris function. In vitro cell experimentation established biocompatibility. Further studies determined minimal DEA leaching, thus confirming these findings. Our photo-responsive contact lens mimics natural iris functionality and may provide an improved treatment option for patients with permanent iris damage.

Commercial Relationships: Farah Shareef, None; David Szlachta, None; Genesis Contreras, None; Andrew Chen, None; Dimitri Azar, None; Michael Cho, None

290-6

Compare Pigment Location, Surface Roughness and the Dynamic Coefficient of Friction (dCOF) of 5 Different Limbal Ring Hydrogel Contact Lenses

Charis Lau [1] Kathrine Osborn-Lorenz [1] Meredith Jansen [1] Danielle Boree [1] Samuele Tosatti [2] Lenora Copper [1]

1. Johnson & Johnson Vision Care, Inc, Jacksonville, FL, United States. 2. SuSoS AG, Dubendorf, Switzerland.

Purpose: Depending on manufacturing methods, limbal ring contact lenses (CLs) can have the pigment located either on the lens surface or enclosed within the lens. The surface characteristics and the differences between the non-pigmented and the pigmented regions of 5 limbal ring CLs, [1-Day Acuvue® Define® with LACREON® etafilcon A with PVP 58% (1DAD), Eye Coffret 2-HEMA, EGDMA 38% (ECO), Naturelle™ hilafilcon B 59% (NAT), Lacelle® 1-day hefilcon A 42% (LC1), and Lacelle® Colors 2-week hefilcon A 42% (LC2)] were compared.

Methods: Scanning Electron Microscopy (SEM) was used to determine the location and depth of pigment particles. Atomic Force Microscopy (AFM) was used to determine the surface roughness of each lens in root mean square (RMS) units at 2 different lens locations, central clear (CC) and peripheral pigmented (PP) on either the front-surface (FS) or back-surface (BS) as applicable. The lubricity (dCoF) was determined with a Basalt-MUST micro-tribometer. A linear mixed model for repeated measures was used for the analysis of the RMS and dCOF to compare all lenses.

Results: 1DAD had pigment particles enclosed at an average depth of 10.4µm (range, 0.6-28.9) below the FS. ECO, NAT, LC1 and LC2 had depths and ranges of 2.8(0.5-5.6), 1.3(0.0-1.7), 1.9(0.4-4.9) & 1.6(0.1-5.4) respectively. The mean RMS surface roughness (nm) between CC & PP regions were 5.5 vs. 9.5 for et-L, 4.5 vs. 54.6 for ECO, 11.5 vs. 30.5 for NAT, 6.4 vs. 45.0 for LC1 and 12.6 vs. 46.8 for LC2 respectively. The RMS difference between the 2 regions were significant for all lens tested ($p<0.001$). ECO, LC1, & LC2 had significantly rougher pigmented surface than both 1DAD ($p<0.001$) and NAT ($p<0.012$). NAT was significantly rougher than 1DAD ($p<0.001$). The average dCoF for the CC & PP regions were 0.024 vs. 0.016 for 1DAD, 0.149 vs. 0.581 for ECO, 0.029 vs. 0.065 for NAT, 0.049 vs. 0.574 for LC1, and 0.105 vs. 0.547 for LC2. 1DAD & NAT had significantly lower dCoF ($p<0.001$) in the pigmented regions compared to the other lenses. 1DAD had significantly lower dCOF than NAT ($p<0.001$).

Conclusions: Pigment affects lens surface roughness and lubricity. 1DAD with pigment enclosed had the least surface roughness and the lowest dCoF compared to other lenses.

Commercial Relationships: Charis Lau, Johnson & Johnson Vision Care (E); Kathrine Osborn-Lorenz, Johnson & Johnson Vision Care, Inc (E); Meredith Jansen, Johnson & Johnson Vision Care, Inc (E); Danielle Boree, Johnson & Johnson Vision Care, Inc (E); Samuele Tosatti, SuSos AG (E); Lenora Copper, Johnson & Johnson Vision Care, Inc (E)

Glaucoma 1

Moderators

Craig Crosson
The Medical University of South Carolina, Charlston, SC, United States
Tomomi Higashide
Kanazawa University, Graduate School of Medical Science, Kanazawa, Ishikawa, Japan

379-1

Evaluation of vision-specific quality of life using binocular visual field in glaucoma patients

Shiho Kunimatsu-sanuki [1] Kazuko Omodaka [1] Ikumi Takatsu [1] Mineko Ono [2] Yoshimi Suzukamo [2] Toru Nakazawa [1]
1. Ophthalmology, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan. 2. Physical Medicine and Rehabilitation, Tohoku University Graduate School of Medicine, Miyagi, Japan.

Purpose: To investigate the usefulness of binocular visual field in the evaluation of vision-specific quality of life (QOL) in glaucoma patients.
Methods: Fifty-five glaucoma patients from the outpatient clinic of Tohoku University were included. The 25-item National Eye Institute Visual Function Questionnaire was used to evaluate patients' QOL (VFQ25 score). Visual field testing was performed with the Humphrey Field Analyzer 24-2 SITA-S program (HFA 24-2). The eye with better mean deviation (MD) was defined as the better eye. We calculated the integrated visual field (IVF) by merging the two results from the monocular HFA 24-2 test, using the patients' best point-by-point monocular sensitivity, and evaluated the mean sensitivity of the IVF within the superior and inferior hemifields. The relationships between VFQ25 score, visual acuity (VA), better- and worse-eye MD with the superior and inferior IVF parameters were determined with Spearman's rank correlation coefficient.
Results: VFQ25 score correlated significantly with better-eye VA (r=-0.40, P=0.002), worse-eye VA (r=-0.44, P=0.001), better-eye MD (r=0.31, P=0.020) and worse-eye MD (r=0.33, P=0.013). The strongest correlation was found between VFQ25 score and inferior IVF (r = 0.53, P<0.0001).
Conclusions: Inferior IVF was the most accurate predictors of QOL in glaucoma patients.
Commercial Relationships: Shiho Kunimatsu-sanuki, None; Kazuko Omodaka, None; Ikumi Takatsu, None; Mineko Ono, None; Yoshimi Suzukamo, None; Toru Nakazawa, None

380-2

Partial abrasion of inner retinal layers in frequent eye rubbing patients

Ritsuko Yamada [1, 2] Tadayuki Nishide [1] Kazuro Yabuki [3] Atsuya Takayama [4] Nobuhisa Mizuki [1]
1. Department of Ophthalmology, Yokohama City University School of Medicine, Yokohama, Japan. 2. Radioisotope Institute St. Marianna University School of Medicine, Kawasaki, Japan. 3. Department of Ophthalmology, Saiseikai Yokohama-shi Nanbu Hospital, Yokohama, Japan. 4. Department of Orhtopedics, Keisuikai Kanazawa Hospital, Yokohama, Japan.

Purpose: Sharma N, et al has stated that the commonly proposed pathogenesis includes the release of inflammatory mediators due to eye rubbing which may alter the corneal collagen and lead to corneal ectasis. McMonnies CW has reported that eye rubbing is some of the many ways of causing eye pressure spikes and eye-pressure spikes that are large, last a long time or occur frequently, may contribute to the progression of pressure-related eye diseases. Mechanical irritation may relate to the pathogenesis of various eye diseases. In this study, we performed imaging analyses of optical coherence tomography (OCT) in patients with frequent eye rubbing subjects.
Methods: The subjects are 262 eyes of 131 frequent eye rubbing patients (average age+/-SD, 65.4 +/- 18.9 year-old), who confessed bad habits of frequent eye rubbing, and showed OCT images of inner retinal layer abrasion. Comparative studies were performed by using 232 eyes of 116 patients with glaucoma (67.0+/-13.3 year-old), meeting the diagnostic criteria, as a positive control, then 118 eyes of 94 healthy controls (56.4+/-18.8 year-old) as a negative control. The OCT scanning were performed by using 3D OCT-2000(Topcon) including observations from 128 to 256 sequential images in macular regions followed by 3D images, then measurements retinal ganglion cell layer (RGCL), retinal nerve fiber layer (RNFL), and average macular thickness values. Statistical studies were examined by Mann-Whitney U-test. The study is adherent to the Declaration of Helsinki.
Results: The partial abrasion of inner retinal layer including internal limiting membrane, to the RNFL or to the RGCL in frequent eye rubbing patients were observed as OCT images. There were a significant thinning of average RGCL, total RNFL and average macular thickness in frequent eye rubbing subjects and glaucoma patients compared with healthy controls (frequent eye rubbing patients, p<0.0001, p<0.0008, p<0.0001, glaucoma patients, p<0.0001, p<0.0009, p<0.0001, respectively), then no significant differences between those in frequent eye rubbing patients and glaucoma patients.
Conclusions: It's suggested that mechanical irritation included by the eye rubbing may cause damage in inner retinal layers lead to RGCL, RNFL followed by macula thinning.
Commercial Relationships: Ritsuko Yamada, None; Tadayuki Nishide, None; Kazuro Yabuki, None; Atsuya Takayama, None; Nobuhisa Mizuki, None

381-3

Transcorneal electrical stimulation therapy for glaucomatous optic neuropathy

Nga Kwan B. Choy [1] Shiu Ming, Jimmy Lai [1] Wai Yip, Jacky Lee [1]

1. Faculty of Medicine, The University of Hong Kong, Hong Kong, Hong Kong.

Purpose: - To examine the efficacy of transcorneal electrical stimulation (TES) in enhancing the function of the optic nerve in glaucomatous optic neuropathy
- To document any side effects arising from TES to treat a chronic eye disease

Methods: Adult patients were recruited in one hospital in Hong Kong. Patients with bilateral glaucomatous optic neuropathy of similar degree, as determined by optic disc morphology, Humphrey visual field (VF) and/or optical coherence tomography (OCT) were recruited. Any form of glaucoma was included. One eye was randomized to receive TES (treatment arm) by drawing envelop, and the other eye would automatically be in the control arm.

Each patient received TES for a period of 30 minutes once per week over 26 weeks. The studied parameters, including visual acuity, intraocular pressure, anterior segment and posteior segment examination with microscopy, VF, OCT for retinal nerve fibre layer (RNFL) and electroretinography (ERG) using multifocal ERG, were measured once at baseline, every 3 months during the study period, and at 3 and 6 months after the last stimulation.

Outcome measures:

Primary outcome: VF, ERG signal

Secondary outcomes: RNFL thickness

Results: Forteen patients were enrolled into the study and three of them completed at least 3 months treatment and investigations at the time of report.

The 3 patients were from 63-76 years old, 2 males and 1 female.

Regarding the VF, all patients demonstrated improvement in at least one of the parameters (median deviation and visual field index) in the treatment eye at 3 months post-treatment as compared to the baseline. In 2 of the patients, both parameters improved. In contrast, in all the 3 patients, the fellow control eye did not show any improvement in any one of the VF parameters. (Table 1)

All 3 patients also showed improvement in at least 3 out of the 4 ERG signal parameters. (Table 2)

OCT findings were inconsistent.

There were no side-effects reported by any patient during the treatment.

Conclusions: Preliminary data on our glaucoma patients demonstrated potential benefit of TES in terms of improvement in VF and ERG signal. TES can be a promising novel treatment for glaucomatous optic neuropathy.

Further studies on the exact duration, pulse duration and current density to achieve maximal benefit would be neccessary.

Commercial Relationships: Nga Kwan Choy, None; Shiu Ming, Jimmy Lai, None; Wai Yip, Jacky Lee, None

Clinical Trail: UW 12-411

Visual field

		MD (dB)		VFI (%)	
		Baseline	3 months	Baseline	3 months
1	Treatment (L)	-12.49	-12.64	63	67
	Control	-17.33	-31.43	42	0
2	Treatment (R)	-10.30	-5.88	74	85
	Control	-6.92	-7.16	88	87
3	Treatment (R)	-4.30	-3.00	94	97
	Control	-12.85	-13.38	66	62

Table 1, Visual field results
MD, median deviation
VFI, visual field index

Electroretinography

Global Flash mfERG (100%)		DC amplitude (nV/deg²)				IC amplitude (nV/deg²)			
		Superior		Inferior		Superior		Inferior	
		Baseline	3 months	Baseline	3 months	Baseline	3 months	Baseline	3 months
1	Treatment (L)	12.4	8.8	10.4	14.4	9.7	13.1	12.8	14.2
2	Treatment (R)	7.9	10.5	6	8.1	17.1	19.5	13.7	19.7
3	Treatment (R)	1.6	1.8	1.2	4.9	3	3.3	5.5	4.6

382-4

Valproic Acid Protects Retinal Neurons from NMDA-Induced Neurotoxicity via Stimulation of Neuronal TrkB Receptors

Atsuko Kimura [1] Kazuhiko Namekata [1] Xiaoli Guo [1] Takahiko Noro [1] Chikako Harada [1] Takayuki Harada [1]

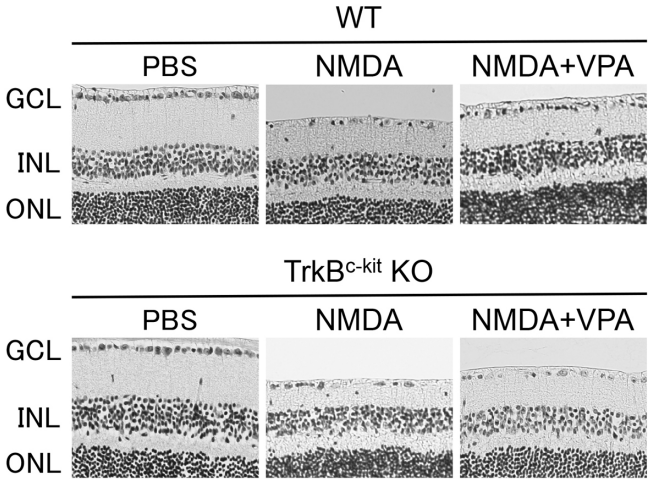
1. Visual Research Project, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan.

Purpose: Valproic acid (VPA) is widely prescribed for treatment of epilepsy, mood disorders and migraines. Recent evidence suggests that it exerts neuroprotective effects in neurodegenerative diseases including Alzheimer's disease. The aim of this study was to examine the neuroprotective effects of VPA against *N*-methyl-D-aspartate (NMDA)-induced neurotoxicity in the retina and elucidate underlying mechanisms.

Methods: C57 BL/6J and $TrkB^{c\text{-}kit}$ KO mice, whose TrkB, a receptor for BDNF, was deleted from RGCs, received intravitreal injection of PBS, VPA (75 mM), NMDA (1 mM) or NMDA+VPA (1 mM+75 mM). Retinal degeneration was monitored over 2 weeks *in vivo* using optical coherence tomography. Histological analyses of the retina were performed using haematoxylin and eosin staining, and retrograde labelling. Visual function was investigated by multifocal electroretinogram. Müller glial cells were stimulated with VPA (0.1 and 1 mM for 24 or 48 h) and processed for quantitative real-time PCR for determination of neurotrophic factor expressions. Oxidative stress was examined by detection of 4-hydroxy-2-nonenal protein and iNOS mRNA expressions.

Results: *In vivo* imaging revealed that VPA suppressed NMDA-induced retinal degeneration and histological analyses demonstrated that retinal cell death, particularly RGC death, is reduced with VPA treatment. Visual impairment caused by NMDA neurotoxicity was ameliorated in VPA-treated mice, indicating neuroprotective effects of VPA with functional significance. VPA stimulated upregulation of BDNF and NGF in Müller cells and the neuroprotective effects of VPA was significantly decreased in $TrkB^{c\text{-}kit}$ KO mice.

Furthermore, NMDA-induced oxidative stress levels were decreased in VPA-treated retinas.
Conclusions: We demonstrate that VPA protects retinal neurons from NMDA neurotoxicity and that VPA-mediated neuroprotection involves upregulation of neurotrophic factors in Müller cells and stimulation of neuronal TrkB signalling. We provide direct evidence for the role of neuronal TrkB using conditional knockout mice. Our findings emphasize the importance of glia-neuron cross-talk in neuroprotection and raise an interesting possibility that VPA may be effective for treatment of glaucoma.
Commercial Relationships: Atsuko Kimura, None; Kazuhiko Namekata, None; Xiaoli Guo, None; Takahiko Noro, None; Chikako Harada, None; Takayuki Harada, None
Support: Ministry of Education, Culture, Sports, Science and Technology of Japan Grant 26861479



VPA-mediated protection of cells in the ganglion cell layer (GCL) is suppressed in TrkB$^{c\text{-}kit}$ KO mice. INL, inner nuclear layer; ONL, outer nuclear layer.

383-5

Spermidine stimulates neuroprotection and optic nerve regeneration following optic nerve injury

Takahiko Noro [2, 1] Kazuhiko Namekata [2] Xiaoli Guo [2] Atsuko Kimura [2] Yuriko Azuchi [2] Tadashi Nakano [1] Hiroshi Tsuneoka [1] Takayuki Harada [2]
1. Ophthalmology, Jikei University School of Medicine , Tokyo, Japan. 2. Visual Science Project, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan.

Purpose: Glaucoma is a progressive optic neuropathy characterized by axonal injury, retinal ganglion cell (RGC) loss, and visual field defects. Polyamines, such as spermidine, are organic cations required for cell growth, cell differentiation, and synthesis of DNA, RNA, and proteins. Moreover, spermidine was reported to play key roles in mediating protection against oxidative damage. The purpose of this study was to evaluate the effects of spermidine on the neuroprotective effect and axon regeneration after optic nerve injury (ONI).
Methods: C57 BL/6J mice at 8 weeks old were used for creating an ONI model with surgical forceps. ONI mice were administered with or without spermidine (30mM) in drinking water. Retinal degeneration was monitored over 2 weeks *in vivo* using spectral-domain optical coherence tomography. Optic nerves were harvested on day14 after ONI. To estimate the total number of regenerating axons, axonal outgrowth was quantified by counting cholera toxin B positive axons beyond the injury site. Histopathological analyses of the retina was performed using haematoxylin and eosin staining. Phosphorylation of p38 mitogen-activated protein kinase (MAPK) in the retina was investigated by western immunoblot analyses and immunohistochemistry. The mRNA expression level of inducible nitric oxide synthase (iNOS) was determined by real-time PCR analyses.
Results: Sequential *in vivo* retinal imaging revealed that spermidine suppressed ONI-induced retinal degeneration and histological analyses demonstrated that the number of surviving RGCs in spermidine-treated mice was significantly higher than that in untreated mice. Immunoblot and immunohistochemical analyses revealed that spermidine significantly suppressed the phosphorylaion of p38 MAPK in RGCs. The iNOS mRNA expression level was significantly reduced with spermidine treatment. Furthermore, spermidine increased the number of regenerating axons in the ONI model.
Conclusions: Our results show that spermidine protects RGCs and increases the number of regenerating axons following ONI. Spermidine may protect RGCs by suppressing p38 MAPK-induced apoptosis and the production of iNOS in the retina. Our results suggest a possibility that spermidine treatment may be effective for glaucoma.
Commercial Relationships: Takahiko Noro, None; Kazuhiko Namekata, None; Xiaoli Guo, None; Atsuko Kimura, None; Yuriko Azuchi, None; Tadashi Nakano, None; Hiroshi Tsuneoka, None; Takayuki Harada, None

384-6

A common, protein-coding variant in the SIX6 gene confers POAG risk by altering DNA binding properties

Michael Hauser [1, 2] Joshua Schipper [6] Shelby Strickland [1] Megan Carnes [4] Yangfan Liu [5] Edwin Oh [5] Nicholas Katsanis [5] Allison Ashley-Koch [1] Raluca Gordan [3] R. R. Allingham [2]
1. Medicine, Duke University, Durham , NC, United States. 2. Ophthalmology, Duke University, Durham, NC, United States. 3. Biostatistics and Bioinformatics, Duke University, Durham, NC, United States. 4. Biology, North Carolina State University, Raleigh, NC, United States. 5. Cell Biology, Duke University, Durham, NC, United States. 6. Center for Genomic and Computational Biology, Duke Univsrsity, Durham, NC, United States.

Study Group: NEIGHBORHOOD Investigators

Purpose: We have recently shown that variants in *SIX6* are not only associated with POAG risk but also reduce optic nerve volume in a zebrafish complementation model and affect RNFL thickness in primary open angle glaucoma (POAG) cases. SIX6 is a homeobox protein that binds DNA, regulating the expression of multiple

downstream genes, thereby playing a major role in ocular development. *SIX6* is also expressed in adults, but little is known about its function there. In this study we examined functional DNA binding characteristics of a common coding variant, Asn141His (rs33912345). Loci that display differential binding are evaluated for association with POAG.

Methods: The DNA binding properties of Asn141 SIX6 and His141 SIX6 protein were tested *in vitro* using custom DNA microarrays. GST-tagged SIX6 protein is detected using fluorescent antibodies to the epitope tag. Binding affinity is determined by fluorescence intensity. Association of loci with POAG was evaluated through our published NEIGHBOR/GLAUGEN genome-wide association study.

Results: We first tested DNA binding of the two SIX6 isoforms using "Universal Protein Binding Arrays," which contain all possible 8-mers in multiple different sequence environments. These arrays showed a significant difference in DNA binding properties, as shown in Figure 1. The technical replicate assays of Asn141 binding demonstrate the high reproducibility of this assay. We subsequently designed a "Genomic Context Protein Binding Array" that contains 36-mers from the human genome that include the consensus SIX6 binding motifs in their natural genomic contexts. This array identified multiple loci that display large differences in DNA binding affinity between the two SIX6 isoforms. We are now testing these loci for association with POAG in the large NEIGHBOR/GLAUGEN genome-wide association study.

Conclusions: The common His141variant of SIX6 is strongly associated with POAG risk. Humans carrying this risk allele have reduced RNFL thickness, and thus a reduced number of retinal ganglion cells, highlighting a possible mechanism for increased POAG risk. We hypothesize that the altered DNA binding properties of SIX6 protein carrying the His141 amino acid substitution is mediating this reduction in retinal ganglion cells through transcriptional misregulation of downstream targets either developmentally or in adults.

Commercial Relationships: Michael Hauser, None; Joshua Schipper, None; Shelby Strickland, None; Megan Carnes, None; Yangfan Liu, None; Edwin Oh, None; Nicholas Katsanis, None; Allison Ashley-Koch, None; Raluca Gordan, None; R. Allingham, None

Support: NIH Grant EY023646

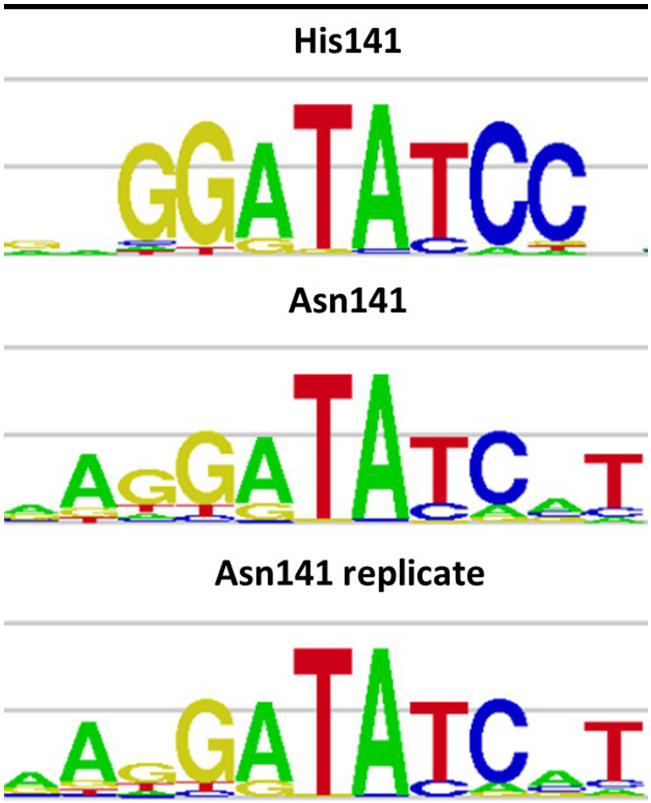


Figure 1. Binding sites for SIX6 protein isoforms.

Glaucoma 2

Moderators

Paul Kaufman
University of Wisconsin School of Medicine & Public Health, Madison, WI, United States
Yoshiaki Kiuchi
Hiroshima University, Hiroshima, Hiroshima, Japan

385-1

Role of the Renin-Angiotensin System in Trabecular Meshwork of Glaucoma

Erdal Tan Ishizuka [1] Atsuhiro Kanda [1] Yasuhiro Shinmei [1] Satoshi Kinoshita [1] Yoko Dong [1] Saori Inafuku [1] Yoshiaki Tagawa [1] Kousuke Noda [1] Susumu Ishida [1]
1. Department of Ophthalmology, Hokkaido University Graduate School of Medicine, Sapporo, Japan.

Study Group: Laboratory of Ocular Cell Biology and Visual Science

Purpose: The renin-angiotensin system (RAS), a known important controller of systemic blood pressure (circulatory RAS), plays distinct roles in the regulation of growth, inflammation and pathological conditions in various organs including the eye (tissue RAS). In glaucoma, increased resistance to aqueous humor drainage through the trabecular pathway consisting of trabecular meshwork (TM) causes the increase in intraocular pressure that results in damage to the retinal neurons. Polymorphisms in angiotensin II receptor gene have been reported to associate with the risk of glaucoma in the Japanese population. In this study, we investigated the role of RAS in TM cells.
Methods: Protein localization of RAS components [prorenin, (pro)renin receptor, angiotensinogen, angiotensin II type 1 receptor (AT1R)] in TM surgically excised from primary open-angle glaucoma and neovascular glaucoma patients were analyzed using immunohistochemistry. Gene expression levels (e.g., tight junction, matrix metalloproteinase) in human TM cell lines stimulated with angiotensin II (Ang II) or prorenin were analyzed by real-time PCR.
Results: (Pro)renin receptor and AT1R immunoreactivities were observed in TM from patients with glaucoma, co-localized with prorenin and angiotensinogen, respectively. Gene expressions of RAS components were detected in TM cell lines by reverse transcription PCR. Real time-PCR analysis revealed that Ang II or prorenin stimulation in human TM cell lines significantly upregulated mRNA expression levels of several genes, which were suppressed with pre-treatment of (pro)renin receptor or AT1R blocker.
Conclusions: Our data using clinical samples and *in vitro* experiments suggest that tissue RAS is associated with pathological events of glaucoma in trabecular meshwork.
Commercial Relationships: Erdal Tan Ishizuka, None; Atsuhiro Kanda, None; Yasuhiro Shinmei, None; Satoshi Kinoshita, None; Yoko Dong, None; Saori Inafuku, None; Yoshiaki Tagawa, None; Kousuke Noda, None; Susumu Ishida, None
Support: Creation of Innovation Centers for Advanced Interdisciplinary Research Areas Program, Takeda Science Foundation, and JSPS KAKENHI-24791823

386-2

Correlation Between Serial Scleral and Corneal Pneumatonometry

Debbie S. Kuo [1] Yvonne Ou [1] Bennie Jeng [2] Robert Bhisitkul [1] Jay M. Stewart [1] Jacque Duncan [1] Ying Han [1]
1. Ophthalmology, University of California, San Francisco, San Francisco, CA, United States. 2. Ophthalmology, University of Maryland, Baltimore, MD, United States.

Purpose: Significant corneal pathology or the presence of keratoprostheses can limit the use of standard techniques for intraocular pressure (IOP) measurements taken over the cornea. Therefore, we studied scleral pneumotonometry as an alternative to corneal pneumotonometry using serial measurements of corneal and scleral IOP over a wide range of IOPs in patients receiving intravitreal injections, which transiently increase IOP.
Methods: We recruited adult patients receiving intravitreal anti-VEGF injections from the UCSF Retina for this prospective, observational study between August and November 2013. Central corneal thickness was measured by pachymetry. Serial measurements of corneal and temporal scleral pneumatonometry (baseline, immediately and 10, 20 and 30 minutes post-injection) were collected. Correlation analysis and a Bland-Altman plot were used to evaluate reliability and agreement between scleral and corneal IOP. A linear mixed model was used to determine the relationship between measurements and perform covariate analyses.
Results: Thirty-three patients (mean age 74.1 ± 13.4), of whom 15% had glaucoma and 52% were pseudophakic, were included in the study. Scleral and corneal IOP by pneumatonometry was strongly correlated (r, 0.94, $p < 0.001$). Scleral IOP averaged 9.0 mmHg higher than corneal IOP (95% limits of agreement, -1.5 to 19.5 mmHg). A linear mixed model resulted in the following equation, scleral IOP = 0.97 x corneal IOP + 10.0. Age, central corneal thickness, laterality, glaucoma and lens status did not impact this relationship.
Conclusions: Scleral pneumatonometry is strongly correlated to corneal pneumatonometry, though biased toward higher values. Changes in scleral and corneal pneumatonometry demonstrated a nearly 1:1 linear relationship over a range of physiologically and pathologically relevant IOPs, showing that differences between serial scleral measurements reflect differences between serial corneal measurements. Therefore, in patients for whom corneal measurements are unreliable or unattainable, scleral pneumatonometry should be considered a useful tool for diagnosing and treating glaucoma to prevent blindness.
Commercial Relationships: Debbie Kuo, None; Yvonne Ou, None; Bennie Jeng, None; Robert Bhisitkul, None; Jay Stewart, None; Jacque Duncan, None; Ying Han, None
Support: NIH-NEI EY02162 Core Grant for Vision

Research & Research to Prevent Blindness unrestricted grant to UCSF Department of Ophthalmology

387-3

Outcomes of Combined subconjunctival with subscleral Ologen implant in glaucoma filtering surgery

Dewang Angmo [1] Talvir Siddhu [1] Meenakshi Wadhwani [1] Shreyas Temkar [1] Tanuj Dada [1]

1. Ophthalmology, Dr RP Centre for Ophthal Sciences, AIIMS , Delhi, New Delhi, India.

Purpose: To study the outcomes of combined subconjunctival with subscleral Ologen implant in glaucoma filtering surgery

Methods: A prospective interventional case series consisting of 23 patients(27 eyes), underwent fornix based trabeculectomy with insertion of ologen both subsclerally and subconjunctivally with low dose MMC (0.1mg/mlx1min). The inclusion criteria included patients(pts) with primary glaucoma aged ≥18yrs with IOP>21mmHg; advanced glaucomatous field defects on 30-2 SITA Standard Humphrey visual field (mean deviation worse than − 12 dB) &advanced cupping (≥0.9:1). Subjects with secondary glaucoma, aphakia/pseudophakia&previous operated surgery were excluded.

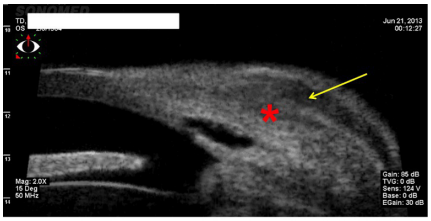
Pts were reviewed at 1wk, 1mth, 3mths, 6mths postoperatively & thereafter every 6mths for BCVA, applanation tonometry, bleb morphology &leaks, ASOCT&UBM of the bleb, antiglaucoma medications& any complications. Complete success was defined as IOP ≤15mmHg with no antiglaucoma medication& Qualified success as IOP≤15mmHg on antiglaucoma medications.

Results: The average age of pts was 46.22 ± 14.8yrs(18-68yrs). The Male:Female ratio was 17:6. The diagnosis were varied with 7pts having Juvenile open angle glaucoma, 4pts-Primary open angle glaucoma, 9pts-Primary angle closure glaucoma, 2pts-steroid induced glaucoma and 1pt with pigment dispersion glaucoma. The highest mean preop IOP was 38.30 ± 6.6mmHg. The average follow up was 15.22 ± 5.8mths. Postoperatively, IOP decreased to 12.38 ± 1.7mmHg;($p<0.0001$). Complete success was noted in 92.6% eyes (25/27), Qualified success in 7.4% (2/27) &there were no failures. The preop&postop BCVA in LogMAR was 0.30 ± 0.20 & 0.30 ± 0.18 ($p=0.31$). The average no. of ocular hypotensive medications used pre-operatively was 4.2 ± 0.51 &post-operatively 0.07 ± 0.27; ($p<0.0001$).

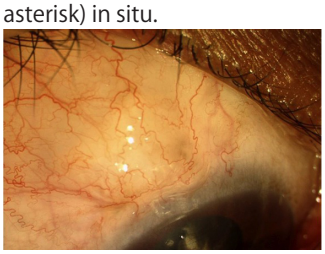
2 pts had shallow anterior chamber in initial postop day, which was reformed and subsequently patients did well. One pt had choroidal detachment, 2 pts required resutring of conjunctiva in initial postop days and 1 pt required needling.

Conclusions: Trabeculectomy with implantation of Ologen implant both subsclerally and subconjunctivally appears to offer encouraging results in terms of IOP control in primary adult glaucoma.

Commercial Relationships: Dewang Angmo, None; Talvir Siddhu, None; Meenakshi Wadhwani, None; Shreyas Temkar, None; Tanuj Dada, None



Postop UBM at 3mths showing well elevated bleb with subconjunctival ologen (yellow arrow)&subscleral ologen (red asterisk) in situ.



Postop clinical photograph at 12mths showing elevated bleb with mild vascularity.

388-4

Effects of ranibizumab on cultured human Tenon's fibroblast, An in vitro study

Siti Munirah [1] Sushil Kumar Vasudevan [1] Siti Hamimah S.Abdul Kadir [1] Zakaria Bannur [1] Jonathan Crowston [2]

1. Faculty of Medicine, Brain and Neuroscience Communities of Research, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia. 2. Centre for Eye Research Australia, Melbourne, VIC, Australia.

Purpose: Minimizing excessive scar formation at the filtering bleb, which are primarily mediated by human Tenon' s fibroblast (HTF) migration and proliferation has become the major determining factor for a successful trabeculectomy. The discovery of anti-Vascular Endothelial Growth Factors (Anti-VEGF) as a potential anti-fibrotic agent has opened a new window in wound healing management post-surgery. Even with numbers of published data reporting the effectiveness of these agents in improving the bleb morphology and maintaining ideal IOP, its mechanism of actions on HTF remains unclear. This study was conducted to understand the effects of ranibizumab on extracellular matrix components and growth factor expression by HTF.

Methods: The effect of ranibizumab on HTF proliferation and cell viability was determined using MTT assay (3-(4,5-dimethylthiazone-2-yl)-2,5-diphenyl tetrazolium). Ranibizumab at concentrations ranging from 0.01 to 0.5mg/ml were administered for 24, 48 and 72 hours in serum and serum free conditions. Supernatants and cell lysates from samples were further assessed for extracellular matrix component; collagen Type 1 and fibronectin and transforming growth factor-β1 and transforming growth factor - β 2 mRNA and protein level using quantitative real time polymerase chain reaction (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA).

Results: At 48-hours, ranibizumab at 0.5mg/ml, significantly induced cell death under serum-free conditions ($p<0.05$). Ranibizumab induced significant reduction of collagen Type 1 and TGF-β1 mRNA, but not fibronectin and TGF- β 2. On the other hand, expression of these four proteins does not seem to be affected by

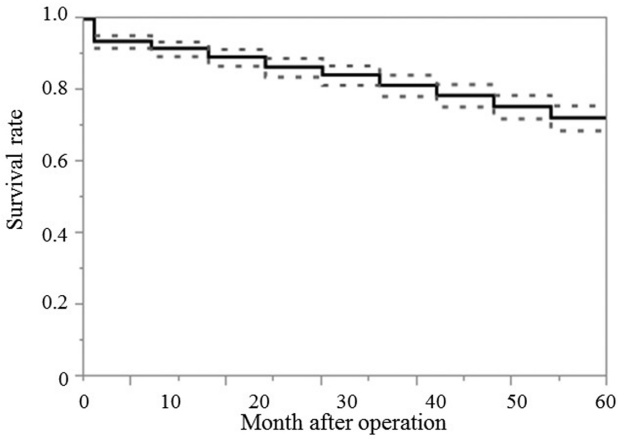
ranibizumab treatment.
Conclusions: We highlighted ranibizumab as a potential candidate in managing scarring following trabeculectomy. The discrepancy in all mRNA and protein level tested showed that ranibizumab might alter the wound healing through distinct molecular mechanisms. Considering numerous stimulatory and inhibitory factors released by HTF, we suggest the synergistic use of ranibizumab with other anti-fibrotic agent to minimize filtering bleb scar thus enhancing the success of trabeculectomy.
Commercial Relationships: Siti Munirah, None; Sushil Kumar Vasudevan, None; Siti Hamimah S.Abdul Kadir, None; Zakaria Bannur, None; Jonathan Crowston, None
Support: The Ministry of Science, Technology and Innovation (MOSTI), Malaysia.Project code, 100-RMI/SF 16/6/2.

389-5

Visual Impairment after Trabeculectomy with Adjunctive Mitomycin C and Its Risk Factors

Kenji Kashiwagi [1] Satoshi Kogure [1] Fumihiko Mabuchi [1] Tatsuya Chiba [1] Tetsuya Yamamoto [2] Yasuaki Kuwayama [3] Makoto Araie [4]
1. Ophthalmology, University of Yamanashi, Chuo, Japan. 2. Ophthalmology, University of Glfu, Gifu, Japan. 3. Ophthalmology, Fukushima Eye Clinic, Osaka, Japan. 4. University of Tokyo, Tokyo, Japan.

Purpose: To report the 5-year incidence of visual impairment after mitomycin C-augmented trabeculectomy and the risk factors of visual impairment as determined from the Collaborative Bleb-Related Infection Incidence and Treatment Study (CBIITS).
Methods: This is a prospective, observational cohort study. Among glaucoma patients registered to the CBIITS, patients with eyes meeting the following qualifications were eliminated from the study, having a bleb-related infection; an onset and/or deterioration of any diseases resulting in visual impairment with no relation to trabeculectomy during the follow-up period; eyes with < 3/60 for blindness analysis; eyes with < 20/60 for low vision analysis and visual acuity loss analysis.
Patients were followed up at 6-month intervals for 5 years. The entry data and follow-up data were analyzed to determine the incidence of blindness, low vision, and visual acuity loss during the follow-up period; risk factors for visual impairment were analyzed.
Results: A total of 950 eyes of 950 glaucoma patients or 725 eyes of 725 glaucoma patients were subjected to blindness analysis or low vision and visual acuity loss analysis, respectively. During the study period, visual acuity significantly deteriorated; 10.8% (95%CI, 9.0%-13.0%) of patients became blind, including 1.3% with no light perception, 12.6% (95% CI:10.5%-15.3%) judged as low vision, and 28.8% (95%CI, 25.6%-32.2%) judged as visual acuity loss. A statistical analysis revealed that glaucoma subtypes, poor pre-operative visual function, and post-operative complications are the main risk factors for visual impairment.
Conclusions: The incidence of visual impairment after mitomycin C-augmented trabeculectomy and its risk factors are presented. Ophthalmologists should consider these results before using mitomycin C-augmented trabeculectomy.
Commercial Relationships: Kenji Kashiwagi, None; Satoshi Kogure, None; Fumihiko Mabuchi, None; Tatsuya Chiba, None; Tetsuya Yamamoto, None; Yasuaki Kuwayama, None; Makoto Araie, None



Kaplan-Meier survival analysis showed the incidence of visual acuity loss of all subjects. Dot lines indicate upper and lower 95% confidential intervals.

390-6

Association of Lens Vault Related Parameters with Angle Closure

Monisha Nongpiur [1,2] Mingguang He [3] Tin Aung [1,2]
1. Glaucoma, Singapore Eye Research Institute, Singapore, Singapore. 2. Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore. 3. State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China.

Purpose: To investigate the relationship between lens vault (LV) related parameters (anterior vault [AV], relative anterior vault [rAV], and relative lens vault [rLV]) and angle-closure.
Methods: We recruited 2047 subjects aged 50 years or more from a community polyclinic. All participants underwent gonioscopy, A-scan biometry, and anterior segment optical coherence tomography (ASOCT, Visante, Carl Zeiss Meditec, Dublin, CA). Customized software (Zhongshan Angle Assessment Program, Guangzhou, China) was used to measure LV and anterior chamber depth (ACD) on horizontal ASOCT scans. LV was defined as the perpendicular distance between the anterior pole of the crystalline lens and the horizontal line joining the two scleral spurs. AV was calculated as ACD+LV; rAV was calculated by dividing the AV by axial length; and rLV was calculated by dividing the LV by AV. An eye was considered to have angle-closure if the posterior pigmented trabecular meshwork was not visible for >180° on non-indentation gonioscopy with the eye in the primary position.
Results: Complete data on 1464 subjects were available for analysis. Of these, 315 (21.5%) had angle closure. Significant differences between open-angles and angle-closure eyes were found for LV (0.39 ± 0.25mm vs 0.77 ±

0.19mm, p<0.001), AV (3.12 ± 0.17mm vs 2.98 ± 0.16mm, p<0.001), and rLV (0.124 ± 0.08 vs 0.260 ± 0.06, p<0.001), but no significant differences were found for rAV (0.129 ± 0.005 vs 0.129 ± 0.008, p=0.45). LV and rLV increased significantly with age (p for trend <0.001 for both), but no significant trend was noted for AV (p=13) and rAV (p=12) with increase in age. After adjusting for age, gender, axial length, and anterior chamber width, a smaller AV was significantly associated with angle-closure (odds ratio [OR] 10.12.5 and 95% confidence interval [CI], 5.7-17.6), comparing highest to lowest quartile). There was low correlation between AV and LV (Pearson' s correlation coefficient [PCC], -0.08), but moderate correlation between AV and anterior chamber width (PCC, 0.64).

Conclusions: The AV, which represents the portion of the anterior segment that is located anterior to the plane of the angles, is significantly smaller in eyes with angle closure, and its magnitude remains stable with advancing age.

Commercial Relationships: Monisha Nongpiur, None; Mingguang He, None; Tin Aung, None

Support: National Medical Research Council Singapore Grants No CSA/004/2008

Visual Neuroscience - Paper

Moderators

Neeru Gupta
University of Toronto, Toronto, Ontario, Canada
Shuichi Yamamoto
Chiba University, Graduate School of Medicine, Chiba, Chiba, Japan

397-1

Higher Room Luminance Increases Multifocal Electroretinogram Amplitude and Implicit Time

Muhamad Syukri Mohamad Rafiuddin [1] Ai Hong Chen [1, 2]

1. Advanced Electrophysiology Centre for Vision, Optometry, Universiti Teknologi MARA, Puncak Alam, Selangor, Malaysia. 2. iROViS, Drug Discovery & Health Community of Research, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia.

Purpose: Luminance had major role in determining the amplitudes of multifocal electroretinogram (mfERG). International Society for Clinical Electrophysiology (ISCEV) in 2012 had suggested that room luminance would be appropriate for multifocal electroretinogram testing without specifying the luminance amount. The purpose of this study is to investigate the effects of different room luminance to the amplitudes and implicit times of multifocal electroretinogram.
Methods: A total of 26 healthy emmetropic subjects were investigated using multifocal electroretinogram and its procedures were conformed to ISCEV 2012 multifocal electroretinogram standard. Room luminance was set at three different intensities; low luminance at 50 lux, moderate luminance at 200 lux and high luminance at 350 lux. Room luminance was measured using Digital Light Meter. All data were analysed using Statistical Package for Social Science (SPSS) version 20.
Results: We had found that only 1 parameter, which is P1 implicit time at peripheral eccentricity was significantly affected by differences in room luminance. Meanwhile, the amplitudes between different luminances had no significant difference. When we directly compared the low luminance to high luminance, we had found that 6 out of 20 parameters for mfERG had significance differences. From these significant values, our study found that amplitudes had increased with higher luminance while implicit times were slower in low luminance.
Conclusions: We suggest that room luminance must be regulated during multifocal electroretinogram testing. Performing mfERG in moderate luminance at 200 lux may result in more reliable dataset.
Commercial Relationships: Muhamad Syukri Mohamad Rafiuddin, None; Ai Hong Chen, None
Support: Fundamental Research Grant Scheme (FRGS) reference number 600-RMI/ST/FRGS 5/3/Fst (45/2011), Ministry of Education, Malaysia.

398-2

Optical coherence tomography (OCT) and electrophysiological assessment in retinal vein occlusion with macular oedema treated with intravitreal triamcinolone acetonide (IVTA)

Dr J L Goyal [1] Dr Richa Agarwal [1] Dr Ritu Arora [1] Dr B Ghosh [1]

1. Ophthalmology, Guru Nanak Eye Centre , New Delhi, India.

Purpose: To evaluate changes in visual acuity, optical coherence tomography and pattern ERG over 3 months in acuteretinal vein occlusion after IVTA
Methods: 20 patients with fresh retinal vein occlusion (branch and central) of less than 1 month duration were included in this prospective interventional study. They received 1mg of intravitreal triamcinolone acetonide (IVTA). Pre-injection the patients underwent a comprehensive ophthalmological examination which included assessment of best corrected visual acuity (BCVA), central macular thickness on OCT, contrast sensitivity (CS) on Pelli Robson chart and pattern ERG. Post-injection the patients were followed at 1 week, 6 weeks and 12 weeks and same parameters were evaluated at each follow up visit.
Results: Mean logMAR BCVA changed from 1.24 ± 0.63 to 0.74 ± 0.47 and CS changed from 0.44 ± 0.44 to 0.78 ± 0.32 at last follow up visit. At 3 months, mean central macular thickness decreased from 497.07 ± 199.80μ to 341.92 ± 118.07μ. There was no statistically significant change in the PERG following IVTA. The amplitude of P50 wave changed from 3.39 ± 2.00 to 3.22 ± 2.63μV. N95/P50 ratio changed from 2.36 ± 3.82 to 3.04 ± 5.07. There was no correlation between visual acuity, central macular thickness and pattern ERG.
Conclusions: Intravitreal acetonide is effective as a short term treatment of macular edema due to retinal venous occlusion, improving both visual acuity and macular thickness. The retinal function as assessed by PERG did not show any significant change before and after treatment suggesting limited role of PERG as a prognostic indicator for visual outcome in these cases.
Commercial Relationships: Dr J L Goyal, None; Dr Richa Agarwal, None; Dr Ritu Arora, None; Dr B Ghosh, None

399-3

Reduced full-field electroretinogram in early post-operative days in eyes with idiopathic epiretinal membrane

Atsuhiro Tanikawa [1] Iyo Ueda [1] Ryota Sakurai [1] Tadashi Mizuguchi [1] Akira Nakamura [1] Yoshiaki Shimada [1] Masayuki Horiguchi [1]

1. Ophthalmology, Fujita Health University School of Medicine, Toyoake, Aichi, Japan.

Purpose: To evaluate the effect of vitrectomy on retinal function in early post-operative days in eyes with

idiopathic epiretinal membrane (ERM), using full-field electroretinograms (ERGs) recorded with skin electrodes.
Methods: The ERGs were elicited from 10 eyes of ERM with skin electrodes, by using pulse reference power line noise reduction system (PuREC, Mayo, Nagoya, Japan). The amplitude of each component in the ERG waveform was compared among responses recorded before surgery and 1 day, 1 week and 1 month after surgery. Photopic negative response (PhNR) amplitude was measured from the baseline to the negative trough at 65msec. The results for each time point were compared by one-way repeated measures analysis of variance. Transconjunctival sutureless 25-gauge vitrectomy was performed with phacoemulsification and IOL implantation. ERM was removed, followed by internal limiting membrane peeling without any staining.
Results: There were no serious complications in all cases. Relative amplitudes (mean ± SE), to the baseline, elicited 1 day, 1 week and 1 month after surgery of each component were as follows, dark adapted 0.01 ERG; 69.9 ± 6.0 %, 119.2 ± 12.9 % and 108.2 ± 5.8 % ($P < 0.001$), dark adapted 3.0 ERG a-wave; 81.0 ± 5.7 %, 109.5 ± 9.1 % and 111.8 ± 8.7 % ($P < 0.001$), dark adapted 3.0 ERG b-wave; 74.9 ± 6.2 %, 116.2 ± 7.8 % and 115.1 ± 9.6 % ($p < 0.001$), light adapted 3.0 ERG a-wave; 97.4 ± 6.5 %, 104.5 ± 12.9 % and 124.6 ± 7.7 % ($p = 0.009$), light adapted 3.0 ERG b-wave; 80.5 ± 5.0 %, 108.3 ± 12.7 % and 113.2 ± 8.6 % ($p = 0.002$), light adapted 3.0 ERG PhNR; 89.2 ± 7.9 %, 78.8 ± 14.0 % and 79.1 ± 10.0 % ($P = 0.212$), flicker ERG; 86.2 ± 6.2 %, 106.4 ± 11.7 % and 103.5 ± 7.1 % ($p = 0.067$), respectively.
Conclusions: All ERG components showed a reduction in amplitude on the first post-operative day. All except PhNR recovered 1 week after surgery, however, PhNR revealed a tendency to further reduction. Decreased mean PhNR amplitude continued 1 month after surgery. These results suggest that there are some functional impairments in the inner retina after vitrectomy.
Commercial Relationships: Atsuhiro Tanikawa, None; Iyo Ueda, None; Ryota Sakurai, None; Tadashi Mizuguchi, None; Akira Nakamura, None; Yoshiaki Shimada, None; Masayuki Horiguchi, None

400-4

VEGFR2 is required for maintaining tissue homeostasis in adult retina

Masaharu Ohbayashi [1, 2] Yuichiro Ogura [2] Akiyoshi Uemura [1]

1. Department of Retinal Vascular Biology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan. 2. Department of Ophthalmology and Visual Sciences, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan.

Purpose: In mouse retinas, vascular endothelial growth factor receptor 2 (VEGFR2) is transiently expressed in retinal progenitor cells during development, and is constitutively expressed in Müller glia in adults. In this study, we assessed the roles of VEGFR2 in retinal neurons and Müller glia in retina-specific *Vegfr2*-knockout mice.
Methods: In mouse retinas, vascular endothelial growth factor receptor 2 (VEGFR2) is transiently expressed in retinal progenitor cells during development, and is constitutively expressed in Müller glia in adults. In this study, we assessed the roles of VEGFR2 in retinal neurons and Müller glia in retina-specific *Vegfr2*-knockout mice.
Results: Despite the absence of VEGFR2 expression in retinal neurons and Müller glia in *Dkk3Cre:Vegfr2-flox* mice, normal retinal architectures were formed with normal ERG response. However, after 2 months of age, the number of photoreceptor cells was progressively reduced, leading to the thinning of neural tissues and regression of retinal vessels. Accordingly, ERG response was completely lost at 6 months of age.
Conclusions: Our results indicate that VEGFR2 in Müller glia is indispensable for maintaining photoreceptor cells in adult retinas, and further evokes a caution for potential risks of intravitreal anti-VEGF therapy.
Commercial Relationships: Masaharu Ohbayashi, None; Yuichiro Ogura, None; Akiyoshi Uemura, None

401-5

Application of Flicker ERG with Newly Devised RETevalTM for Prematurely Born Babies

Akio Tabuchi [1] Tsutomu Yamashita [1] Tomoko Yokoyama [3] Nobumasa Takahashi [2] Ikuko Nojima [2] Mizue Takasugi [2] Noriko Kimura [2] Masao Yoshikawa [4]

1. Sensory Science, Kawasaki University of Medical Welfare, Kurashiki, Japan. 2. Pediatrics, Fukuyama Medical Center, Fukuyama, Japan. 3. Ophthalmology, University of Hiroshima, Hiroshima, Japan. 4. Mayo com., Inazawa, Japan.

Purpose: In order to estimate of the retinal function in prematurely born babies (PBB), we applied a flicker electroretinogram (flicker ERG) with newly devised RETeval^{TM}. Although a fundus examination of PBB by an ophthalmoscope and/or a wide angle fundus camera (ex. Ret Cam) has been reliable methods for understanding of the retinal condition, especially for a management of the retinopathy of prematurity(ROP), we cannot tell its retinal function from these retinal findings. Zetterstroem(1954) has been reported that the flicker ERG in normally born infants could be recorded under a strong light stimulation. We showed the flicker ERG of PBB who were examined for the management of ROP in the NICU of the Fukuyama Medical Center, and the flicker ERG of PBB even with very low birth weight and also with very short pregnant period could be recorded by the RETeval.
Methods: Subjects were twenty-seven PBB who underwent fundus examinations for management of ROP in the NICU of the Fukuyama Medical Center. The birth weights were 1,368 ± 509g (mean ± SD), the pregnant periods, 30.6 ± 2.8 w, and the gender, male ;18, female ; 9. Seven of the subjects (25.9%) were impossible to record because of incorporation. Recording was performed just before the fundus examination with dilated pupils. For the flicker ERG, we used RETeval^{TM} (LKC Technology, Inc., USA). It was non-invasive, full field ERG cone b-wave flicker response with 28.3 Hz, and the white light intensity of 3 cds/m^2 under 30 cd/m^2 of background (according to ISCEV standard). One-piece adhesive skin electrodes taped below each eyelid. Examination times (two-session) were 10-20 sec/eye. This clinical study was done under the permission of the ethic committee of Fukuyama Medical

Center (No.31).
Results: 39 eyes of 20 subjects showed meaningful flicker ERG. The amplitude (uV) were ranged from 1.0 to 11.3 (3.7 ± 2.2) with increasing as the gestational period was increasing (r=0.4843, p=0.0024), on the other hand, the latency (msec) were ranged from 26.6 to 40.9 (34.4 ± 2.7) with decreasing tendency as that was increasing(r=-0.2716).
Conclusions: We successfully recorded the flicker ERG of PBB. Shorter gestational period of PBB, smaller amplitude and longer latency of the flicker ERG were recorded. The flicker ERG may indicate on prematurity of PBB from a view point of the retinal function.
Commercial Relationships: Akio Tabuchi, None; Tsutomu Yamashita, None; Tomoko Yokoyama, None; Nobumasa Takahashi, None; Ikuko Nojima, None; Mizue Takasugi, None; Noriko Kimura, None; Masao Yoshikawa, None

Eye Movements/Strabismus/Amblyopia/Neuro-ophthamology - Paper

Moderators

Erwin D. Palisoc
Manila Central University College of Medicine, Caloocan, Philippines
Atsushi Miki
Kawasaki Medical School, Kawasaki, Okayama, Japan

402-1

Orbital Septum-Sparing Dorsal Approach for Transcutaneous Simultaneous Advancement of the Levator Aponeurosis and Müller Muscle

Ari Shinojima [1] Mitsuko Yuzawa [1]
1. Ophthalmology, Nihon University Hospital, Tokyo, Japan.

Purpose: Identifying the levator aponeurosis can be difficult, especially in the presence of abundant fat, when the orbital septum is opened. Furthermore, if previous surgery has left severe eyelid tissue adhesions, levator aponeurosis identification becomes difficult. To overcome this problem, we performed the aponeurotic ptosis operation by the following method and evaluated the results.
Methods: Patients over 60 years of age diagnosed with aponeurotic ptosis were enrolled in this study. All subjects had a margin reflex distance (MRD) less than 2.0 mm, and levator function (LF) exceeding 6.0 mm. Twenty-eight eyes of 16 patients (5 men, 8 eyes; 11 women, 20 eyes) underwent the following operation. The levator complex of the Müller muscle and the levator aponeurosis at the fornix 7-8 mm from the edge of the upper tarsal plate were grasped simultaneously from the dorsal side using 6-0 vicril®. Three parts in total were adjusted and sutured at one-third the height of the upper tarsal plate. Mean follow-up was 5.2 ± 2.4 (range, 3-12) months. Mean age was 76.0 ± 7.5 (range, 61-86) years. We analyzed MRD, LF, and postoperative satisfaction for each eye, regardless of whether the ptosis surgery was unilateral or bilateral, by visual analog scale (VAS) for comparison with preoperative expectations.
Results: Preoperatively, MRD was 0.39 ± 0.69 mm (range; 0-2 mm) and LF was 9.2 ± 2.5 mm (range; 6-14 mm). Postoperatively, MRD was 3.8 ± 0.71 (range; 3-5 mm) and LF was 13.0 ± 3.2 (range; 8-15 mm). Statistically significant improvements were obtained in MRD ($P < 0.001$) and LF ($P < 0.001$) (both, n=28). The MRD increase did not differ significantly between postoperative satisfactory (group 1, n=21) and unsatisfactory (group 2, n=7) results (p=0.06). LF change also failed to reach statistical significance (p=0.65). VAS score satisfaction did not differ significantly between the unilateral (A, n=4) and bilateral (B, n=12) groups (p=0.52).
Conclusions: Our operative method increased MRD and LF. However, the MRD and LF increases were not statistically significant as compared to the VAS score. Furthermore, the VAS scores did not differ significantly between unilateral and bilateral ptosis operations. Aesthetic elements and/or mental problems may affect satisfaction with results.
Commercial Relationships: Ari Shinojima, None; Mitsuko Yuzawa, None

403-2

Automated Pupillograph as a Screening Tool in Ophthalmology Clinic

Chaithra Aroor [1] Dr Rohit Shetty [1] Narendra KP [1] Bhujang Shetty [1]
1. Narayana Nethralaya, Bangalore, India.

Study Group: Control and Cases

Purpose: To evaluate the efficacy of an automated pupillograph as a screening tool in ophthalmology clinic.
Methods: We enrolled 110 subjects in the study (80 normals and 30 patients with Glaucoma) .We measured the pupillary reactions in all the patients using RAPDx Expanded Pupil Diagnostics (Konan Medical USA, Inc., Irvine, CA) and compared the same with Neutral Density Filters (NDF). In addition the 30 Glaucoma patients also underwent analysis of Macular Ganglion cell (mGCC) thickness using a Spectral domain -Optical Coherence Tomography(Optovue RTVue XR AVANTI).
Results: The pupillary reactions assessed by NDF was compared with that of RAPDx and we found statistically significant correlation ($p<0.001$) between the both.
Mean amplitude of pupillary reactions on RAPDx (0.14) correlated moderately with the mGCC thickness, however mean latency of the pupillary reactions (0.12) showed a weak correlation.
Conclusions: This pilot study concludes that RAPDx is comparable to NDF in measuring RAPD and can be used interchangeably.
The log-scaled RAPD amplitudes correlated moderately with the differences in mGCC thickness, but the log-scaled RAPD latencies showed a weaker correlation.
RAPDx may be used as a screening tool in Ophthalmology clinic.
Commercial Relationships: Chaithra Aroor, None; Dr Rohit Shetty, None; Narendra KP, None; Bhujang Shetty, None

404-3

Role of Zinc (Zn) in Ethambutol-induced Neuroretinopathies, Functional and Morphologic assessment in rabbit model

Erwin D. Palisoc [1] Hitoshi Ishikawa [2, 3] Ken Asakawa [2, 3] Kimiya Shimizu [3] Kimiyo Mashimo [3]
1. Department of Ophthalmology, Manila Central University College of Medicine, Caloocan city, Philippines. 2. Department of Orthoptics and Visual Science, Kitasato University School of Allied Health Sciences, Sagamihara city, Kanagawa, Japan. 3. Department of Ophthalmology, Kitasato University School of Medicine, Sagamihara city, Kanagawa, Japan.

Purpose: This study aims to evaluate functional and morphologic differences as well as to characterize histopathologic changes in the retina, optic nerve, and chiasm of pigmented rabbits secondary to the administration of Ethambutol (EMB) and compare it with

those given Zinc (Zn) supplementation.
Methods: A total of six pigmented rabbits were used. EMB was orally administered to all rabbits, initially at 110 mg/kg/day followed by 180 mg/kg/day, for a maximum of 20 weeks. Three of these rabbits were also given Zn supplementation at a dose of 8 mg/kg/day. Subjects were examined using infrared pupillometer, standard ERG, ophthalmoscopy and fundus photos were taken at an interval of 2 weeks. Serial serum Zn concentrations were also determined. All the EMB-treated rabbits were euthanized and the globe including the optic nerve and chiasm were harvested. Histopathologic examination of harvested tissues was done afterwards. Both Light and Electron microscopy were used for examination.
Results: Rabbits who are given higher doses of EMB alone over a prolonged duration of time, showed both decreased constriction rate and pupillary diameter from the baseline in the infrared pupillometer using red stimulus, but normal response in the bright flash ERG exam. Optic disc changes were also seen through fundus examination. Histopathologically, observable decrease in the retinal ganglion cell density, demyelination and glial reaction in the optic nerve and chiasm were also noted; On the other hand, those rabbits given EMB with Zn, only the outer retina and choroid were affected wherein depigmentation in the choroid, damaged retinal pigmented epithelium, and degeneration of the bipolar cells were observed. Moreover, no significant changes were observed in both ERG and infrared pupillometric tests.
Conclusions: The results of this study suggest that the retina, optic nerve and chiasm were markedly damaged both morphologically and functionally in rabbits with EMB-induced toxic neuroretinopathies. However, those given Zn supplementation were minimally affected by the toxic doses of EMB. Thus, Zn can be used as a prophylactic medication to avoid the occurrence of ocular toxicity from use of EMB as treatment for tuberculosis.
Commercial Relationships: Erwin Palisoc, None; Hitoshi Ishikawa, None; Ken Asakawa, None; Kimiya Shimizu, None; Kimiyo Mashimo, None

405-4

Incidence and risk factors of inferior rectus injury in pediatric orbital blow-out floor fractures

Yan Tong Koh [2] Shantha Amrith [1] Errol W. Chan [1]
1. Ophthalmology, National University Hospital, Singapore, Singapore. 2. Ophthalmology, Tan Tock Seng Hospital, Singapore, Singapore.

Purpose: Inferior rectus (IR) injuries in pediatric orbital blow-out floor fractures are rare. The aim of this study was to evaluate the incidence, features and risk factors of IR injury in this group.
Methods: A retrospective case review on sequential cases of pediatric orbital blow-out fractures (age <18 years) from 2000 to 2013 in a tertiary ophthalmic center in Singapore. Subjects were excluded if there was rim involvement, multiple fractures, or poor quality computed tomography (CT) images. All cases underwent surgical repair. Demographic and clinical characteristics were collected. Fracture extent was assessed and classified into 4 groups, (A1) floor lateral to infraorbital canal; (A2) floor medial to the canal; (A3) involving the inferomedial strut, and (A4) medial blowout fracture. Fracture area was defined from a medial anatomic landmark of the ethmoidomaxillary suture to the medial and lateral border of the fracture in each CT image displaying the fracture. The anteroposterior extent of the fracture was determined by comparing CT images displaying the fracture to those with the orbital floor. Univariate analyses were performed, and $p<0.05$ was taken to be significant.
Results: A total of 48 subjects were identified, with 5 and 43 cases with and without IR injury respectively (incidence = 10.4%). The mean age was 16.4 ± 1.1 years (range 15-18) and 11.9 ± 2.6 years (range 5-15) for subjects with and without IR injury respectively ($p=0.002$). Majority were males in both groups. Mean time to surgery was 10.8 ± 10.8 days (range 0-40) and 12.8 ± 9.6 days (range 3 – 24) for subjects with and without IR injury respectively ($p>0.05$). Eyes with IR injury were more likely to have restriction in both up and downgaze, than eyes without IR injury (100% versus 27.9% $p=0.003$). The mean fracture area of subjects with and without IR injury were 1.95 ± 0.7 cm^2 and 0.83 ± 0.5 cm^2 respectively ($p=0.01$). There were no significant differences in the proportion of subjects with symptoms suggestive of oculocardiac reflex, and fracture extent in both groups ($p>0.05$).
Conclusions: The incidence of IR injury in pediatric orbital blow-out fractures is low. Risk factors for IR injury include older age, restriction in up and downgaze, and larger fracture area.
Commercial Relationships: Yan Tong Koh, None; Shantha Amrith, None; Errol Chan, None

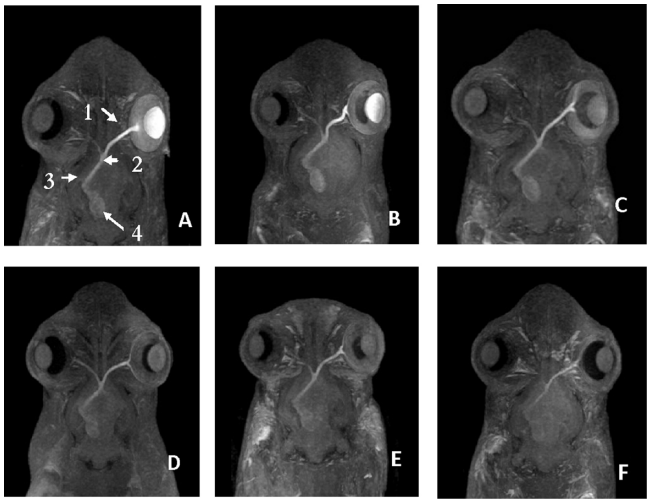
406-5

Dose and Time Response of Mn^2+-enhanced Magnetic Resonance Imaging for Tracing Visual Pathway in Rabbits

Yuntao Hu [1] Weiling Wang [4] Hui Xu [2] Xiaodong Sun [3] Zhizhong Ma [1]
1. Ophthalmology, Peking University Third Hospital, Beijing, China. 2. Radiology, Peking University Third Hospital, Beijing, China. 3. Ophthalmology, Affiliated First People's Hospital of Shanghai Jiao Tong University, Shanghai, China. 4. Ophthalmology, Hospital of Ningxia Medical University, Yinchuan, China.

Purpose: To explore time and dose response of Mn^{2+}-enhanced MRI for tracing visual pathway in vivo using intravitreal $MnCl_2$ injection.
Methods: 36 rabbits were randomly divided into six groups (each group, n=6). 25 μ l $MnCl_2$ in various concentrations (2, 5, 10, 15, 20, 40 mmol/L) was injected into vitreous of left eyes for each group respectively. MRI was performed to examine the imaging of visual pathway at 4, 6, 8, 12, 24 hours, and 2, 4, 7 days after intravitreal $MnCl_2$ injection. The signal-noise ratio (SNR) of MRI in visual pathway was calculated and compared among different groups and time points.
Results: 2 mmol/L of $MnCl_2$ just could induce optical nerve enhancement, but no image of lateral geniculate body and superior colliculus enhanced was found. In 5 - 40 mmol/L groups, the visual pathway from retina to contralateral

superior colliculus were enhanced significantly (Fig1). However, no signal was enhanced in visual cortex in each group. The images were enhanced strongest at 24 h, and then the signals attenuated gradually from 24 h to 7 days.
Conclusions: Manganese-enhanced MRI for tracing visual pathway is related with time and dose of intravitreal Mn^{2+} injection.
Commercial Relationships: Yuntao Hu, None; Weiling Wang , None; Hui Xu , None; Xiaodong Sun, None; Zhizhong Ma, None
Support: National Basic Research Program of China (973 Grant 2011CB707506); Seed Fund of Peking University Third Hospital (NO.YZZ08-9-13) ; Linghu Fund of Peking University Third Hospital (NO.64508-01).



407-6

The 10-year Effectiveness of Botulinum (A Toxin Therapy) on Esotropia in Children

Patcharapan Bhaktikamala [1] Supaporn Theingtrisorn [1]
1. Ophthalmology, Prince of Songkhla University , Hatyai, Songkhla, Thailand.

Purpose: To Evaluate long term s and complications of botulinum A toxin treatment of Esotropia in children
Methods: A cohort study in which 15 patients had a history of Esotropia, and joined in treatment with botulinum A toxin, between September 1998 and February 2002 . The data was reviewed from medical records , clinical examination and laboratory investigations were done . Mean time follow-up was 11.9 years (min,max 11.38, 10.96). Success was defined as alignment with 10 PD (prism diopter) of orthophoria with or without a history of muscle surgery. The data was analysed by the Wilcoxon Signed Ranks Test.
Results: Of the twenty-two patients that joined the treatment between September 1998 and February 2002, only 15 patients (68.2%) were included in this study. The patients included 9 females (60%) and they had a history of muscle surgery after botulinum A toxin injection (were 13 patients) (86.6%). Mean age at examination was 13.19 years (min, max 11.3, 16.11). Median pretreatment deviation was 35 PD (18, 60). Motor success was achieved in 79% of patients and patients a deviation of ET 30 - <60 PD had the highest success rate (6 patients) (67%). Gross stereopsis was suppressed in all patients
Conclusions: In a Cohort study (following a 10-year post botulinum A toxin therapy) found the botulinum A toxin treatment did not succeed in controlling ocular alignment or to improve gross stereopsis but almost all of the patients that were adjuvant by surgical treatment had motor success. Botulinum A toxin injection may be an alternative or primary treatment for children with small- to moderate-angle infantile esotropia, which cannot tolerate muscle surgery in an early period of life.
Commercial Relationships: Patcharapan Bhaktikamala, None; Supaporn Theingtrisorn , None

Physiology/Pharmacology - Paper

Moderators

Xiaorong Li
Tianjin Medical University Eye Hospital & Eye Institute, Tianjin, China
Nobuhisa Naoi
Miyazaki University, School of Medicine, Miyazaki, Miyazaki, Japan

408-1

Enhanced Ocular Delivery of Anticancer Drugs by Blocking P-gp Drug Efflux Transporters in Blood Ocular Barriers, An Experimental Study

Santosh K. Patnaik [3] Nabanita Haldar [3] Bhavna Chawla [4] Vasantha Thavaraj [1] Nihar R. Biswas [2] Thirumurthy Velpandian [3]
1. RHN, ICMR, New Delhi, India. 2. IGIMS, Patna, Bihar, India. 3. Ocular Pharmacology & Pharmacy, Dr.R.P.C, AIIMS, New Delhi, New Delhi, India. 4. Dr. R.P.C , AIIMS, New Delhi, New Delhi, India.

Purpose: To evaluate the effect of P-glycoprotein on the intraocular disposition of its substrate etoposide after intravenous administration in the presence of P-gp blocker (GF-120918) in healthy Rabbits.
Methods: New Zealand albino rabbits were used with the approval of Institutional animal ethics committee (IAEC). Considering the triple drug combination chemotherapy used in retinoblastoma, in this experiment etoposide, carboplatin and vincristine were co-administered to the rabbits. Etoposide was used at a dose of 5 mg/kg and carboplatin at the dose of 18.7 mg/kg were mixed in normal saline and infused over the period of one hour. At the end of infusion vincristine was infused as IV bolus at the dose of 0.05mg/kg. All animals received the same protocol and in the P-gp blocker group, GF120918 at the dose of 3.5 mg/kg was administered as IV bolus injection 30 min prior to the commencement of triple drug combination for etoposide and carboplatin intravenous administration. Samples were stored at -80 ☒C until the analysis by LC-MS/MS.
Results: There was a significant increase in ocular tissue level concentration of etoposide in the blocker pre-treatment group as compared to the non-blocker group. Ocular tissue disposition of etoposide after IV administration was significantly altered in the presence of P-gp blocker (GF-120918). The results were statistically significant $P<0.05$.
Conclusions: Our findings suggested that P-gp blocker significantly increased the levels of etoposide in ocular tissues. P-gp blockade could be beneficial in the treatment of retinoblastoma
Commercial Relationships: Santosh Patnaik, None; Nabanita Haldar, None; Bhavna Chawla, None; Vasantha Thavaraj, None; Nihar Biswas, None; Thirumurthy Velpandian, None

409-2

The application of exosomes derived from mesenchymal stem cells in retinal diseases

Xiaorong Li [1]
1. Tianjin Medical University Eye Hospital & Eye Institute, Tianjin, China.

Purpose: To investigate the therapeutic role of exosomes derived from mesenchymal stem cells (MSCs) in retinal injury and inflammation using the animal model of retinal laser injury, retinal detachment (RD) and experiment autoimmune uveitis (EAU).
Methods: MSC-derived exosomes were purified from supernatants of human umbilical cord MSCs by sequential centrifugations and ultrafiltrations, and determined by proteome analysis. Exosomes were intravitreal injected into mice model of retinal injury created by multi-wavelength argon laser and rat model of RD induced by subretina sodium hyaluronate injection, and periocular injected into the rat model of EAU. Electrophysiology (ERG) and H&E staining were performed continuously to evaluate the structure and function of the retina for all three models. TUNEL staining was done to detect apoptosis, RHO and GFAP expression was detected by immunohistochemistry for both laser injury and RD models, and the gene expressions of c-met, MCP-1 and ICAM-1 were measured by RT-PCR for laser-injury mice.
Results: Both the histology and ERG in exosomes treated group were significantly ameliorated at different phase compared with those in control group for three models. Remarkable less apoptotic cells, decreased GFAP and increased RHO expression were detect in the retina after exosome therapy in both laser injury and RD models. Exosome injection significantly reduced C-met, MCP-1 and ICAM-1 expressions after laser injury. In EAU models, T1, Th17 T cells as well as macrophage infiltration significantly decreased in the eyes after exosome therapy.
Conclusions: MSC-derived exosomes could limit retina damage, inhibit apoptosis and suppress inflammatory response in retina laser injury, RD and EAU model, and therefore provide an attractive potential therapeutic agent for retinal injury and inflammation.
Commercial Relationships: Xiaorong Li, None

410-3

eNOS-related mechanotransduction changes in aged porcine angular aqueous plexus cells

Yuan Lei [1,5] William D. Stamer [2] Jihong Wu [1,5] Xinghuai Sun [3,4]
1. Research Centre, Eye and ENT Hospital of Fudan University, Shanghai, China. 2. Duke University, Durham , CA, United States. 3. Department of Ophthalmology and Vision Science, Eye and ENT hospital, Shanghai Medical College, Fudan University, Shanghai, China. 4. State Key Laboratory of Medical Neurobiology, Institutes of Brain Science, Fudan University, Shanghai, China. 5. Shanghai Key Laboratory of Visual Impairment and Restoration,

Eye and ENT hospital, Shanghai Medical College, Fudan University, Shanghai, China.

Purpose: To investigate effects of aging on endothelial nitric oxide synthase (eNOS) expression and signaling in angular aqueous plexus (AAP, functional equivalent to human Schlemm' s canal) cells subjected to shear stress.
Methods: AAP cells were isolated differentially from porcine outflow tissues using puromycin selection. Cell aging was induced by culturing cells in hyperoxia condition (40% O_2, 5% CO_2) for 14 days. AAP cells grown in chamber slides were exposed to a shear stress of 8 dynes/cm^2 for 24 hours. Expression of eNOS, eNOS-phospho Thr495, eNOS-phospho Ser1177 and Akt-phospho was tested by western blot analysis and immunofluoresence staining. Nitric oxide levels were measured using the Griess assay.
Results: Compared to control, eNOS levels in aged cells was significantly reduced by 60% ($p<0.05$, n=6). Phosphorylation of eNOS at Ser1177 and Akt at Ser473 was 63% and 80% lower in aged cells respectively, whereas phosphorylation of the eNOS inhibition site (Thr495) increased by 6.1 fold ($p<0.05$, n=6). Shear stress (8 dynes/cm^2 for 24 hours) increased eNOS abundance (total protein and at cell borders) and phosphorylation at Ser1177 by 1.7 and 1.8 fold respectively ($p<0.05$, n=6), whereas aged cells were unresponsive. In control cells exposed to shear stress, NO concentration was 1.8 fold higher than the static group ($p<0.05$, n=4); however aged cells were unresponsive to shear stress (4.3 ± 1.3 mM vs. 4.1 ± 1.4 mM).
Conclusions: Aged AAP cells appear compromised in their mechanotrasduction machinery involving eNOS, the protein product of the gene, NOS3, polymorphisms of which impart a risk for the development of glaucoma.
Commercial Relationships: Yuan Lei, None; William Stamer, None; Jihong Wu, None; Xinghuai Sun, None
Support: National Science Foundation China (81100662, 81371015), Shanghai Municipal Health Bureau Young Outstanding Scientist Program (XYQ2013083), 211 Project of Fudan University (EHF158351), Scientific Research Foundation for the Returned Overseas Chinese Scholars (State Education Ministry), Research to Prevent Blindness Foundation (EY022359, WDS).

411-4

Pulse-waveform analysis of choroidal blood flow in acute central serous chorioretinopathy

Michiyuki Saito [1] Wataru Saito [1] Kiriko Hirooka [1] Yuki Hashimoto [1] Shohei Mori [1] Kousuke Noda [1] Susumu Ishida [1]

1. Department of Ophthalmology, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido, Japan.

Purpose: To quantitatively investigate the characteristics of macular choroidal blood flow using pulse-waveform analysis of laser speckle flowgraphy (LSFG) in patients with acute central serous chorioretinopathy (CSC).
Methods: This retrospective, observational case series includes 20 eyes of 20 patients with acute CSC. All subjects underwent LSFG in addition to comprehensive ophthalmologic examinations at baseline and 6 months later. On the LSFG color map, the macula was enclosed in a square and automatically divided into 25 equal segments. Each grid segment was classified based on the presence of delayed choroidal filling during early-phase indocyanine green angiography (ICGA). The average regional macular mean blur rate (MBR) and parameters of the waveform analysis, i.e., the blowout time (BOT), skew, and acceleration time index (ATI), were calculated for each area with or without delayed choroidal filling, and compared between baseline and at 6 months after baseline.
Results: Subretinal fluid spontaneously resolved in all eyes within 6 months. The average MBR significantly decreased in each area with or without delayed choroidal filling during the 6-month follow-up (P = 0.0046 and P = 0.000095, respectively). The BOT significantly increased at 6 months in both areas (P = 0.00011 and P = 0.00021, respectively). Skew values significantly decreased at 6 months in both areas (P = 0.00045 and P = 0.0057, respectively). At baseline, the BOT was significantly lower and the skew significantly higher in the area with choroidal filling delay than in the area without it (P = 0.029 and P = 0.014, respectively). The ATI did not change in each area during the 6-month follow-up.
Conclusions: The baseline skew and BOT values suggested circulatory disturbance at the acute stage of CSC, particularly in areas with delayed choroidal filling. Our results indicate that these parameters may be useful new indexes to evaluate the activity in choroidal lesions in acute CSC.
Commercial Relationships: Michiyuki Saito, None; Wataru Saito, None; Kiriko Hirooka, None; Yuki Hashimoto, None; Shohei Mori, None; Kousuke Noda, None; Susumu Ishida, None

412-5

RPE65 Retinal Gene Therapy Update and the Frequency of the RPE65 Mutations in the Asia-Pacific Region, Implications for Increased Genetic Testing and Preparation for Future Treatments

Daniel C. Chung [1]

1. Scheie Eye Institute, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, United States.

Purpose: To evaluate the frequency of inherited retinal degeneration due to autosomal recessive *RPE65* gene mutations within the Asia-Pacific region and to report the findings from Phase I/II clinical trials and the study design of the ongoing Phase III trial.
Methods: A systematic literature review of peer reviewed publications originating from institutions and cohorts within the Asia Pacific region, that report mutational analysis for the *RPE65* gene were collected. Data referring to inclusion criteria, cohort size and number with *RPE65* mutations were noted. Clinical trial and design data were obtained from the prior Phase I/II and ongoing phase III *RPE65* gene therapy trials being conducted at the Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA.
Results: A comprehensive literature search identified 12 reports of mutational analysis on cohorts of Leber Congenital Amaurosis (LCA) and/or autosomal recessive

retinitis pigmentosa (arRP) diagnosed subjects from the Asia-Pacific region. A total of 445 unrelated probands were identified, 348 LCA and 97 arRP. Overall, 27 subjects had *RPE65* mutations (27/445, 6.0%), and if further delineated, 5.1% of arRP subjects (5/97), and 6.3% of LCA subjects (22/348). The current status of the RPE65 gene therapy clinical trials consists of 12 subjects (ages 8-44), with confirmed *RPE65* mutations, each receiving a subretinal injection of an adeno-associated virus type 2 (AAV2) encoding the human wildtype *RPE65* gene in the worse seeing eye, in a dose escalation Phase I/II trial. In the subsequent follow-on trial, 11 of the 12 subjects received a sub-retinal injection in the contralateral non injected eye. Endpoints included pupillometry, full field senstivity, visual field testing, contrast sensitivity, visual acuity and mobility testing. Initial results show excellent safety profiles and efficacy. Current Phase III study is a multi-center, randomized trial.

Conclusions: Results from the Phase I/II *RPE65* gene therapy clinical trials have shown efficacy and safety. The Phase III trial is ongoing and is now fully enrolled. The frequency of *RPE65* mutations in LCA and arRP are significant and would indicate a growing need for genetic screening to identify individuals from the Asia-Pacific region, who would benefit from future *RPE65* gene therapy treatments.

Commercial Relationships: Daniel Chung, None
Support: Hope for Vision, CHOP Grant 225120000-01-06, FM Kirby Foundation
Clinical Trail: NCT01208389

Clinical/Epidemiologic Research - Paper

Moderators

Mark Radford
Queensland Eye Institute, South Brisbane, Queensland, Australia
Hidetoshi Kawashima
Jichi Medical University, Shimotsuke, Tochigi, Japan

413-1

Teacher led screening for early identification of vision problems among children

Subodh Gnyawali [1] Dipesh Bhattarai [2]
1. Academics and Research, BP Eye Foundation, Kathmandu, Kathmandu, Nepal. 2. Ophthalmology, BP Eye Foundation, Kathmandu, Kathmandu, Nepal.

Purpose: One out of every ten children in primary level schools is reported to have vision problems. Vision problems can have a major impact on the development of a young child. Early identification and intervention minimize the effects of a vision loss on a child's development and provides the best opportunity for effective, inexpensive treatment. There is no system of vision screening at the time of admission to school or any time during their school years. Mid Term Review of Vision 2020, Nepal has recommended teachers as an alternative to fill the existing gap in human resources and to address a huge unmet need for early identification of vision problems among children. BP Eye Foundation took the initiative of training 128 teachers from 64 primary level schools in three districts. They were trained by optometrists on taking visual acuity, recording and referral. A total of 19,200 children were screened by the teachers and 3,840 children were referred to nearest eye health facility where they were taken care of by trained eye health professional. The purpose of this research was to validate the screening done by teachers so as to augment screening services in remote locations of the country which are not accessible to health service system of the country.
Methods: Twenty percent of children referred by trained teachers for eye examination were randomly selected and rechecked for their visual acuity by optometrists at the children's respective schools. Another 20% of children who were reported to have normal visual acuity by teachers were also selected randomly and reassessed by optometrists to observe any difference. Children who needed a treatment were provided with the same on site.
Results: Among 768 children reported to have vision problem by the trained teachers 676 (88%) were found to have a vision problem. Among another 768 children reported normal by the teachers, 85 (11%) were found to have a vision problem by the optometrists. Sensitivity of the screening test was found to be 88.8% and the specificity 88.1%.
Conclusions: Vision screening done by trained teachers is a valid test to identify children with vision problems because of the tests high sensitivity and specificity values. As schools are suitable places for mass screening and easier for follow ups periodic screening program at schools led by teachers would help in the early identification of children with vision problems.
Commercial Relationships: Subodh Gnyawali, None; Dipesh Bhattarai, None

414-2

Diabetic retinopathy in newly diagnosed type 2 diabetes mellitus patients in urban slums of Mumbai, India is 6.32%

Sunita Mohan [2] Radhika Krishnan [2] Arvind K. Singh [2] Uthra Satagopan [2] Ashwini Rogye [2] Manish Sonawane [2] Ravina Gaonkar [2] Sundaram Natarajan [1] Govindasamy Kumaramanickavel [1] Catherine McCarty [3]
1. Aditya Jyot Eye Hospital, Mumbai, India. 2. Aditya Jyot Foundation for Twinkling Little Eyes, Mumbai, India. 3. Essentia Institute of Rural Health, Duluth, MN, United States.

Study Group: Aditya Jyot Diabetic Retnopathy in Urban Mumbai Slums Study (AJDRUMSS)

Purpose: To identify the proportion and risk factors for diabetic retinopathy (DR) in newly diagnosed diabetes (NDD) with type 2 diabetes mellitus (T2DM) in urban slums of Mumbai, India.
Methods: A cross-sectional study was conducted in non-randomly selected slums of seven wards of Mumbai. A door-to-door survey, fasting blood sugar (FBS) estimation was conducted at the level of field followed by complete ophthalmic evaluation. Dilated (1% tropicamide drops), bilateral, seven-field fundus photography was obtained for each study subject. The photographs were graded using the Early Treatment Diabetic Retinopathy (ETDRS) adaptation of the modified Airlie House classification.
NDDs from this study were analysed for the current paper. Risk factor measurements including gender, blood pressure (BP), body mass index (BMI), anthropometry were obtained for each subject. Grade of retinopathy was defined as the higher grade of the two eyes. Chi-squared test with Yates correction was used to estimate the association between presence of any retinopathy and other variables.
Results: There were 95 NDD subjects. None of the study subjects had proliferative retinopathy. The mean age was 55.4years (± 9.8 yrs), with male, female ratio being 55:40. The prevalence of diagnosed retinopathy in NDD was 6.32% (95% CI 2.6%-13.8%). Association of DR with age of subject, gender, BMI, history of hypertension, socio-economic status, literacy status, polyuria, polydipsia and weight loss was found statistically non-significant (p>0.05).
Conclusions: Diabetic retinopathy was diagnosed in about one out of sixteen NDD subjects with T2DM (6.32%) in urban slums of Mumbai. None of the traditional risk factors were found significantly associated with DR, indicating early screening of NDD for DR irrespective of presence/absence of known risk factors. However studies with larger sample size of NDD are needed to ascertain the findings of this paper.
Commercial Relationships: Sunita Mohan, None; Radhika Krishnan, None; Arvind Singh, None; Uthra Satagopan,

None; Ashwini Rogye, None; Manish Sonawane, None; Ravina Gaonkar, None; Sundaram Natarajan, None; Govindasamy Kumaramanickavel, None; Catherine McCarty, None
Support: World Diabetes Foundation (WDF08-338)

415-3

Association of Early Microvascular Damage in the Eye and Kidney with Risk of Cardiovascular Events in a Multi Ethnic Asian Population

Carol Cheung [1] Wanfen Yip [1] Charumathi Sabanayagam [1] Tien Yin Wong [1]
1. Singapore Eye Research Institute, Sinapore, Singapore.

Purpose: Retinal microvascular signs may provide insights into the structure and function of systemic small vessels that are associated with development of cardiovascular diseases (CVD). We examined the combined effect of retinal microvascular abnormality and microalbuminuria in predicting incident CVD events in a multi-ethnic Asian cohort.
Methods: We conducted a prospective, population-based study in a multi-ethnic Asian cohort (Malay, Chinese and Indian) aged ≥40 years at baseline using data from the Singapore Malay Eye Study and the Singapore Prospective Study. Retinal microvascular abnormalities were assessed from baseline retinal photographs following standardized protocols. Retinopathy was graded using the modified Airlie House classification system. Retinal arteriolar caliber and venular caliber were measured using a computer-assisted program. Incident CVD (fatal and nonfatal) event was defined as newly diagnosed clinical stroke or acute myocardial infarction (AMI) or CVD death documented by National Registry of Diseases Office, Singapore, by record linkage.
Results: A total 3496 participants were free of prevalent stroke and AMI at baseline. During the follow-up (median 5.8 years), 126 (3.60%) participants developed CVD event. In Cox proportional-hazards models adjusting for established risk factors (age, gender, race, systolic blood pressure, diabetes, smoking, total cholesterol, HDL cholesterol and anti-hypertensive medication), presence of retinopathy (hazard ratio [HR] 2.03, 95% confidence interval (CI), 1.29 to 3.19) and wider retinal venular caliber (HR 1.20, 95% CI, 1.01 to 1.41, per SD decrease) were independently associated with risk of CVD event. Persons with retinal microvascular abnormalities and microalbuminuria were close to 7 times more likely to have incident CVD (HR, 6.67, 95% CI, 2.70 to 16.46), compared with persons with neither retinal vascular abnormalities nor microalbuminuria.
Conclusions: Presence of retinal microvascular abnormalities (retinal venular widening and presence of retinopathy) predicts the risk of CVD in an Asian population. We further showed that the risk of CVD is increased in people with coexistent of presence of retinal microvascular abnormalities and microalbuminuria.
Commercial Relationships: Carol Cheung, None; Wanfen Yip, None; Charumathi Sabanayagam, None; Tien Yin Wong, None

416-4

Knowledge Attitudes and Practice of common Eye Diseases and Presenting Vision in Rural Bangladesh, The Bangladesh Population-based Diabetes and Eye Study (BPDES)

Fakir M Amirul Islam [1,2] Rahul Chakrabarti [1] Christine Critchley [1]
1. Statistics, Data Science and Epidemiology, Swinburne University of Technology, Melbourne , VIC, Australia. 2. Organisation for Rural Community Development , Narail, Bangladesh.

Purpose: To assess the Knowledge, Attitudes and Practice (KAP) amongst the general community regarding common eye diseases in rural Bangladesh.
Methods: Data were collected from a cluster random sample of 3104 adults residing in a rural district in Bangladesh. Participants underwent a KAP questionnaire survey regarding assessing common eye diseases, socio-demographic and medical history.
Results: Participants were aged between 30 and 89 years with a mean (standard deviation) age of 51 (11.8) years. 65.5% were female and47% had no schooling. The majority (90%) had heard of blurred vision, only 4% heard of diabetic retinopathy and 58% people did not know vision loss could be prevented. Older age, people with no schooling and lower socio-economic status (SES) were associated with poorer KAP. A lower proportion (57%) of people with no schooling reported that they knew that vision loss could be prevented compared to those with School Secondary Certificate (SSC) or above (72%), $p<0.001$. Any level of education compared to no schooling (β (95% confidence interval (CI), 0.165 (0.080, 0.250), any level of access to financial resources compared to insufficient financial resources most of the time (β (95% CI), 0.290 (0.199, 0.382) reported significantly more knowledge, after multivariate adjustments for covariates.
Conclusions: Knowledge of common eye diseases is reasonably good, but knowledge of some less frequent diseases and the effect of some diseases is very limited in rural Bangladesh. Public health programs to increase knowledge of eye diseases, their risk factors and managing diseases is required to assist people living in rural Bangladesh.
Commercial Relationships: Fakir M Amirul Islam, None; Rahul Chakrabarti, None; Christine Critchley, None

417-5

Dietary Nutrients and Age-Related Cataract in the Age-Related Eye Disease Study I, A study of Prevalence, Incidence, and Progression to Cataract Extraction

Lauren Doss [1] Tanya Glaser [1] JohnPaul SanGiovanni [1] Elvira Agron [1] Emily Chew [1]
1. Clinical Trials Branch, Division of Epidemiology and Clinical Applications, National Eye Institute, National Institutes of Health, Bethesda, MD, United States.

Purpose: To evaluate relationships of dietary nutrient intake with baseline status and incidence of age-related

cataract in the Age-Related Eye Disease Study I (AREDS I).

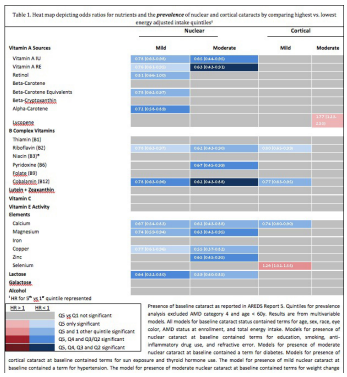
Methods: In this nested case-control and prospective study from a multi-center natural history study and phase III randomized clinical trial, we estimated baseline nutrient intake with a validated food frequency questionnaire and ascertained cataract presence, severity, and progression from annual photographs across a 12 year period. We applied repeated measures logistic regression to examine the baseline nutrient-cataract relationships, and Cox regression in the incident analysis. Analysis of cataract prevalence included 6129 eyes of 3115 participants collected over 10.4 years. Analyses of cataract incidence included 6046 eyes of 3073 participants over a 10.5 year period.

Results: Intake of vitamin A, riboflavin, B6, B12, copper, zinc, magnesium, calcium, and lactose was associated with decreased baseline prevalence of at least moderate nuclear cataract (see Figure 1 for prevalence data). Vitamin E (Odds Ratio 0.65 (0.43,0.97)) and Niacin (Odds Ratio 0.79 (0.51,1.20)) were associated with decreased incidence of nuclear cataract.

Conclusions: The results from our study suggest that dietary intake of certain nutrients may affect opacification of the lens. Specifically intakes of vitamin A, riboflavin, B12, copper, calcium, magnesium, and lactose were associated with a decreased baseline prevalence of mild and moderate nuclear cataract. Consideration of these findings in the context of those from applied biochemical research in the lens will help characterize the putative role of nutrients in cataract pathogenesis, treatment, and prevention.

Commercial Relationships: Lauren Doss, None; Tanya Glaser, None; JohnPaul SanGiovanni, None; Elvira Agron, None; Emily Chew, None

Clinical Trail: NCT00594672



Heat map depicting odds ratios for nutrients and the ***prevalence*** of nuclear and cortical cataracts by comparing highest vs. lowest energy adjusted intake quintiles

Retina 3

Moderators

Sundaram Natarajan
Aditya Jyot Eye Hospital/Maharashtra University of Health Sciences, Mumbai/Nashik, Maharashtra, India
Toshiaki Kubota
Oita University Faculty of Medicine, Oita, Oita, Japan

458-1

Changes in visual acuity and health related quality of life among patients with diabetic macular edema with and without intra-vitreal bevacizumab treatment

Dipesh Bhattarai [5] Jyoti Khadka [1] Anita Shrestha [2] Madan P. Upadhyay [3] Subodh Gnyawali [3] Sagun Narayan Joshi [4]
1. Flinders University, Adelaide, SA, Australia. 2. NHSSP, Kathmandu, Nepal. 3. B. P. Eye Foundation, Kathmandu, Nepal. 4. Department of Ophthalmology, Institute of Medicine, Kathmandu, Nepal. 5. Internship, Non Communicable Disease, WHO country office for Nepal, Kathmandu, Nepal.

Purpose: Bevacizumab is comparatively a new medicine being used in treatment of diabetic macular edema. Diabetic retinopathy with macular edema is the most potentially blinding and visually disabling complication of diabetes globally, hence leading to decreased Health Related Quality of Life (HRQOL). This study was conducted to determine the efficacy of intra-vitreal bevacizumab injection in improving visual acuity and HRQOL in patients with diabetic macular edema.
Methods: A prospective hospital based quasi experimental study was conducted in Nepal among 180 patients with diabetic macular edema. Among them 90 patients (treatment group) received intra-vitreal bevacizumab injection along with laser treatment while the other 90 patients received laser treatment only (control group). Baseline and one month follow up assessment of visual acuity, central macular thickness and HRQOL (generic and vision specific HRQOL, using SF12 and INDVFQ questionnaire respectively) were done in both groups and the findings were compared.
Results: Significant improvement in visual acuity was observed in the treatment group i.e., from 0.70 ± 0.33 to 0.60 ± 0.32 Log MAR (p value <0.05) with statistically significant reduction in central macular thickness i.e., from 486.29 ± 163.93 μm to 420.89 ± 124.92 μm (p value < 0.05). Similarly, vision specific HRQOL improved significantly in treatment group i.e., median score from 54 to 50.5 (p value <0.05) while it did not improve in control group. There was no significant change in the score of generic HRQOL in both groups. The change in vision specific HRQOL was found to be positively correlated with the change in visual acuity (ρ =0.41).
Conclusions: Intra-vitreal bevacizumab injection significantly improved the visual acuity in patients with diabetic macular edema which was found to be positively associated with improvement in vision specific HRQOL.

Commercial Relationships: Dipesh Bhattarai, None; Jyoti Khadka, None; Anita Shrestha, None; Madan Upadhyay, None; Subodh Gnyawali, None; Sagun Narayan Joshi, None
Clinical Trail: 66(6-11-E)2/070/071

459-2

Whole Exome sequencing reveals two novel mutations in the FAM161A gene causing autosomal recessive retinitis pigmentosa in Indian population

Yu Zhou [1] Zhi-lin Jiang [1] Lu-lin Huang [1] Sundaresan Periasamy [2] Xian-jun Zhu [1]
1. Key Laboratory for Human Disease Gene Study and Institute of Laboratory Medicine, Cheng Du, Si Chuan, China. 2. Department of Genetics Aravind Medical Research Foundation, Aravind Eye Hospital, Tamilnadu, India.

Study Group: Sichuan Provincial Key Laboratory for Human Disease Gene Study and Institute of Laboratory Medicine

Purpose: Retinitis pigmentosa (RP) is a heterogenous group of inherited retinal degenerations caused by mutations in at least 50 genes. The *FAM161A* gene was identified as the causative gene for RP28, an autosomal recessive form of RP. In this study, we performed a clinical and exome sequencing study of three consanguineous marriage Indian families RP-252, RP-184 and RP-182 with patients affected with retinitis pigmentosa.
Methods: A detailed patient history of the index patients and their relatives were recorded regarding disease onset, symptoms and progression. All RP patients recruited in this study were under normal ophthalmological examinations. DNA samples were collected from the index patients and their relatives and subjected to high resolution whole exome sequencing. After calling reads, mapping and filtering against multiple databases, Sanger sequencing was performed to assessment of the mutations.
Results: All of the RP patients recruited showed typical characteristics of retinitis pigmentosa disease, night blindness outset, peripheral vision impairment and progressive decrease of rod and cone photoreceptor function. By whole exome sequencing we identified several homozygous genomic regions one of which included the recently identified *FAM161A* gene mutated in RP28-linked autosomal recessive RP. Sequencing analysis revealed a novel homozygous nonsense mutation, c.1959G>T/p.E653D was in patient IV:1 of family RP-184 and a novel homozygous frame shift mutation p.R592fsX2 was in both patients of family RP-252 and RP-182.
Conclusions: In conclusion, we identified two novel homozygous mutations c.1959G>T/p.E653D and p.R592fsX2 of RP28-linked RP gene *FAM161A* in India population.

Commercial Relationships: Yu Zhou, None; Zhi-lin Jiang, None; Lu-lin Huang, None; Sundaresan Periasamy, None; Xian-jun Zhu, None
Support: National Natural Science Foundation of China (81400437)

460-3

Innovative Stem Cell Therapy for the Treatment of Retinitis Pigmentosa

Wei He [1]

1. He University Eye Hospital, Shenyang, China.

Purpose: Human retinal progenitor cells (hRPCs) are expandable in vitro and represent a possible therapy for retinal degenerative diseases. This study aimed to investigate the therapeutic potential of hRPCs transplanted into a Royal College of Surgeons (RCS) rat model of retinal degeneration and the mechanisms of hRPCs transplantation for the protection of visual function.
Methods: Congenic RCS rats at 21 days old were used in this study and divided into three groups. Group A (n=14) received a vitreous injection of 5 μl of hRPC cell suspension (about 2 × 105cells). Group B (n=14) received a vitreous injection of 5 μl of vehicle only (HBSS). In both groups, the left eyes (OS) were the treatment eyes, while the right eyes (OD) served as controls and were untreated. Lastly, group C (n=6) was kept as a baseline control without vitreous injection. To monitor the efficacy of hRPCs in preserving vision, ERG studies were performed at 2, 4, 8, 12 weeks post-treatment before the animals were sacrificed for histology and gene chips analysis. Following ERG recordings, rats were sacrificed and retinal cross-sections were prepared for histological evaluation of transplant survival and preservation of the ONL. Or the rats were sacrificed and total RNA was eluted for Microarray analysis.
Results: Bright-flash ERG responses were tested at 2, 4, 8, 12 weeks post-transplantation of hRPCs by measuring the electrical activity of the outer (a-wave) and inner (b-wave) retina. hRPCs-grafted animals achieved the significant b-waves changes over background performance compared to vehicle-injected animals(P4W=0.0108<0.05, P8W=0.0355<0.05, P12W=0.0267<0.05) after 4 and 8 weeks post-transplantation. The histological analysis shows that the outer nuclear layers are completely atrophied in vehicle-injected and untreated animals, but there are still remaining some outer nuclear layer cells in hRPCs-grafted animals.
Conclusions: In RCS rat model of retinal degeneration, intravitreal injection of hRPCs could protect the retinal photoreceptor cells in RCS rats; The protective effect may due to the function of nutritional factors generated by the hRPCs ; Using hRPCs-secreted nutritional factors may be a way for the treatment of retinal degenerative diseases; The detail mechanisms are being investigated .
Commercial Relationships: Wei He, None

461-4

Changes of cytokines levels in aqueous humor at the induction phase of aflibercept treatment in age-related macular degeneration

Tomohito Sato [3] Masaru Takeuchi [3] Yoko Karasawa [3] Masataka Ito [1] Toshio Enoki [2]

1. Developmental Anatomy and Regenerative Biology, National Defense Medical College, Tokorozawa, Japan. 2. Ophthalmology, Enoki Eye Clinic, Sayama, Japan. 3. Ophthalmology, National Defense Medical College, Tokorozawa, Japan.

Purpose: To investigate changes of proinflammatory cytokines at induction phase of aflibercept treatment in exudative age-related macular degeneration (AMD).
Methods: Nine eyes of 9 patients with exudative AMD who had undergone intravitreal injection of aflibercept (IVA) were studied. Undiluted aqueous humor samples were collected at the time of initiation and before third IVA. PDGF-BB, IL-1 β , IL-1r α , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17A, Eotaxin, bFGF, G-CSF, GM-CSF, IFN- γ , IP-10, MCP-1, MIP-1 α , MIP-1 β , RANTES, TNF- α and VEGF in the aqueous humor were measured by the Bio-Plex kit® (Bio-Rad Laboratories, Inc.). The cytokines with values below limits of detection were assigned negative or a numerical value of 0 pg/ml for statistical analysis. The positive rates and levels of cytokines in the aqueous humor were measured.
Results: In the aqueous humor of before third IVA, the positive rates and cytokines levels of IL-12 and VEGF were lower than those of the time of initiation. There was no significant difference in other cytokines without IL-12 and VEGF between the time of initiation and before third IVA. In correlations of VEGF and other cytokines, there was the positive correlation between VEGF and IL-12 both the time of initiation and before third IVA. There was no significant correlation between VEGF and other cytokines without IL-12 both the time of initiation and before third IVA.
Conclusions: The present study indicated that the level of IL-12 known as a T cell stimulating factor was decreased by IVA, and positively correlated with the level of VEGF in the aqueous humors of AMD patients. The findings suggest that IVA induces an inhibition of Th1 immune induction at the induction phase of aflibercept treatment in AMD patients.
Commercial Relationships: Tomohito Sato, None; Masaru Takeuchi, None; Yoko Karasawa, None; Masataka Ito, None; Toshio Enoki, None

462-5

Mechanisms of action of human iPSC-derived neural progenitor cells on treating retinal degenerative disease

Yuchun Tsai [1] Bin Lu [1] Dhruv Sareen [1] Clive Svendsen [1] Shaomei Wang [1]

1. Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, United States.

Purpose: Progression in stem cell-based therapy has been shown to be promising for treating retinal degenerative diseases. An ideal cell type to treat retinal degeneration should fulfill the following criteria, efficient preservation of vision, unlimited cell sources, no ethical problems, and no immune rejection. Recently, we have found that human neural progenitor cells derived from induced pluripotent stem cells (iNPCs) can preserve both photoreceptors and visual functions after grafting into the subretinal space of the Royal College Surgeon (RCS) rats. Noteworthy, grafted cells migrated along the subretinal space and a layer of extracellular matrix-like (ECM) material was observed in area where iNPCs were distributed. Here, we study

whether ECM plays a key role in vision protection offered by iNPCs.
Methods: iNPCs monolayer was co-cultured underneath retinal explants from adult RCS (P42-56) or wild type rats. 7 days after co-culture, explants were fixed and the photoreceptor cell survival were examined by DAPI staining and TUNEL assay. The iNPCs were collected and the mRNA expression of ECM related genes were analyzed by human extracellular matrix and adhesion molecules PCR Array (QIAGEN). The conditioned medium which might contain ECMs secreted by iNPCs was examined by multiplexed ECM detection assays (tebubio). Cell migration was analyzed by Cultrex cell migration assay.
Results: The adult RCS rat retinal explant was more susceptible to cell death compared with wild type at day 7 and co-cultured with iNPCs reduced the cell death of photoreceptors. The mRNA expression of matrix metallopeptidase 2 (MMP2), MMP 9, selectin, secreted phosphoprotein 1, E-cadherin, and Integrin β 4 were significantly upregulated, whereas collagen, laminin, and connective tissue growth factor were shown to be downregulated. MMP2 and MMP9 were identified to be more potent in the conditioned medium. Secretion of MMP2 and MMP9 by iNPCs promoted iNPCs cell migration *in vitro*.
Conclusions: The results underscore the potential therapeutic mechanisms underlying the improvement of further stem cell-based therapy to treat age-related macular degeneration and other degenerative retinal diseases.
Commercial Relationships: Yuchun Tsai, None; Bin Lu, None; Dhruv Sareen, None; Clive Svendsen, None; Shaomei Wang, None
Support: The Knight Templar Eye Foundation carer start grant

463-6

rAAV.sFlt-1 gene therapy human trial for wet age related macular degeneration

Elizabeth P. Rakoczy [1] Choo-May Lai [1] Aaron L. Magno [2] Matthew Wikstrom [1] Martyn A. French [3] Steven D. Schwartz [4] Mark S. Blumenkranz [5] Thomas W. Chalberg [6] Mariapia A. Degli-Espoti [1] Ian J. Constable [1]
1. Centre for Ophthalmology and Visual Sciences, The University of Western Australia, Perth, WA, Australia. 2. Molecular Ophthalmology, Lions Eye Institute, Nedlands, WA, Australia. 3. Scool of Pathology and Laboratory Medicine, The University of Western Australia, Perth, WA, Australia. 4. Ophthalmology, University of California, Los Angeles, Los Angeles, CA, United States. 5. Ophthalmology, Stanford University, Palo Alto, CA, United States. 6. Avalanche Biotechnologies, Menlo Park, CA, United States.

Purpose: To assess whether a single subretinal rAAV. sFlt-1 injection is safe for the treatment of wet age related macular degeneration and whether it results in an improvement of vision over a period of 1 year.
Methods: Six subjects received 100 ul injection of rAAV.sFlt-1 (3 low dose, 10E10 vg and 3 high dose, 10E11 vg; 2 control subjects were treated only with protocol ranibizumab. All subjects received 0.5 mg ranibizumab at baseline and Day 30 followed by rescue based on prespecified criteria. Laboratory tests included haematology, renal and hepatic function, electrolytes, urine protein and IgM, IgG, IgA and lymphocyte subset analysis. In addition, assays for anti-AAV antibodies, neutralizing antibodies, and ELISpot were also performed. Ophthalmic safety was assessed by biomicroscopic examination, IOP, indirect ophthalmic examination, OCT, color fundus photography and fluorescein angiography.
Results: Subretinal injection of rAAV.sFLT-1 was highly reproducible. Clinical laboratory assessments generally remained unchanged from baseline. Four of six (66%) treatment group patients required zero rescue injections, and two of six (33%) required only one rescue injection, while Control group patients required an average of three injections during the 52-week follow up period. Mean Best Corrected Visual Acuity (BCVA) increased by 7 ☒ 5 letters in the Treatment group and decreased by 3 ☒ 5 letters in the Control group. No adverse events were related to gene therapy.
Conclusions: rAAV.sFLT-1 was safe and well tolerated. Preliminary efficacy, based on the maintenance of BCVA, was observed. These results support ocular gene therapy as a potential long-term treatment option for wet AMD.
Commercial Relationships: Elizabeth Rakoczy, Avalanche Biotechnologies (C), Avalanche Biotechnologies (P); Choo-May Lai, Avalanche Biotechnologies (F), Avalanche Biotechnologies (P); Aaron Magno, Avalanche Biotechnologies (F); Matthew Wikstrom, None; Martyn French, None; Steven Schwartz, Avalanche Biotechnologies (I), Avalanche Biotechnologies (P); Mark Blumenkranz, Avalanche Biotechnologies (I), Avalanche Biotechnologies (P); Thomas Chalberg, Avalanche Biotechnologies (E), Avalanche Biotechnologies (I), Avalanche Biotechnologies (P); Mariapia Degli-Espoti, None; Ian Constable, Avalanche Biotechnologies (F), Avalanche Biotechnologies (P)
Support: National Health and Medical Research Council of Australia
Clinical Trail: NCT01494805

Visual Psychophysics/Physiological Optics - Paper

Moderators

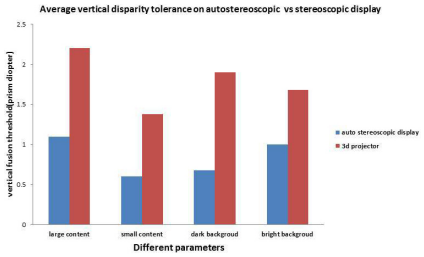
Hiroshi Uozato
Niigata University of Health and Welfare, Niigata, Niigata, Japan
Yasuki Ito
Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan

464-1

Human vertical disparity tolerance differences between 3D stereoscopic and autostereoscopic displays

Xinzhu Sang [1] Di Zhang [2] Peng Wang [1] Jean Louis de Bougrenet de la Tocnaye [2]
1. State Key Laboratory of Information Photonics and Optical Communications, Beijing University of Posts and Telecommunications, Beijing, China. 2. Optics Department, Telecom Bretagne, Brest, France.

Purpose: Vertical disparity is an important factor to test our resilience to binocular disparity perception, in relief images displayed in different 3D display devices because exacerbating the capability of our fusion process and being at the origin of a potential fatigue. Varied methods have been proposed to assess the 3D content quality based on different display platforms, with respect to the impact factors including horizontal disparity, disparity velocity, luminance contrast et al. Here we used the vertical disparity as a test parameter because it is more sensitive for human vision than the horizontal disparity which is extensively considered in 3D comfort evaluation. For this reason, a comprehensive comparison for the two main 3D display systems was carried out based on vertical disparity tolerance tests.
Methods: Various images were depicted on 3D projectors and autostereoscopic TV. 29 subjects (mean age = 22.8 years) with normal vision were divided into two groups to participate the tests. Since the two mechanisms have different properties, factors that will affect the viewing experiences are not the same. Here we considered the common factors that are important for both, including the image background, the 3D content, the room lighting, the viewing distance and the vertical disparity velocity. Vertical disparity fusion amplitude was recorded as reference to assess the tolerance.
Results: The human vertical disparity threshold for 3D stereo was larger than for auto-stereoscopic TV. Parameters concerned in our experiments affected subject performance differently. The significant factors impacting both systems were, image background (autostereoscopic TV, $P=0.045$; projector, $p = 0.031$), 3D contents (autostereoscopic TV, $P=0.016$; projector, $P<0.001$) and the disparity velocity (autostereoscopic TV, $P=0.044$; projector, $P < 0.001$). For the viewing distance and room lighting, they had effects on 3D projector ($P<0.001$) but not on autostereoscopic TV.
Conclusions: Our study compared two different mechanisms for stereoscopic displays. A better vertical disparity tolerance has been obtained with 3D projectors. Thus, discrepancies on different platforms should be noticed when 3D contents were generated or specific parameters were measured.
Commercial Relationships: Xinzhu Sang, None; Di Zhang, None; Peng Wang, None; Jean Louis de Bougrenet de la Tocnaye, None



Averaged vertical fusion threshold for each group were calculated corresponding to different test parameters.

465-2

Attempts to measure longitudinal chromatic aberration with polychromatic eccentric photorefraction

Yun Chen [1] Arne Ohlendorf [2] Frank Schaeffel [1]
1. Institute for Ophthalmic Research,University Tuebingen, Tuebingen, Germany. 2. ZEISS Vision Science Lab, Institute for Ophthalmic Research, University Tuebingen, Tuebingen, Germany.

Purpose: Infrared eccentric photorefraction is widely used to measure refractive errors in animal models and humans (Schaeffel et al. 1993). If photorefraction is performed using a flash of white light, generated by white LEDs, a separate analysis of the R,G and B channel of the video camera could basically provide LCA.We have studied calibration issues, the effect of variations in fundal reflectance due to macular pigment and the effect of higher order aberrations of the eye.
Methods: Calibration of white light photorefraction was done simultaneously in the R,G,and B channel by holding trial lenses (-4D to +4D) in front of subjects' eyes. Pupil size was controlled by a 5mm artificial pupil.10 eyes of Caucasian were calibrated in a dim room under 3 conditions:1) in the fovea 2)10 degrees off-axis in the temporal retina 3)in the fovea but after 10 mins exposure to bright light. Higher order aberrations were measured by WASCA wavefront Analyzer (Carl Zeiss,Germany). Macular pigment optical density(MPOD) was measured by a psychophysical procedure (MPOD Tinsley,UK). Ocular parameters were correlated with the calibration factors (= conversion factors, converting the brightness slope in the pupil into refractive error; Schaeffel et al 1993).
Results: Conversion factors were highly variable among subjects and much more variable than in infrared light, from 3.81 to 13.83 in condition 1, 2.26 to 8 in 2, and 7.64 to 15.97 in 3. There were many significant correlations to ocular parameters, conversion factors fovea to temporal retina ($R=0.831$ in R, 0.843 in G, 0.757 in B,$df=9,p<0.01$), conversion factors to spherical aberration (R from 0.816 to 0.757,$df=9,p<0.01$), and to refractive error of the subjects

in the fovea (R from 0.843 to 0.827,df=9,p<0.01). Conversion factors were highly correlated to MPOD in G and B in the fovea but not at 10 degree in the temporal retina.
Conclusions: A number of ocular parameters influence the calibration of white light photorefraction, like MPOD, refractive error, and spherical aberration. While the effect of the first two variables may trace back to variations in fundal reflectance (using the non-linear video system response curve in different ranges which causes different brightness slopes), the correlation to spherical aberration is unexpected and requires further studies. These factors have to be controlled before polychromatic photorefraction can be used to measure LCA.
Commercial Relationships: Yun Chen, None; Arne Ohlendorf, Car Zeiss Vision International GmbH (E); Frank Schaeffel, None
Support: OpAL (Optical and Adaptational Limits of Vision); OpAL is an Initial Training Network funded by the European Commission under the Seventh Framework Program (PITN-GA-2010-264605)

466-3

Ultra-High Resolution Spectral-Domain Polarization-Sensitive Optical Coherence Tomography for Retinal Imaging

Barry Cense [1] Bas Biezemans [1] Ernesto Garcia Ruiz [2]
1. Center for Optical Research and Education, Utsunomiya University, Utsunomiya, Tochigi, Japan. 2. National Instruments Japan Corporation, Nagoya, Japan.

Purpose: In glaucoma, a decrease in the birefringence (an optical property) of the retinal nerve fiber layer can be an early indicator of the disease. Macular diseases such as age-related macular degeneration and diabetic retinopathy are known to disrupt the organization of Henle' s fiber layer. Since the organization is a source of phase retardation, a decrease in retardation may be a sensitive indicator of macular disease. In comparison to standard spectral-domain polarization-sensitive OCT (SD-PS-OCT), ultra-high resolution SD-PS-OCT provides structural information as well as retardation and fast axis orientation data at an unprecedented axial resolution, which may be important for the early detection of retinal diseases.
Methods: Two new ultra-high resolution spectral-domain polarization-sensitive OCT systems were designed. One design used a Wollaston prism to split the two orthogonal states and image them adjacent on the same line scan detector, while in the other a beam displacer was used to offset the two orthogonal states on two lines of a dual line scan camera. Images were obtained from the macula and the optic nerve. Data was analyzed using Stokes vector analysis, to retrieve the double pass phase retardation. The coherence length, sensitivity and roll-off were quantified on a mirror in a model eye.
Results: Using the Wollaston-prism system, a coherence length of 3.0 μm was measured in tissue (n = 1.38). With 69.6 nW incident on the spectrometer and a spectrometer efficiency of 16%, the measured sensitivity was 91.25 dB, which is 1 dB below the theoretical sensitivity of a spectral-domain polarization-sensitive system. The sensitivity roll-off was 10 dB over the first mm. Images obtained from the macula and optic nerve showed double pass phase retardation data similar to data obtained with PS-OCT systems, however, at an higher axial resolution.
Conclusions: PS-OCT permits mapping of the two-dimensional double-pass phase retardation distribution in the macular region and optic nerve with an axial resolution of ~3μm.
Commercial Relationships: Barry Cense, Topcon (F), Utsunomiya University (P); Bas Biezemans, None; Ernesto Garcia Ruiz, None

467-4

Label-free Imaging of the Retina

Cong Ye [1] Sicong He [2, 3] Qiqi Sun [2, 3] Jianan Y. Qu [2, 3] Christopher K. Leung [1]
1. Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Kowloon, Hong Kong. 2. Department of Electronic and Computer Engineering, Hong Kong University of Science and Technology, Kowloon, Hong Kong. 3. Center for System Biology and Human Health, Hong Kong University of Science and Technology, Kowloon, Hong Kong.

Purpose: This study aims at investigating the feasibility of label-free imaging of the retina and retinal ganglion cells (RGCs) using a multimodal nonlinear optical (NLO) microscope.
Methods: An NLO microscope integrating stimulated Raman scattering (SRS), two-photon excitation fluorescence (TPEF), and second-harmonic generation (SHG) was devised. Immediately before the NLO imaging, unfixed flat-mounts of the retina-choroid-sclera complex were freshly prepared from eyes of ICR, Thy1-YFPG transgenic mice (10-80% of RGCs labeled with yellow fluorescent protein (YFP)), and Thy1-YFPG mice after retrograde-labeling of RGCs with DiI. Images were acquired for analysis.
Results: TPEF (NADH, all-trans-retinol, and lipofuscin), SRS vibrational, and SHG signals were collected to visualize the structures with different depths of the retina-choroid-sclera complex. As shown in Fig.1, SRS imaging had a better performance in revealing the morphologies of the retinal neurons and their neurites, while TPEF and SHG signals were better in visualizing the retinal pigmented epithelium and sclera, respectively. SRS imaging of the RGCs was further validated using Thy1-YFPG mice and retrograde-labeling. It was evident in Fig.2 that the RGCs and nerve fiber bundles were clearly shown in SRS images and corresponding well with the YFP-labeled (upper panel) and retrograde-labeled (lower panel) RGCs in TPEF images, indicating label-free TPEF and SRS imaging could be a powerful tool to visualize individual RGC.
Conclusions: NLO imaging has been demonstrated to be feasible in *ex vivo* label-free imaging of the retina. Further study is required to explore its potential in imaging the retina *in vivo*.
Commercial Relationships: Cong Ye, None; Sicong He, None; Qiqi Sun, None; Jianan Y. Qu, None; Christopher K. Leung, None

Fig.1 Representative TPEF and SRS images of the flat-mount of the retina-choroid-sclera complex dissected from an ICR mouse eye. (a1)-(a2) TPEF and SRS images of the ganglion cell layer; (b1)-(b2) inner plexiform layer; (c1)-(c2) inner nuclear layer; (d1)-(d2) outer nuclear layer; (e1)-(e2) retinal pigmented epithelium; (f1)-(f2) sclera; (g) paraffin section of the posterior part of an ICR eye stained with hematoxylin-eosin showing the locations of the TPEF and SRS images (a)-(f). The field of view of (a)-(f) is 100μm × 100μm. Scale bar in (g): 50μm.

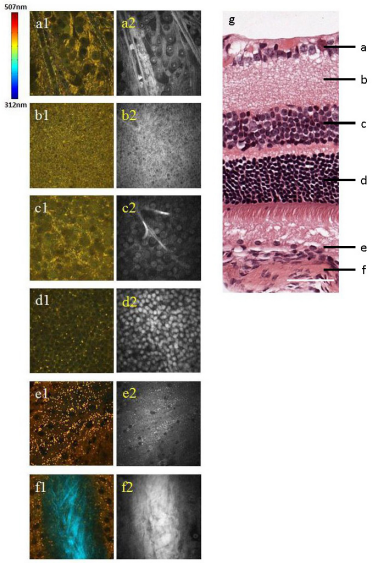
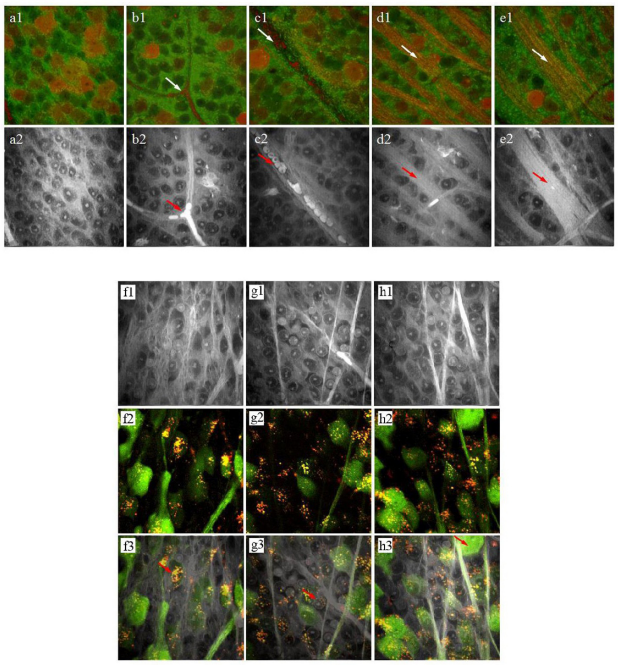


Fig.2 Representative TPEF and SRS images of the retinal ganglion cells (RGCs) and retinal nerve fiber bundles of the flat-mounts of the retina-choroid-sclera complex dissected from a Thy1-YFPG transgenic mouse eye (upper panel) and a Thy1-YFPG mouse eye after retrograde labeling of RGCs with DiI (lower panel). Upper panel: (a1)-(e1) TPEF images; green: NADH; orange: yellow fluorescent protein (YFP); red: hemoglobin; (a2)-(e2) SRS images. Arrow in (b&c): red blood cells. Arrow in (d&e): retinal nerve fiber bundles. Lower panel: (f1)-(h1) SRS images; (f2)-(h2) TPEF images of the YFP (green) and DiI (yellow) signals; (f3)-(h3) overlaying images of SRS, YFP, and DiI signals. Arrow in (f3): co-localization of SRS, YFP, and DiI signals in one RGC. Arrow in (g3): co-localization of SRS and DiI signals in one RGC. Arrow in (h3): co-localization of SRS and YFP signals in one RGC. The field of view of all images is 100μm × 100μm.



468-5

Functional visual acuity of early presbyopia

Yusaku Katada [1] Kazuno Negishi [1] Kazuhiro Watanabe [1] Yuta Shigeno [1] Megumi Saiki [1] Hidemasa Torii [1] Minako Kaido [1] Kazuo Tsubota [1]

1. Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan.

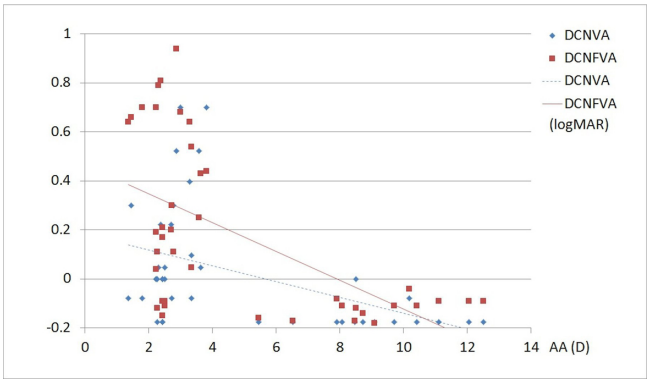
Purpose: To evaluate the visual function in early presbyopia subjects using functional visual acuity (FVA) test.

Methods: This study included twenty-seven eyes of 27 healthy older volunteers (mean age, 44.1 ± 2.6 years) and fifteen eyes of 15 healthy young volunteers (mean age, 28.4 ± 4.8 years). Distance corrected visual acuity (DCVA), distance corrected near visual acuity (DCNVA), subjective amplitude of accommodation (AA), distance and near pupil diameter were measured. Distance functional visual acuity (DFVA), distance corrected near functional visual acuity (DCNFVA) were measured using the FVA Measurement System (AS-28, Kowa Co Ltd, Aichi, Japan). The standard Schirmer test without topical anesthesia and the standard tear break up time measurement was also performed.

Results: There is no significant difference in DFVA between older and young subjects. However, the DCNFVA was significantly lower than DCNVA in older subjects ($P < 0.001$), and the difference between DCNVA and DCNFVA (the subtraction of DCFVA from DCNVA) was significantly larger in older subjects than that in young subjects ($P = 0.009$). A significant linear negative correlation was observed between DCNVA and AA ($r = -0.601$, $P < 0.001$), DCNFVA and AA ($r = -0.692$, $P < 0.001$) in older subjects. According to the regression equation, the decrement of DCNFVA for the decrement of 1 diopter in AA was greater than that of DCNVA for the all subjects. Stepwise regression analysis revealed that AA and near pupil diameter were significant prognostic factors for DCNFVA in the model. Tear function parameters were not adopted into the regression model.

Conclusions: DCNFVA can detect decreased near visual performance in early presbyopia more sharply than conventional near visual acuity, and can detect the masked presbyopia with normal near visual acuity. DCNFVA might be a good index for decreased accommodation amplitude in early presbyopia.

Commercial Relationships: Yusaku Katada, None; Kazuno Negishi, None; Kazuhiro Watanabe, None; Yuta Shigeno, None; Megumi Saiki, None; Hidemasa Torii, None; Minako Kaido, US patent no, 7470026 (P); Kazuo Tsubota, US patent no, 7470026 (P)



Correlation between the distant corrected near visual acuity (DCNVA) and amplitude of accommodation (AA) ($r = -0.601$, $p < 0.001$), and distant corrected near functional visual acuity (DCNFVA) and AA ($r = -0.692$, $p < 0.001$). Significant linear correlations were observed in the both combinations, and the slope of the linear regression between DCNFVA and AA was steeper than that between DCNVA and AA.

469-6

Accommodative Movements in the Anterior and Posterior Segment, Implications for Presbyopia and Glaucoma

Paul L. Kaufman [1, 2] Julie Kiland [1] Gregg Heatley [1] Timothy Michael Nork [1] Jared McDonald [1] Alexander Katz [1] Elke Lütjen-Drecoll [3] Mary Ann Croft [1]

1. Ophthamology and Vis. Sci., University of Wisconsin, Madison, WI, United States. 2. Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, WI, United States. 3. Institute of Anatomy II, University of Erlangen–Nuremberg, Erlangen, Nuremburg, Germany.

Purpose: We seek to better understand human accommodative amplitude and presbyopia.

Methods: A stimulating electrode was inserted into the Edinger-Westphal nucleus (midbrain) to induce accommodation in seven iridectomized rhesus monkeys (ages 5 to 27 years old). Accommodation was measured (Hartinger) and ultrasound biomicroscopy (UBM; 50, 35, 20 MHz), endoscopy and fundus photography were utilized to image the various intraocular structures during accommodation in phakic and aphakic eyes. Various contrast agents (i.e., triamcinolone, fluorescent microspheres) were used to enhance visualization of the intraocular structures and fluid movements during accommodation.

Results: The peripheral capsule moves forward while the central capsule bows backward in the aphakic eye. The PVZ INS-LE zonula which, attaches anteriorly to the posterior lens equator and posteriorly to the vitreous zonule insertion zone, moves forward during accommodation in the presence/absence of the lens or the lens capsule. There are accommodative/disaccommodative movements of the elastic choroid network and of the vitreous that occur throughout the posterior segment and extend all of the way back to the region of the optic nerve. The movements are choroid are ten-fold greater in the region of the ora serrata than in the region of the optic nerve. All of these movements decline with age. In addition, there was an age-related aggregation of vitreous fibers peripherally and these vitreous fiber aggregates were in close juxtaposition with and were attached to the vitreous zonule.

Conclusions: There are extralenticular-zonular structures and vitreous movements that are linked to accommodation. The age-related increase in vitreous fibers attached to the vitreous zonule may contribute to posterior restriction of the ciliary muscle and vitreous zonule. Age-related immobility of the ciliary muscle due to posterior restriction may limit accommodation and physically stress the optic nerve head as we age. Eliminating such restrictions may restore mobility of the muscle, facilitate the function of accommodating intraocular lenses (IOLs), and mitigate accommodative stress to the optic nerve region. Identification and study of all the intraocular structures associated with accommodation will increase understanding of how the eye accommodates earlier in life, and change how we understand and treat presbyopia and perhaps glaucoma.

Commercial Relationships: Paul Kaufman, Refocus Group Inc. (C), Alcon (C), Johnson and Johnson (C), Z-Lens, LLC (C); Julie Kiland, None; Gregg Heatley, None; Timothy Michael Nork, None; Jared McDonald, None; Alexander Katz, None; Elke Lütjen-Drecoll, None; Mary Ann Croft, Refocus Group (C), Refocus Group (R)

Support: CR, C, F. Support, NEI (RO1 EY10213 & R21 EY018370 to PLK, Core Grant for Vision Research Grant # P30 EY016665), RPB, NIH Grant # 5P51 RR 000167 to WNPRC, OPREF.

Immunology/Microbiology - Paper

Moderators

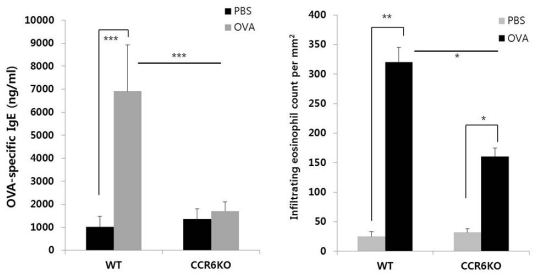
Justine Smith
Flinders University, Adelaide, South Australia, Australia
Atsuki Fukushima
Kochi Medical School, Nankoku, Kochi, Japan

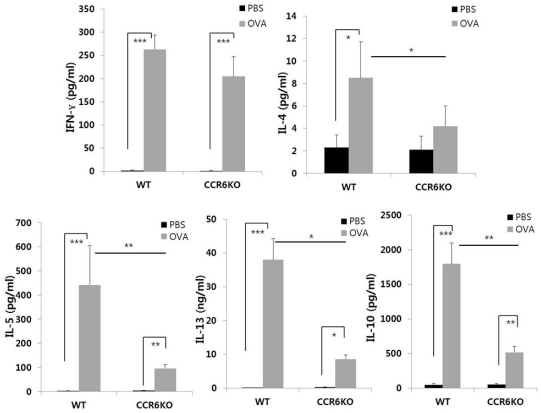
562-1

The C-C Chemokine receptor 6 (CCR6) is crucial for Th2-driven allergic conjunctivitis

So-Hyang Chung [1] Choun-Ki Joo [1]
1. Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea (the Republic of).

Purpose: Allergic conjunctivitis from an allergen-driven Th2 response is characterized by conjunctival eosinophilic infiltration. Although CCL20-CCR6 axis has been reported to play a proinflammatory role in several murine models of autoimmune diseases including allergic diseases, their underlying mechanism need to be investigated. We here examined whether CCL20-CCR6 axis could play a role in the development of allergic conjunctival inflammation using murine experimental allergic conjunctivitis (EAC) model induced by ovalbumin (OVA) allergen.
Methods: Mice were challenged with two doses of OVA via conjunctival sac after systemic challenge with OVA in alum. Several indicators for allergy were comparatively evaluated in wild-type and CCR6 KO EAC mice.
Results: Wild-type mice challenged with OVA via conjunctival sac following systemic challenge with OVA in alum had severe allergic conjunctivitis. The absence of CCR6 suppressed IgE-mediated and conjunctival inflammation (Fig.1). Reduced allergic inflammation was ascribable to reduced cytokine responses from Th-2 type and Treg were in draining lymph node although Th-1 type responses and dendritic cell subsets are not affected by the absence of CCR6 (Fig.2).
Conclusions: Our findings suggested that CCR6 might be crucial for optimal development of Th2 immune responses and further allergic conjunctival inflammation in EAC model.
Commercial Relationships: So-Hyang Chung, None; Choun-Ki Joo, None
Support: This work was supported by a grant of the Korea Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI13C0016).





563-2

Regulatory T Cell Dysfunction in High-Risk Corneal Transplantation

Takenori Inomata [1] Jing Hua [1] Sang-Mok Lee [2] Tina Shiang [1] Homer Chang [1] Qiang Zhang [1] Reza Dana [1]
1. Ophthalmology, Schepens Eye Research Institute/ Massachusetts Eye and Ear Infirmary/ Harvard Medical School, Boston, MA, United States. 2. Ophthalmology, Hallym University College of Medicine, Gyeonggi-do, Korea (the Republic of).

Purpose: To determine kinetics and function of $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) and peripherally induced Neuropilin-1 $(Nrp\text{-}1)^-$ Tregs in high-risk (HR) vs. low-risk (LR) corneal transplants.
Methods: To induce inflamed high-risk graft beds, intrastromal corneal sutures were placed two weeks prior to transplantation in mice (n=5). Ipsilateral draining lymph nodes (DLNs) and corneas were harvested 14 days after transplantation. Angiogenesis and lymphangiogenesis in the graft were assessed by CD31 and LYVE-1 immunofluorescence staining, respectively. DLNs were analyzed for IFN-γ^+CD4^+ T effector cells, Tregs, $Nrp\text{-}1^-$Tregs and their expression of inhibitory molecules such as CTLA-4 and PDL-1. Secretory cytokines (IL-10, Latent TGF-β, and IFN-γ) were measured using ELISA. The suppressive function of Tregs on T effector cells was evaluated using an *in vitro* proliferation assay, and proliferation of T effector cells was determined via BrdU incorporation.
Results: Frequencies of Tregs among $CD4^+$ T cells were significantly reduced in HR compared to LR recipients (p=0.024), whereas the frequencies of T effector cells were significantly increased in HR compared with LR recipients (p=0.038). Frequencies of $Nrp\text{-}1^-$ Tregs among $CD4^+$ T cell were significantly reduced in HR recipients compared with LR recipients (11%, p<0.01). CTLA-4 and PDL-1 expression was significantly decreased with in $Nrp\text{-}1^-$Tregs of HR compared with LR recipients (CTLA-4; p=0.038, PDL-1; p=0.0459). IL-10 expression among $Nrp\text{-}1^-$Tregs of HR recipients was significantly lower compared with LR recipients (p<0.001). Latent TGF-β and IFN-γ among $Nrp\text{-}1^-$Tregs were significantly higher in HR compared with LR recipients (p<0.01). The suppressive function of Tregs

and Nrp-1-Tregs from HR hosts was significantly reduced compared with Tregs and Nrp-1-Tregs from LR recipients (Tregs; $p<0.001$, Nrp-1-Tregs; $p<0.05$). The formation of blood vessels and lymphatics was significantly increased in HR grafts compared with LR grafts (blood vessels, $p<0.0001$, lymphatics, $p=0.047$).

Conclusions: Impaired induction and function of peripherally induced Nrp-1- Tregs after transplantation in inflamed (HR) graft beds correlates with the loss of graft tolerance and subsequent rejection.

Commercial Relationships: Takenori Inomata, None; Jing Hua, None; Sang-Mok Lee, None; Tina Shiang, None; Homer Chang, None; Qiang Zhang, None; Reza Dana, None

Support: NIH Grant, EY 12963

564-3 Withdrawn

CD73/adenosine axis contributes to the immunosuppressive ability of mesenchymal stem cells in experiment autoimmune uveitis

Xiaomin Zhang [1]

1. Tianjin Medica University Eye Hospital & Eye Institute, Tianjin, China.

Purpose: To investigate the role of CD73/adenosine axis in the immunosuppressive ability of mesenchymal stem cells (MSCs) in experimental autoimmune uveitis (EAU).

Methods: EAU was induced in C57BL/6 mice by adoptive transfer of IRBP 1-20 specific T cells. AD-MSCs were injected intravenously into the mice on day 10 after transfer. Alternatively, MSCs were pretreated with APCP, a CD73 inhibitor, before infusion to block the function of CD73 on MSCs. Clinical characteristics, histopathological changes and electroretinography were observed. In vitro, the proliferation and differentiation of T cells from both human and mouse were assessed, and the changes of CD73 expressions on human and murine T cells on co-culture with MSCs were analyzed by flow cytometry.

Results: MSC treatment significantly reduced the severity of EAU, protected the structure and function of retina, while APCP-pretreated MSCs lost most of the therapeutic effect. In vitro, CD73 on MSCs could cooperate with CD39 on T cells to produce adenosine, an immunosuppressive factor, resulting in inhibition of T cell proliferation. MSCs could also increase the ratio of Treg/ Th17 cells via CD73 expression. On co-culture with MSCs, CD73 expression on CD4+ T cells increased. TGF- β 1 was the only cytokine that contribute to CD73 up-regulation, and blocking TGF-β 1 produced by MSCs abrogated this effect.

Conclusions: CD73/adenosine axis mediated the immunomodulatory function of MSCs in autoimmune uveitis. Our findings imply an additional and novel mechanism that participates in the immunomodulatory function of MSCs for the treatment of autoimmune diseases.

Commercial Relationships: Xiaomin Zhang, None

565-4

Epigenetic Therapy for Ocular Inflammation

Lai Wei [1]

1. State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China.

Purpose: Uveitis is responsible for up to 20% of all blindness. $CD4^+$ T cell mediated ocular inflammatory diseases have been treated with corticosteroids, immunosuppressive agents and biologics targeting key inflammatory cytokines. However, many patients are resistant or intolerant to current therapies. Recent advance in epigenetic therapies in cancer provides a new outlook for treating ocular inflammation. We therefore investigated whether an epigenetic therapy drug, Zebularine, can potentially control the inflammation model of human retinal pigment epithelium (RPE) cells *in vitro*.

Methods: Illumina High-throughput sequencing technology was used to profile the genome-wide modification patterns of H3K4me3 in RPE cells. Real-time PCR was performed to measure the RNA expression of genes of interest in RPE cells.

Results: To understand the molecular mechanisms by which Zebularine may control ocular inflammation, we investigated the effects of Zebularine on the human RPE cells' response to inflammatory cytokines. Using Illumina high-throughput sequencing technology, we profiled the genome-wide modification patterns of H3K4me3 in RPE cells treated with or without Zebularine in combination with IL-1b and TGF-b. The overall H3K4me3 level was significantly reduced by Zebularine. In addition, IL-1b mediated inflammatory gene induction, such as IL-6 expression, was significantly reduced by Zebularine in RPE cells.

Conclusions: Our results suggest that Zebularine may reduce inflammatory response of RPE cells by controlling overall H3K4me3 patterns as well as key cytokines and transcription factors responsible for the ocular inflammation in RPE cells.

Commercial Relationships: Lai Wei, None

566-5

Characteristic T-cell populations in the vitreous fluid of eyes with viral infection-associated uveitis

Kazuichi Maruyama [1] Tohru Inaba [2] Sunao Sugita [3] Hiroshi Kunikata [1] Manabu Mochizuki [4] Toru Nakazawa [1]

1. Ophthalmology, Tohoku University Graduate School of Medicine, Sendai, Japan. 2. Infection Control and Laboratory Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan. 3. RIKEN Center for Development Biology, Kobe, Japan. 4. Ophthalmology, Tokyo Dental and Medical University, Tokyo, Japan.

Purpose: Previously, we found that the T-cell population in the vitreous fluid of sarcoidosis patients had a high CD4/CD8 ratio and a high %CD4. However, the T-cell population in patients with other types of granulomatous uveitis is still not well understood. Here, we analyzed the

T-cell population in granulomatous uveitis associated with viral infection, and compared our findings with those from sarcoidosis granulomatous uveitis.
Methods: Prospective clinical case series. This study enrolled 18 eyes of 18 viral infection patients (13 male and 4 female) and 53 patients with definitive sarcoidosis. In viral infection, diagnoses included 4 eyes of 4 patinets with varicella zoster virus (VZV)-associated acute retinal necrosis (ARN), 2 eyes of 2 patients with herpes simplex virus (HSV) type 1-associated ARN, 2 eyes of 2 patients with HSV-2-associated ARN, 5 eyes of 5 patients with cytomegalovirus (CMV)-associated retinitis, and 1 eye of 1 patient with human T-lymphotropic virus (HTLV)-1. Vitreous samples from the uveitis patients were analyzed with flow cytometry and PCR.
Results: The CD4/CD8 ratio was lower in the viral infection-associated uveitis vitreous samples than the sarcoidosis samples (1.21 ± 0.24 vs. 24.25 ± 3.91). Moreover, the %CD8 was higher in the viral infection-associated uveitis vitreous samples than the sarcoidosis samples (45.06 ± 4.22 vs. 6.91 ± 0.63).
Conclusions: The vitreous CD8 T-lymphocyte subset in viral-associated uveitis has a high diagnostic value for uveitis.
Commercial Relationships: Kazuichi Maruyama, None; Tohru Inaba, None; Sunao Sugita, None; Hiroshi Kunikata, None; Manabu Mochizuki, None; Toru Nakazawa, None

567-6

Analysis of cytokines related to helper T cells in the vitreous of endogenous and infectious uveitis

Masaki Shibata [1] Tomohito Sato [1] Yoko Karasawa [1] Masataka Ito [1] Masaru Takeuchi [1]
1. Ophthalmology, National Defense Medical College, Tokorozawa, Saitama, Japan.

Purpose: To investigate Th cell related-cytokines elevated in the vitreous of endogenous uveitis (EU), and to compare with those of acute retinal necrosis (ARN).
Methods: Vitreous fluids were obtained from consecutive 24 eyes of 22 EU patients and 4 eyes of 4 ARN patients who were undertaken vitrectomy from April 1, 2012 to February 31, 2014 in National Defense Medical College. As the control, vitreous fluids randomly obtained from 15 eyes of 14 patients with epiretinal membrane (ERM) were used. The vitreous levels of IL-1b, IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IFN-g, soluble CD40 ligand (sCD40L), and TNFa were measured by Bio-Plex® assay. The positive rates and the vitreous levels were first compared between ERM and EU groups, and then the cytokines detected in the vitreous of EU were compared with those of ARN group.
Results: The positive rates of IL-1b, IL-4, IL-10, IL-17A, IL-17F, IL-21, IL-25, IL-31, IFN-g, and sCD40L in EU patients were higher than those of ERM patients, in which those of IL-1b, IL-10, IL-31, IFN-g, and TNFa were significant. In addition, IL-6, IL-10, IL-31, IFN-g, and TNFa were significantly higher in EU patients at the vitreous levels. On the other hand, the positive rates of IL-1b, IL-10, IL-17F, IL-25, IL-31, IFN-γ, sCD40L, and TNF α were higher in ARN patients compared with EU patients, in which there were significant differences in those of IL-1b, IL-10, and IL-31. However, the positive rates of IL-4, IL-17A, IL-21, and IL-22 were higher in EU patients than ARN patients, although those were not significant. The vitreous levels of IL-1b, IL-6, IL-10, IL-31, IFN-γ, sCD40, and TNF α were significantly higher in ARN patients than EU patients, while those of IL-4, IL-17A, IL-21, and IL-22 were vice versa as well as the positive rates.
Conclusions: It was suggested that Th1-, Th2-, and Th17-cells were involved in the development of EU, and Th1- and Treg cells were in that of ARN, and that the severity of ocular inflammation would be associated with the activity of Th1 cells mediated by inflammatory cytokines.
Commercial Relationships: Masaki Shibata, None; Tomohito Sato, None; Yoko Karasawa, None; Masataka Ito, None; Masaru Takeuchi, None, (F)

Biochemistry/Molecular Biology - Paper

Moderators

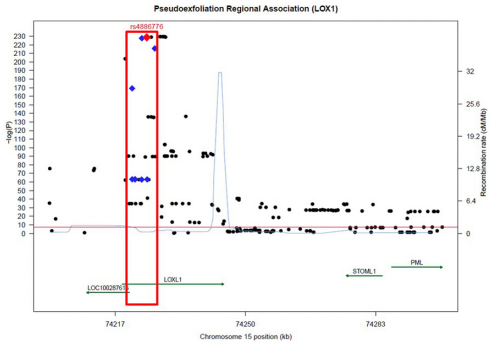
Akira Murakami
Juntendo University Graduate School of Medicine, Bunkyo, Tokyo, Japan
Kazushige Tsunoda
National Institute of Sensory Organs, Meguro, Tokyo, Japan

568-1

Genetic variants associated with exfoliation glaucoma affect promoter activity of the LOXL1 antisense gene

R Rand Allingham [1] Khor Chiea Chuen [3] Mineo Ozaki [2] Susan Williams [4] Allison Ashley-Koch [1] Inas Aboobakar [1] Tin Aung [3] Michael Hauser [1]
1. Duke University, Durham, NC, United States. 2. University of Miyazaki, Miyazaki, Japan. 3. National University of Singapore , Singapore, Singapore. 4. University of Wittswatersrand, Johannesburg, South Africa.

Purpose: Exfoliation glaucoma (XFG) is the most common identifiable form of open-angle glaucoma in the world. The LOXL1 gene is significantly associated with increased risk of XFG in populations worldwide. In a South African exfoliation dataset, the most strongly associated variants are located within the first intron of LOXL1. This region contains the promoter for the LOXL1 antisense RNA (LOXL1-AS1), a regulatory RNA for LOXL1. In this study we 1) evaluate whether these highly associated intronic variants alter the promoter activity of LOXL1-AS1 and 2) compare the peak region for LOXL1 association observed in the South African dataset with a Japanese case/control exfoliation dataset.
Methods: Using a dual luciferase reporter assay, we determined the effects of 3 selected SNPs (rs1550437, rs6495085, and rs6495086), all of which are highly associated with XFG in the South African dataset. The 3-SNP 1630 bp risk and protective haplotypes were cloned and promoter activity was tested in a HEK293 cell line. SNPs in the LOXL1 region that is highly associated with exfoliation in the South African exfoliation dataset were also analyzed for association in a Japanese exfoliation case/control dataset (1,578 exfoliation cases and 1,215 controls) that were genotyped with Illumina OmniExpress microarray (717,991 SNPs).
Results: Data from the dual-luciferase reporter assay indicated that the haplotype of all three non-risk alleles, which is the most common haplotype in the South African dataset, resulted in 28% reduction of promoter activity ($p=2.9x10^{-5}$) compared to the risk haplotype. Association for rs1550437 and rs6495085 was $P = 7.8 X 10^{-170}$ and $8.2 X 10^{-64}$, respectively. (rs6495086 was monomorphic). A locus zoom plot (figure) demonstrates that the region of peak association in the Japanese dataset (SNPs, black circles) matches that seen in South Africans (SNPs blue diamonds, region within red box).
Conclusions: The region of highest association with LOXL1 in South African and Japanese exfoliation cases is similar. We observed that XFG-associated variants may alter promoter activity for LOXL1-AS1. Altered LOXL1-AS1 expression may regulate the expression of LOXL1, which is a reasonable pathophysiologic mechanism for disease. Future directions include testing full risk and non-risk haplotypes to determine the impact of overexpression or knockdown of LOXL1-AS1 on LOXL1 expression and activity.
Commercial Relationships: R Rand Allingham, None; Khor Chiea Chuen, None; Mineo Ozaki, None; Susan Williams, None; Allison Ashley-Koch, None; Inas Aboobakar, None; Tin Aung, None; Michael Hauser, None
Support: TGF Foundation Grant (MAH)



569-2

Taflotan sine reveals beneficial effects on the tear film and ocular surface, a proteomic study

Franz H. Grus [1] Sebastian Funke [1] Sabine Beck [1] Katrin Lorenz [1] Marion Kotterer [1] Dominik Wolters [1] Norbert Pfeiffer [1]
1. Ophthalmology, University Medical Center, Experimental Ophthalmology, Mainz, Germany.

Purpose: After therapeutic switch from IOP lowering Xalatan (latanoprost) to preservative-free Taflotan sine, the ocular surface health status should be assessed by proteomic analyses in a 6 month longitudinal study. ´
Methods: Schirmer tears of POAG patients (N=19) switching from Xalatan to Taflotan® sine were analyzed by use of a gel-based LC ESI Orbitrap XL mass spectrometric workflow to reveal protein candidates responding to Taflotan® sine application in a representative sample pool (N=3/ 0, 2, 12, 24 weeks). Longitudinal analysis of tear proteins was realized considering linear and non-linear analysis methods. Revealed candidate proteins as well as established dry eye markers and cytokines were selected for antibody based microarray validation assays. Finally, candidate proteins were examined for localization and corresponding biological functions.
Results: By use of the LC ESI workflow more than 1000 proteins in POAG patient tears could be identified. Regression analysis ($p<0.05$, $R^2 \geq 0.9$) demonstrated response in 12% of tear proteins after the medical switch. Predominantly level declines in the course of Taflotan® sine appliance could be revealed. Thereby annexin 11, cadherin 5, plectin, serotransferrin, kinectin and pyruvate kinase isozymes M1/M2 manifested a distinct approximation to the healthy level. Also dry eye markers

like mammaglobin B and cytokines shifted towards the healthy level. Accordingly a regeneration of the ocular surface indicated by a decline in epithelial leakage proteins, cytokines and dry eye associated proteins could be proposed. However, only a very few of the known biomarkers of dry –eye were affected.

Conclusions: A distinct change in the tear proteomic pattern after a therapeutic switch to preservative-free Taflotan® sine in POAG patients could be demonstrated, whereby the portion of leakage proteins and inflammatory cytokines was shown to be receded in the course of the study reflecting an improvement of ocular surface conditions. Furthermore, according to proteomic results the changes in the ocular surface of POAG patients differs largely from those seen in typical dry-eye patients.

Commercial Relationships: Franz Grus, Santen (F), Santen (R); Sebastian Funke, None; Sabine Beck, None; Katrin Lorenz, None; Marion Kotterer, None; Dominik Wolters, None; Norbert Pfeiffer, None

Support: Supported in part by Santen

570-3

Malondialdehyde is Increased in Wet AMD Patients' Serum and Eyes

Fuxiang Ye [1] Hiroki Kaneko [1] Ryo Ijima [1] Yosuke Nagasaka [1] Kei Takayama [1] Masatoshi Nagaya [1] Hiroko Terasaki [1]

1. Ophthalmology, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan.

Purpose: To examine the level of malondialdehyde (MDA) in the serum and eyes in patients with neovascular age-related macular degeneration (wet AMD).

Methods: Serum from 75 patients (wet AMD, n = 41; control, n = 34) were collected in the department of Ophthalmology, Nagoya University Hospital. Human eyes were purchased from Minnesota Lions Eye Bank (wet AMD, 6 eyes; control, 5 eyes) and San Diego Eye Bank (fixed eyes for staining). The MDA levels in serum and retinal pigment epithelium (RPE) / choroid lysates were measured by MDA-protein adduct ELISA. MDA-protein adduct staining was performed on the sections of wet AMD and control eyes by immunohistochemistry (IHC). All examinations were analysed statistically using the Mann-Whitney U test. Differences were considered to be statistically significant at $P < 0.05$.

Results: MDA level was significantly higher in wet AMD patients' serum than the control samples ($Z = 2.7538$, $P = 0.0055$). MDA was significantly increased in RPE / choroid tissues obtained from wet AMD patients' eyes ($Z = 2.1909$, $P = 0.0303$). Also, increased MDA-protein adduct staining was observed in wet AMD patients' eyes compared to the control eyes.

Conclusions: To our knowledge, our study is the first report showing the increase of MDA not only in the serum, but also in the eyes of wet AMD patients. MDA, a marker of oxidative stress, shows strong relationship with the disease of wet AMD.

Commercial Relationships: Fuxiang Ye, None; Hiroki Kaneko, None; Ryo Ijima, None; Yosuke Nagasaka, None; Kei Takayama, None; Masatoshi Nagaya, None; Hiroko Terasaki, None

Support: This work was supported by a Grant-in-Aid for Young Scientists (A) and a Grant-in-Aid for Challenging Exploratory Research from the Japan Society for the Promotion of Science (H.K.), the Chukyo Longevity Medical and Promotion Foundation (H.K.), the Takeda Science Foundation (H.K.), and the Hori Science and Arts Foundation (F.Y.)

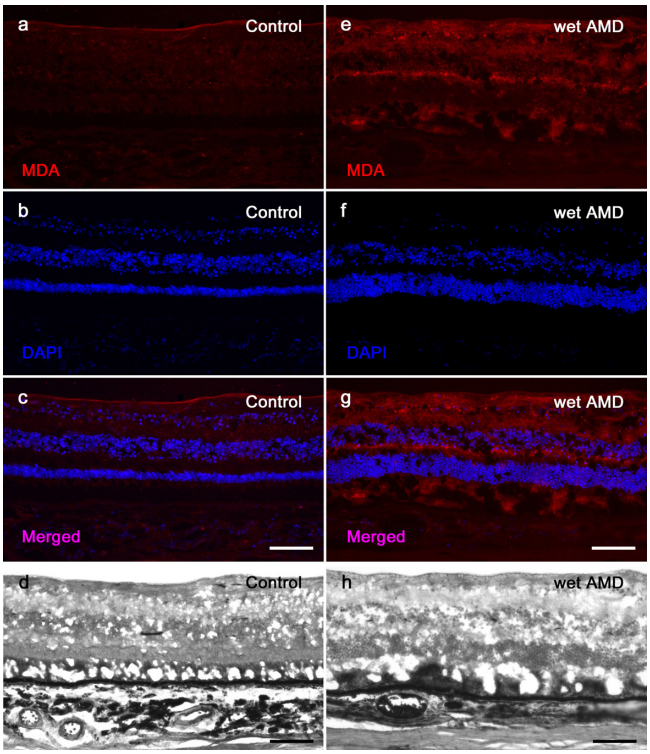


Figure 3. Wet AMD patient's retina showed increased MDA staining.
Cryosections showing retina and RPE / choroid in wet AMD patients (**e-h**) or control subjects (**a-d**). (**a, e**) MDA-protein adduct staining showing increased MDA level in wet AMD retina compared to the control retina. (**b, f**) DAPI staining showing the cell nucleus. (**c, g**) Merged images showed that wet AMD retina showed more MDA staining than the control retina. (**d, h**) Images taken by phase-contrast microscopy showing the derangement of retina in wet AMD eyes compared to the control eyes. Scale bar = 100 μ m

571-4

Loss of function analysis of nicotinamide nucleotide adenylyltransferase 1 gene in developing mouse retina

Akira Murakami [1] Eisuke Arai [1] Yuki Yoshikawa [2] Sumiko Watanabe [2]

1. Department of Ophthalmology, Juntendo Univ Grad School of Medicine, Tokyo, Japan. 2. Department of Molecular and Developmental Biology, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

Purpose: *NMNAT1*, a gene involved in nicotinamide adenine dinucleotide (NAD) synthesis, was found to be responsible for a subset of LCA cases with sever macular atrophy. Recently, we also identified a missense mutation (c.709C>T, p.Arg237Cys) in exon 5 of *NMNAT1* in unrelated two cases of LCA. Interestingly, in both cases, the mutant allele was hemizygousity which was due was

to a deletion in exon 4/5 or exon 5. These findings suggest that the one allele of the mutant may be sufficient for retinal development but not to maintain retina after the birth. We are also presuming that expression level of wild and/or mutant *NMNAT1* allele is important for proper development and maintenance of retina. To elucidate the function of *NMNAT1* in developing normal retina and the pathophysiology of LCA, we employed a mouse retinal explant cultural system as in vitro model of retinal development.

Methods: As for a loss-of-function analysis, we constructed plasmid based sh-RNA for Nmnat1, and the plasmid was electroporated into embryonic mouse retinal explants. The explants were cultured and examined by immunohistochemistry.

Results: Loss-of-function of Nmnat1 in developing mouse retina resulted in severe perturbation of retinal development. High dose of sh-Nmnat1 expressing retina could not survive more than 3 days in culture; however low dose of sh-Nmnat 1 expressing retina incompletely differentiated and failed to form outer plexiform layer.

Conclusions: In vitro perturbation of expression levels of Nmnat1 in developing mouse retina suggested that down-regulation of Nmnat1 showed various phenotypes depend on the expression levels. Phenotype analyses of the retinal explants with combinatorial transfection of sh-RNA and wild-/mutant-Nmnat1 are undergoing.

Commercial Relationships: Akira Murakami, None; Eisuke Arai, None; Yuki Yoshikawa, None; Sumiko Watanabe, None

572-5

Modulation of miRNA-7 trafficking and ubiquitin protein ligase A (UBE2A)-mediated amyloidogenesis by the circular RNA (circRNA) ciRS-7 in age-related macular degeneration (AMD)

Walter Lukiw [2, 1] Yuhai Zhao [3, 1] Aileen Pogue [5] Surjyadipta Bhattacharjee [4]

1. Neurology and Ophthalmology, LSU Neuroscience Center, New Orleans, LA, United States. 2. Neurology and Ophthalmology, LSU Neuroscience Center, New Orleans, LA, United States. 3. LSU Neuroscience Center, New Orleans, LA, United States. 4. LSU Neuroscience Center, New Orleans, LA, United States. 5. Alchem Biotech, Toronto, ON, Canada.

Purpose: A naturally occurring family of circular RNAs (circRNAs) are highly represented in the eukaryotic transcriptome. Recently characterized, traditional methods of RNA detection/analysis requiring a free 5 ′ or 3 ′ ribonucleotide terminus may have significantly underestimated circRNA abundance and significance. Intrinsically resistant to exonucleolytic decay, circRNAs are enriched in mammalian brain and retina. Specific small non-coding RNAs such as the evolutionary ancient human microRNA-7 (hsa miRNA-7; chr 9q21.32; ~23 nt; http://www.mirbase.org/cgi-bin/mirna entry.pl?acc=MI0000263; a known, important post-transcriptional regulator of phagocytosis) are associated with a circRNA for miRNA-7 (ciRS-7) in the same tissues. ciRS-7 contains about ~70 tandem anti-miRNA-7 sequences; ciRS-7 (~1400 nt) thereby acts as a kind of endogenous, competing, anti-complementary miRNA "sponge" to adsorb, and hence quench, normal miRNA-7 function. Here we have investigated a circRNA-miRNA-mRNA regulatory circuit in AMD that appears to be involved in amyloidogenesis.

Methods: DNA & miRNA arrays, Northern blot hydridization, the circularity-sensitive RNaseR; RT-PCR; Western immunoanalysis

Results:

We provide initial evidence of a mis-regulated miRNA-7-circRNA system in sporadic AMD. Deficits in ciRS-7, and ciRS-7 "sponging activities" might be expected to increase ambient miRNA-7 levels in AMD-affected retina, as is observed, to ultimately contribute to the down-regulation of selective miRNA-7-sensitive messenger RNA (mRNA) targets. The presence of up-regulated miRNA-7, due to a deficiency in ciRS-7 "sponging" effects, was shown to down-regulate AMD-relevant targets, such as, for example, the ubiquitin protein ligase A (UBE2A; miRNA-7-UBE2A mRNA energy of association, E_A = − 22.86 kcal/mol). UBE2A, an autophagic, phagocytic protein essential in the clearance of amyloid peptides is depleted in AMD retina.

Conclusions: This circRNA-miRNA-mRNA regulatory system appears to represent another important layer of epigenetic control over pathogenic gene expression in progressive degenerative disorders such as AMD. Indeed, our ideas on specialized forms of RNA in the CNS continue to evolve, and technological advancement, refinement and recent discoveries continue to challenge our basic perceptions of retinal nucleic acid signaling in health and disease.

Commercial Relationships: Walter Lukiw, None; Yuhai Zhao, None; Aileen Pogue, None; Surjyadipta Bhattacharjee, None

Support: an unrestricted departmental grant from Research to Prevent Blindness (RPB) and NIH Grants NEI EY006311, NIA AG18031 and NIA AG038834

573-6

HPS6 mutations identified by whole-exome sequencing in two sisters with ocular albinism

Yoshihiro Hotta [1] Miho Sato [1] Junya Nakajima [2, 3] Katsuhiro Hosono [1] Daisuke Miyamichi [1] Takashi Negishi [1, 4] Takahito Nomura [1] Noriko Miyake [3] Tsutomu Ogata [5] Naomichi Matsumoto [3]

1. Department of Ophthalmology, Hamamatsu University School of Medicine, Hamamatsu, Japan. 2. Department of Pediatrics, Tokyo Medical University, Tokyo, Japan. 3. Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan. 4. Department of Ophthalmology, Juntendo University School of Medicine, Tokyo, Japan. 5. Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Japan.

Purpose: To report the cases of two sisters with ocular albinism (OA) caused by *HPS6* mutations. They were analyzed all exons and surrounding area of the 5 causative genes (*TYR*, *OCA2*, *TYRP1*, *SLC45A2*, and *GPR143*) for OA and oculocutaneous albinism (OCA) by Sanger sequencing, and no mutation was recognized. In this time,

whole-exome sequencing (WES) identifies gene mutations responsible for Hermansky-Pudlak syndrome (HPS) in the two sisters.

Methods: A 3-year-and-11-month-old girl was referred to us with poor vision. She presented congenital nystagmus, exotropia and iris translucency. Additionally, we examined her younger 5-month-old sister who presented nystagmus with impaired vision. Both sisters had light brown hair and fair skin. Their hair and skin color seemed to be normal for Japanese people and they got suntans in summer. Moreover, their ocular fundus was albinotic with bilateral foveal hypoplasia. In the follow-up period of 56 months, they exhibited only ocular findings without general findings such as bleeding problem. These clinical features strongly suggested the presence of OA in the two sisters.

Written informed consent was obtained from the parents prior to performing the molecular genetic studies. In this study, to identify the causative mutations, we performed WES in the elder sister and her parents. Genomic DNA was extracted from peripheral lymphocytes of the two sisters and their parents using standard procedures. Two candidate genes were extracted from data obtained by WES. The identified possible pathogenic mutations were confirmed using Sanger sequencing.

Results: Novel compound heterozygous mutations of *HPS6* (c.1897delC, mother origin and c.2038C>T, father origin) were identified in the two sisters. *HPS6* is one of the causative genes of HPS which is complicated with OCA and bleeding problem. WES is a comprehensive method for efficient analysis of large amounts of genes. This cost effective method increases throughput and saves time.

Conclusions: We report two sisters with OA caused by *HPS6* mutations. WES is beneficial to diagnosis of an unusual HPS phenotype.

Commercial Relationships: Yoshihiro Hotta, None; Miho Sato, None; Junya Nakajima, None; Katsuhiro Hosono, None; Daisuke Miyamichi, None; Takashi Negishi, None; Takahito Nomura, None; Noriko Miyake, None; Tsutomu Ogata, None; Naomichi Matsumoto, None

Support: The Japan Society for the Promotion of Science (Grant-in-Aid for Scientific Research (C) 26462659), The Ministry of Health, Labour and Welfare of Japan.

Anatomy/Pathology - Paper

Moderators

Toshinori Murata
School of Medicine, Shinshu University, Matsumoto, Nagano, Japan
Hidetoshi Yamashita
Yamagata University School of Medicine, Yamagata, Yamagata, Japan

574-1

Comparison of Posterior Eye Shape using Retinal SDOCT and MRI

Anthony N. Kuo [1] Ryan P. McNabb [1] Sina Farsiu [2, 1] Seang Mei Saw [3] Terri Young [4, 1] Joseph A. Izatt [2, 1]
1. Duke Eye Center, Durham, NC, United States. 2. Biomedical Engineering, Duke University, Durham, NC, United States. 3. National University of Singapore, Singapore, Singapore. 4. Ophthalmology and Visual Sciences, University of Wisconsin, Madison, WI, United States.

Purpose: Posterior eye shape is an important biomarker for understanding developmental differences in the myopic eye and has also been correlated with pathologic myopia. Posterior eye shape is usually measured using magnetic resonance imaging (MRI). Measuring posterior eye shape with spectral domain optical coherence tomography (SDOCT) would be more convenient, but in addition to optical distortions, the field of view of current generation SDOCT is substantially smaller than MRI. In this study, we compared MRI and SDOCT measures of posterior eye shape as described by asphericity.
Methods: Previously acquired 1.5 T MRI (GE and Siemens) and retinal SDOCT (Bioptigen) images of 10 eyes of 5 subjects obtained under an IRB approved protocol were used for this analysis. The spherical equivalent refractive error in these eyes ranged from +1.37 to -5.75. For MRI, the posterior 240° of the eye in the axial equatorial slice was automatically segmented. For SDOCT, the images were corrected for optical and display distortions, and the RPE was automatically segmented. The segmentations of the eyes in both imaging modalities were then fit to an ellipse using least squares. Ellipse fitting of the SDOCT data required a pilot estimate of the equator of the eye based on axial biometry (IOLMaster, Zeiss). Asphericity (Q) was calculated from the fitted ellipse parameters [Atchison et al., 2005]. Paired numerical and categorical differences between MRI and SDOCT measures of asphericity in the same eye were then compared.
Results: Q_{MRI} ranged from -0.16 to 0.29, while Q_{OCT} ranged from -0.09 to 1.58. The mean paired difference between the two modalities (Q_{MRI}-Q_{OCT}) was -0.48 ± 0.49 which was significantly different ($p = 0.01$, Wilcoxon signed rank test). For categorical assignment, MRI classified 8 eyes as oblate ($Q > 0$) and 2 as prolate ($Q < 0$). SDOCT also classified the same 8 eyes as oblate and 2 eyes as prolate ($\kappa = 1.0$).
Conclusions: As would be expected given the difference in region size sampled, MRI and SDOCT numerical measures of asphericity were significantly different. Wide-field scans may be needed for SDOCT to be numerically comparable to MRI. However, despite the smaller field of view, SDOCT was able to correctly assign eyes categorically as prolate or oblate. Thus, readily accessible SDOCT may offer an alternative to more cumbersome MRI for clinical studies requiring categorical assignment of posterior eye shape.
Commercial Relationships: Anthony Kuo, None; Ryan McNabb, None; Sina Farsiu, None; Seang Mei Saw, None; Terri Young, None; Joseph Izatt, Bioptigen (I), Bioptigen (P), Bioptigen (S)
Support: NIH K23-EY021522 (ANK), R01-EY014685 (TLY)

575-2

Induction of apoptosis by resveratrol in human uveal melanoma cells

Sandeep Goswami [1] Arpna Srivastava [1] Bhavna Chawla [2] Neelam Pusker [2] Mandeep S. Bajaj [2] Seema Kashyap [3] Jasbir Kaur [1]
1. Department of Ocular Biochemistry, Dr. Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi, India. 2. Ocular Oncology and Pediatric Ophthalmology Services, Dr. Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi, India. 3. Department of Ocular Pathology, Dr. Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi, India.

Purpose: Uveal melanoma is the highly malignant tumor of the eye and frequently leads to metastatic death. Depending on size and other parameters, traditional treatment of the primary tumor has been enucleation. Despite the advances in treatment, mortality varies considerably, to more than 50% in high-risk patients, primarily due to metastasis to the liver. Therefore, it is critical to develop drugs that are non-toxic and can be administered for potentially extended periods to patients who have diagnosed with primary uveal melanoma. In recent years, resveratrol, a naturally occurring polyphenol highly enriched in grapes, peanuts, red wine, and a wide variety of food sources, has attracted considerable interest because of its cardioprotective, antiaging, and cancer chemopreventive effects. Resveratrol has been shown to inhibit tumor initiation, promotion, and progression in a variety of cell culture systems and animal models of skin and mammary carcinogenesis. The present study was designed to investigate the effect of resveratrol on human uveal melanoma cells.
Methods: Human uveal melanoma cells isolated from tissues of enucleated eyes were treated with resveratrol in a dose and time-dependent manner. Cell viability was measured using MTT assay. The proapoptotic and antiproliferative effects were evaluated by Hoechst stain and flow cytometry respectively. Mitochondrial transmembrane potential was measured as a function of drug treatment using JC-1, whereas the release of cytochrome c from mitochondria was assessed by immunoblotting. Caspase activities were determined by spectrofluorimetry.

Results: Resveratrol induced a dose- and time-dependent decrease in cell viability and inhibited cell proliferation by inducing S-phase arrest and apoptotic cell death in uveal melanoma cells. Preceding cell death, resveratrol evoked a rapid dissipation of mitochondrial transmembrane potential. This was followed by the release of cytochrome c into the cytoplasm and a substantial increase in the activities of caspase-9 and -3. Additionally, in a cell-free system, resveratrol directly induced the depolarization of isolated mitochondria.
Conclusions: These results demonstrate that resveratrol inhibits uveal melanoma cell proliferation and induces apoptosis through activation of the mitochondrial (intrinsic) apoptotic pathway. This may warrant further exploration as an adjuvant to conventional anticancer therapies for uveal melanoma.
Commercial Relationships: Sandeep Goswami, None; Arpna Srivastava, None; Bhavna Chawla, None; Neelam Pusker, None; Mandeep Bajaj, None; Seema Kashyap, None; Jasbir Kaur, None

576-3

NADPH Oxidase-4 as an Indicator of Reactive Oxygen Species Stress in Retinoblastoma

Lata Singh [1] Seema Kashyap [1] Neelam Pusker [2] Seema Sen [1] Jasbir Kaur [3] Anjana Sharma [4]

1. Ocular Pathology, All India Institute of Medical Sciences, New Delhi, India. 2. Ophthalmology, All India Institute of Medical Sciences, New Delhi, India. 3. Ocular Biochemistry, All India Institute of Medical Sciences, New Delhi, India. 4. Ocular Microbiology, All India Institute of Medical Sciences, New Delhi, India.

Purpose: Reactive Oxygen Species (ROS) have been shown to enhance proliferation of cancer cells. NADPH Oxidases (NOX4) are major intracellular source of reactive oxygen species and elevated ROS levels are often found associated with cancer, apoptosis resistance, tumor cell invasion etc. Under oxidative stress conditions, excessive ROS can damage cellular proteins, lipids and DNA, leading to fatal lesions/mutations in cell that contribute to carcinogenesis. Therefore, the purpose of this study is to examine ROS stress by evaluating the expression of NOX4 protein in human retinoblastoma.
Methods: Immunohistochemical (IHC) expression of NOX4 protein was analyzed in 60 prospective cases of primary enucleated retinoblastoma specimens and then validated by western blotting. Cytoplasmic staining was considered as positive and graded as weak/negative, moderate and strong. Expression of this protein was correlated with clinical parameters, tumor differentiation, and various histopathological high risk factors (HRFs).
Results: There was a slight male preponderance (58%) & 13/60 (21.7%) were bilateral. The tumor was poorly differentiated in 45/60 (75%) with extensive necrosis in 37 (61.66%) cases. Calcification was found in 17/60 (28.33%) cases. Massive choroidal invasion was the most frequently observed histopathological high risk factor in 33.3% cases. In addition, optic nerve cut end and retrolaminar invasion was seen in 28.3% cases, iris & ciliary body in 15% whereas scleral invasion was found in 11.66%. One or more than one HRFs were identified in 25/60 cases. NOX4 protein was expressed in 71.6% (43/60) primary retinoblastoma cases by immunohistochemistry. NOX4 was statistically significant with massive choroidal invasion and poor differentiation.
Conclusions: This is the first study to show the expression of NOX4 protein in retinoblastoma tumor. Our results revealed that NOX4 was highly expressed in human retinoblastoma. Therefore, retinoblastoma tumor may exhibit greater ROS stress than normal cells. Investigating NOX4 might be helpful for developing therapy with combination of ROS-eliminating strategies in the management of retinoblastoma patients.
Commercial Relationships: Lata Singh, None; Seema Kashyap, None; Neelam Pusker, None; Seema Sen, None; Jasbir Kaur, None; Anjana Sharma, None

577-4

Correlation of HMG protein with Clinicohistopathological Parameters in Primary Retinoblastoma

Mithalesh K. Singh [1] Lata Singh [1] Seema Kashyap [1] Neelam Pusker [2] Seema Sen [1] Anjana Sharma [3] Bhavna Chawla [2]

1. Ocular Pathology, All India Institute of Medical Sciences, New Delhi, Delhi, India. 2. Ophthalmology, All India Institute of Medical Sciences, New Delhi, Delhi, India. 3. Ocular Microbiology, All India Institute of Medical Sciences, New Delhi, Delhi, India.

Purpose: High Mobility Group proteins (HMG) are more abundant in rapidly dividing and transformed cells. These are a group of newly recognized protein regulating tumorigenesis, and tumor invasion. Expression of HMGA2 is associated with increased rate of metastasis in various cancers. The aim of the study to investigate the expression of HMGA2 protein in primary retinoblastoma cases.
Methods: Prospective analysis was performed on 60 primary enucleated cases over a period of one year. Immunohistochemistry was performed on FFPE sections to study the expression of HMGA2 protein and correlated with clinical and histopathological high risk features (HRFs) such as invasion and differentiation of the tumors. mRNA expression was performed by semi-quantitative Reverse Transcriptase PCR (RT-PCR).
Results: A total of 60 eyes were taken of which 11(18.33%) eyes had bilateral involvement. Ages ranged from 4 - 60months. 46(76.66%) cases were reported as poorly differentiated tumors. Histopathologically, 15(25%) had massive choroid invasion, 18(30%) had optic nerve invasion, 6 cases each had sclera and ciliary body invasion. Strong expressions of HMGA2 were seen in 56.66% cases. RT-PCR was performed on 35 fresh cases in which 62.85% cases show m-RNA expression. Expression of HMGA2 was statistically significant with massive choroid invasion ($p=0.0483$) and with more than one HRFs ($p=0.0192$).
Conclusions: Our study has demonstrated over expression of HMGA2 in primary retinoblastoma tumors. Results depict a strong correlation of HMGA2 protein with massive choroidal invasion and histopath high risk features which indicate poor prognosis. HMGA2 could thus serve as therapeutic target in the management of

retinoblastoma.
Commercial Relationships: Mithalesh Singh, None; Lata Singh, None; Seema Kashyap, None; Neelam Pusker, None; Seema Sen, None; Anjana Sharma, None; Bhavna Chawla, None

578-5

Profiling of MicroRNAs Involved in Retinal Degeneration Caused by Selective Müller Cell Ablation

Sook Hyun Chung [1] Weiyong Shen [1] SoRa Lee [1] Mark Gillies [1]

1. Clinical Ophthalmology and Eye Health , Save Sight Institute , Sydney , NSW, Australia.

Purpose: Dysfunction of Müller cells, the principal glial cells of the mammalian retina, has been implicated in the pathogenesis of several retinal diseases such as diabetic retinopathy and macular telangiectasia. In order to understand the potential contribution of Müller cells to retinal disease better, we have developed a transgenic mouse model in which foci of Müller cell ablation can be selectively induced. MicroRNAs (miRNAs), small non-coding RNAs that are involved in post-transcriptional modulation, have critical functions in various biological processes. The aim of this study was to profile differential expression of miRNAs and to examine changes in their target genes 2 weeks after induced Müller cell ablation in our transgenic model.
Methods: We performed differential expression of miRNAs using the miScript HC PCR array, and target gene prediction was achieved by two databases (TargetScan and mirTarbase). DAVID and KEGG pathway databases were applied for functional analysis of the target genes, and the genes were validated with qRT-PCR, western blot and immunohistochemistry.
Results: We identified 20 differentially expressed miRNAs and their 78 overlapping target genes from the two databases. DAVID and KEGG pathway analysis suggested that the target genes were generally involved in cell apoptosis, p53, neurotrophin, calcium, chemokine and Jak-STAT signalling pathways. qRT-PCR and western blot validation with 7 target genes including Cyclin D2, Caspase 9, insulin-like growth factor 1 (IGF1), IL-1 receptor-associated kinase (IRAK), calmodulin (CALM) and Janus kinase 2 (Jak2), revealed that some are positively, rather than negatively, correlated with their corresponding miRNAs.
Conclusions: Results revealed by miRNA profiling, target gene analysis and validation were generally consistent with our previous findings that selective Müller cell ablation causes photoreceptor degeneration and neuroinflammation. Our data on alterations of miRNAs and their target gene expression after Müller cell ablation provide further insights into the potential role of Müller cell dysfunction in retinal disease.
Commercial Relationships: Sook Hyun Chung, None; Weiyong Shen, None; SoRa Lee, None; Mark Gillies, None
Support: Lowy Medical Research Institute/National Health and Medical Research Council (NHMRC, Australia; APP1028393)/Ophthalmic Research Institute of Australia