Temporal Changes in Lenses of CRYAA101D Transgenic Mice Compared to CRYAAWT Mice Prior to Cataract Development

Om P. Srivastava, Kiran Srivastava, Shylaja Hegde, Roy Joseph.
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Purpose: The purpose was to examine temporal changes in lenses of CRYAA101D mice relative to CRYAAWT (wild type, WT) mice to elucidate potential mechanism of cataract development.

Methods: Because the lenses of mice expressing αA N101D transgene developed mild cortical opacity by the age of 7-months (J. Biol. Chem. 286:11579-11592, 2011), the relative temporal changes in lenses 1-, 3- and 5-months old transgenic mice compared to WT mice were determined. The analyses included comparative changes in fiber cells ultrastructure including cytoskeleton and membranes, and binding of αA101D to membranes and its distribution in water soluble (WS)- and water insoluble (WI)-proteins.

Results: The scanning electron microscopic (SEM) analysis showed altered fiber cell structures in 5-months old lenses of CRYAA101D mice. Immunohistochemical analyses using anti-aquaporin 0-, anti-His (for αA identification)- and anti-phallolidin-antibodies revealed that membranes and cytoskeleton of lenses αA101D mice were altered relative to age-matched WT lenses. Similar comparative analysis showed a greater level of association of αA101D to lens membranes of transgenic mice relative to WT mice, which was further confirmed by EM-immunogold labeling analysis of lens sections. The comparative protein profiles revealed greater levels of water insolubilization and degradation of αA101D in lenses of αA101D mice.

Conclusions: Relative to WT-mice, the lenses of αA101D mice showed altered membranes and cytoskeleton, an increased association of αA101D to membranes and water insolubilization and degradation. These changes might play a role in the development of lens opacity in CRYAA101D mice.

Commercial Relationships: Om P. Srivastava, None; Kiran Srivastava, None; Shylaja Hegde, None; Roy Joseph, None
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Program Number: 1208 Poster Board Number: C0296
Presentation Time: 3:15 PM–5:00 PM

Congenital cataract: functional effects of three CRYBB2 amino acid changes

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Purpose: The purpose of this study was to investigate the impact of three amino acid changes caused by a CRYBB2 gene conversion event that we identified in a large congenital cataract family.

Methods: The CRYBB2 cDNA was generated by RT-PCR using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems), RNA from human retina, CRYBB2-specific primers and the pcdNA3.1 vector. Three mutations, c.433 C>T (p.R145W), c.440A>G (p.Q147R), and c.449C>T (p.T150M), were introduced into the wild type clone using the QuikChange Lightning kit (Agilent). Cultured human lens epithelium (HLE) SRA 01/04 cells were transfected using Lipofectamine 2000 (Life Technologies). Cell lysates containing protease inhibitors were sonicated and spun into separate supernatant and pellet fractions. After SDS-PAGE, Western blot analysis was carried out using a goat polyclonal antibody against CRYBB2 (Santa Cruz Biotechnology).

Results: Missense changes R145W and T150M were found to be conserved across 15 species while Q147R differs in puffer fish (Tetraodon nigroviridis). Protein modeling with PROVEAN and PolyPhen-2 predicted that R145W and T150M may each be deleterious/possibly damaging, while Q147R is predicted to be benign/neutral. ProtScale showed altered hydrophobicity for CRYBB2 containing all three missense changes. Cloned cDNA expression constructs were created containing wild type sequence and each of the three individual changes. We expressed wild type and triple mutant CRYBB2 cDNA in HLE cells. Western blot analysis showed that the wild type CRYBB2 protein is present only in the soluble fraction of the cell extract while the mutant protein is detected only in the pellet.

Conclusions: Informatic analysis suggests that two of the three CRYBB2 missense mutations that we have identified in a congenital cataract family are hitting residues that are conserved across species.
Purpose: To compare ocular UV exposure in Asian and Western facial contours.

Methods: Mannequins, simulating facial skeletons of Western and Asian females aged 40s, embedded with UV-AB sensors (sensitivity 280-400 nm, ALGAN, Japan) at top of head, forehead, and eyes (nasal, center, temporal) with facial reliefs (distance perpendicular to a line connecting forehead and cheek from corneal surface) of 5 mm and 10 mm for Asian and Western, respectively, were set on a grey urethane-coated concrete surface, (UV reflectance approx. 10% comparable to asphalt) and measurement in 8 azimuths were performed from 0° to 40° of solar altitude per 5° on Kanazawa Medical University roof (lat.36.66°N, long.136.65°E, alt. 50 m), October 1, 2013.

Results: UV exposure doses at tops of head for both facial contours were 8-10 mW/cm² at 40° solar altitude (culmination). Maximum ocular doses from the average of 8 azimuths were around 2 mW/cm² and 1 mW/cm² in Asian and Western, respectively. When facing the sun, ocular dose in Asian showed a bimodal curve of about 4 mW/cm² at maximum at around 30° solar altitude, and in Western a chevron curve of 1.7 mW/cm² at maximum culmination. Ocular exposure at 10°, 20°, 30°, and 40° of solar altitude in the average of 8 azimuths in Western showed 41%, 47%, 63%, 65% dose of Asian. Even facing the sun, Western eyes were shadowed due to skeletal geometry at about 10° or more of solar altitude.

Conclusions: Due to differences in facial skeletal geometry, ocular exposure to UV in Asians is greater than in Westerners.

Commercial Relationships: Hiroshi Sasaki, None; Natsuko Hatusaka, None; Naoko Shibata, None; Shinshuke Shibata, None; Yusuke Kurihara, None; Cheng-Yu Tsai, None; Eri Kubo, None

Program Number: 1211 Poster Board Number: C0299
Presentation Time: 3:15 PM–5:00 PM
Investigation of crystalline lens injury threshold by 75 GHz band exposure in rabbit

Purpose: To determine if specific cytokines are elevated in the eye during diabetic cataract formation in a streptozotocin (STZ)-induced diabetic rat model and to assess the direct effects of selected cytokines on lens fiber structure in whole-lens culture.

Methods: Wistar rats (125-150g, n=35) were injected with a single 75 mg/kg intravenous dose of STZ to induce diabetes. Untreated animals served as controls. Animals were euthanized at 1, 2, 3 and 4 weeks post-injection, blood glucose levels (BGL) were recorded and aqueous humor and vitreous humor were collected and frozen. Cytokine analysis was performed using a bead-based immunoassay for the following cytokines: IFN-γ, TNF, IL-1α, IL-2, IL-4, and IL-10, followed by flow-cytometric analysis. For whole lens culture, normal Wistar rat lenses (n=24 animals) were cultured with IL-1α or IL-4 or were untreated (controls). Following either 24 hours (OD) or 48 hours (OS) in culture, lenses were photographed, decapsulated and fixed for further structural analysis.

Results: At 1 week post-injection, mean BGL of STZ-injected animals was 268 mg/dL compared to 102 mg/dL in controls, indicating successful diabetic induction. TNF, IL-1α, and IL-4 were increased during diabetes in either aqueous humor, vitreous humor or both compared to controls. TNF was increased in both aqueous and vitreous humors at approximately 4 weeks after diabetic induction. A marked increase in IL-1α levels was noted in aqueous humor as early as 1 week post-STZ injection. Specifically, aqueous humor samples showed a 5-fold increase by 1 week post-induction and an 8-fold increase by 4 weeks post-induction. An immediate increase in IL-4 was also detected at 1 week post-STZ injection in the vitreous humor; IL-4 concentration remained elevated throughout week 4. Lenses cultured with either IL-1α or IL-4 showed structural alterations such as suturel widening, foci of fiber end disruption and discrete superficial opacities, primarily on posterior surfaces, as early as 24 hours. Lenses cultured in media without cytokine remained discrete superficial opacities, primarily on posterior surfaces, as early as 24 hours. Lenses cultured in media without cytokine remained.

Conclusions: IL-1α and IL-4 increased immediately subsequent to diabetic induction, and treatment of normal lenses with these cytokines induced structural changes consistent with documented changes in diabetic cataracts. These findings suggest that IL-1α and IL-4 may be involved in the initiation of diabetic cataract formation.

Commercial Relationships: Kristin J. Al-Ghoul, None; Fareeha Mahmood, None
Support: Rush Translational Sciences Consortium Pilot Grant (KJA)

Program Number: 1210 Poster Board Number: C0298
Presentation Time: 3:15 PM–5:00 PM
Influence of difference between Asian and Western facial contours on ocular UV exposure

Purpose: To compare ocular UV exposure in Asian and Western facial contours.

Methods: Mannequins, simulating facial skeletons of Western and Asian females aged 40s, embedded with UV-AB sensors (sensitivity 280-400 nm, ALGAN, Japan) at top of head, forehead, and eyes (nasal, center, temporal) with facial reliefs (distance perpendicular to a line connecting forehead and cheek from corneal surface) of 5 mm and 10 mm for Asian and Western, respectively, were set on a grey urethane-coated concrete surface, (UV reflectance approx. 10% comparable to asphalt) and measurement in 8 azimuths were performed from 0° to 40° of solar altitude per 5° on Kanazawa Medical University roof (lat.36.66°N, long.136.65°E, alt. 50 m), October 1, 2013.

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Conclusions: Due to differences in facial skeletal geometry, ocular exposure to UV in Asians is greater than in Westerners.

Commercial Relationships: Hiroshi Sasaki, None; Natsuko Hatusaka, None; Naoko Shibata, None; Shinshuke Shibata, None; Yusuke Kurihara, None; Cheng-Yu Tsai, None; Eri Kubo, None

Program Number: 1211 Poster Board Number: C0299
Presentation Time: 3:15 PM–5:00 PM
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Methods: Wistar rats (125-150g, n=35) were injected with a single 75 mg/kg intravenous dose of STZ to induce diabetes. Untreated animals served as controls. Animals were euthanized at 1, 2, 3 and 4 weeks post-injection, blood glucose levels (BGL) were recorded and aqueous humor and vitreous humor were collected and frozen. Cytokine analysis was performed using a bead-based immunoassay for the following cytokines: IFN-γ, TNF, IL-1α, IL-2, IL-4, and IL-10, followed by flow-cytometric analysis. For whole lens culture, normal Wistar rat lenses (n=24 animals) were cultured with IL-1α or IL-4 or were untreated (controls). Following either 24 hours (OD) or 48 hours (OS) in culture, lenses were photographed, decapsulated and fixed for further structural analysis.

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Conclusions: IL-1α and IL-4 increased immediately subsequent to diabetic induction, and treatment of normal lenses with these cytokines induced structural changes consistent with documented changes in diabetic cataracts. These findings suggest that IL-1α and IL-4 may be involved in the initiation of diabetic cataract formation.

Commercial Relationships: Kristin J. Al-Ghoul, None; Fareeha Mahmood, None
Support: Rush Translational Sciences Consortium Pilot Grant (KJA)
3) regular-cell density area. Exposure to 100 mW/cm² did not induce prominent damage, but mitotic cells were observed in the exposed area. Exposure to 50 mW/cm² resulted in no ocular change or mitotic cells in the exposed area. The average temperatures of cornea during exposure at 200, 100, 50 mW/cm² were as follows: 49.4±0.9°C (200 mW/cm²), 42.2±0.4°C (100 mW/cm²), 38.2±0.2°C (50 mW/cm²), respectively. MTLC color change was observed to rise from the right under the cornea and the convection descended to the upper pupil area or the upper crystalline lens side (200 mW/cm² data).

Conclusions: Exposure to 75 GHz MMW induced not only corneal damage but also lens epithelial cell damage in rabbit eye. It was absorbed by cornea and caused heat transport to the crystalline lens. The present data is evidence of a corneal and lens damage threshold below 100 to 50 mW/cm².

Commercial Relationships: Masami Kojima, Ministry of Internal Affairs and Communications, Japan (F); Cheng-Yu Tsai, Ministry of Internal Affairs and Communications, Japan (F); Yukihisa Suzuki, Ministry of Internal Affairs and Communications, Japan (F); Kensuke Sasaki, National Institute of Information and Communications Technology (E); Kanako Wake, National Institute of Information and Communications Technology (E); Soichi Watanabe, National Institute of Information and Communications Technology (E); Yoshitsugu Kamimura, Ministry of Internal Affairs and Communications, Japan (F); Masao Taki, Ministry of Internal Affairs and Communications, Japan (F); Kazuuyuki Sasaki, Ministry of Internal Affairs and Communications, Japan (F); Hiroshi Sasaki, Ministry of Internal Affairs and Communications, Japan (F)


Program Number: 1212 Poster Board Number: C0300
Presentation Time: 3:15 PM–5:00 PM

Goji berry effects on cataract development in ultraviolet light-irradiated bovine lenses
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School of Optometry and Vision Science, University of Waterloo, Waterloo, ON, Canada.

Purpose: Ultraviolet (UV) light is a factor for the development of cataracts. One mechanism for damage to the lens is via UV light-induced increases in free radicals. The goji berry is a traditional Chinese health supplement that has antioxidant properties. This study was undertaken to determine whether goji berry can prevent or delay UV-induced cataractogenesis.

Methods: Lenses were dissected and cultured in medium for 24 hours. Lenses were placed in medium with or without goji berry extract, then placed into an incubation chamber equipped with UVB light (2.0 J/cm²) for two hours. Control lenses were placed in light-extract, then placed into an incubation chamber equipped with UVB light (2.0 J/cm²) for two hours. Control lenses were placed in light-exposure at 200, 100, 50 mW/cm² were as follows; 49.4 ± 0.4°C (200 mW/cm²), 42.2 ± 0.4°C (100 mW/cm²), 38.2 ± 0.2°C (50 mW/cm²), respectively. MTLC color change was observed to rise from the right under the cornea and the convection descended to the upper pupil area or the upper crystalline lens side (200 mW/cm² data).

Conclusions: Exposure to 75 GHz MMW induced not only corneal damage but also lens epithelial cell damage in rabbit eye. It was absorbed by cornea and caused heat transport to the crystalline lens. The present data is evidence of a corneal and lens damage threshold below 100 to 50 mW/cm².

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Program Number: 1212 Poster Board Number: C0300
Presentation Time: 3:15 PM–5:00 PM

Ocular heat diffusion after threshold in vivo exposure to 1090 nm infrared radiation
Zhaohua Yu1, Karl Schulmeister2, Nooshin Talebi-Zadeh1, Martin Kronschlager1, Per G. Soderberg1. 1Neuroscience/Ophthalmology, Gullstrand Lab, Uppsala, Sweden; 2Seibersdorf Labor GmbH, Seibersdorf, Austria.

Purpose: To investigate the heat flow in the ocular media after in vivo exposure to 1090 nm radiation at the radiant exposure dose intended for cataract induction.

Methods: Altogether 20 six-weeks-old albino Sprague-Dawley rats were anesthetized and the pupils were bilaterally dilated prior to experimental exposure. The animals were randomly divided into two groups of 10. All animals were unilaterally exposed to 6.2 W coherent infrared radiation at 1090 nm with a spot size of 2 mm within the dilated pupil for 8 seconds (1.6 kJ/cm²). The beam was divergent on the cornea generating a close to collimated beam between the lens and the retina to minimize retinal heating. In one group, temperature was recorded with three thermocouple probes placed at the limbus, in the vitreous just behind lens, and on the outer sclera next to the optic nerve, respectively. In the other group, one thermocouple probe was placed at the limbus and another probe on the outer sclera next to the optic nerve. Temperature was recorded from the end of the exposure till temperature descended back to normal.

Results: After the exposure, the temperature decreased exponentially as a function of time asymptotically approaching a minimum. In the three-probes group, the temperature rise at the end of the exposure, expressed as a 95 °C confidence interval for the mean was 11 ± 3 °C at the limbus, 16 ± 6 °C in the vitreous behind lens and 16 ± 7 °C on the sclera next to the optic nerve. Heat diffusivity, estimated as the rate constant was, estimated as a 95 °C confidence interval for the mean, 3 ± 0.4 × 10-5 s¹ in the limbus, 5 ± 1 × 10-5 s¹ in the vitreous behind lens, and 7 ± 2 × 10-5 s¹ on the sclera next to the optic nerve (d.f. = 9). In the two-probes group, the temperature elevation at the end of the exposure was 9 ± 1 °C at the limbus and 26 ± 10 °C on the sclera next to the optic nerve. The heat diffusivity, estimated as a 95 °C confidence interval for the mean, was 3 ± 0.4 × 10-5 s¹ at the limbus and 11 ± 3 × 10-5 s¹ on the sclera next to the optic nerve (d.f. = 9).

Conclusions: An irradiance of 197 W/cm² of 1090 nm for 8 s (1.6 kJ/cm²), in the corneal plane close to collimated between the lens and the retina, induces a temperature increase of about 10 °C at the limbus and about 25 °C close to the retina. It takes about 30 s at the
Commercial Relationships: Zhaohua Yu, None; Karl Schulmeister, None; Nooshin TalebiZadeh, None; Martin Kronsclager, None; Per G. Soderberg, None

Program Number: 1214 Poster Board Number: C0302
Presentation Time: 3:15 PM–5:00 PM
Establishment of a lens epithelial monolayer culture system for the study of glutathione transport

**Xingjun Fan**, **Jeremy Whitson**, **Vincent M. Monnier** 1, **Ulrich Hopfer** 2
1Pathology, Case Western Reserve Univ, Cleveland, OH; 2Physiology and Biophysics, Case Western Reserve University, Cleveland, OH.

**Purpose:** In aging, human lens glutathione (GSH) levels are impaired and accompanied with increased oxidation, protein disulfide formation and protein cross-link. Lens GSH concentrations are believed tightly regulated by two main mechanisms, i.e. by adjusting the rate of synthesis and transporter systems. This was confirmed by our LEGSKO mouse (PLOS ONE 2012(7):e50832). However, the molecular identity of GSH transporters has remained elusive; In order to test the candidate GSH transporters, we are establishing a lens epithelial monolayer culture system to mimic in vivo conditions, since lens has a unique structure with a polarized undifferentiated epithelial cell layer and differentiated fibers.

**Methods:** The GSH (H3-labeled) uptake and transport was tested in homozygous LEGSKO and wild type whole lenses using ex vivo culture and in human lens epithelial cell line (HLE-B3) and primary cultured mouse lens epithelial cells monolayer that were cultured in 24-well Transwell plates. After two weeks of growth, monolayer integrity was monitored by transepithelial electrical resistance (TEER) and the rejection value of Lucifer yellow, a nontransportable fluorescence compound. The monolayer tight junction was characterized by immunocytochemistry using tight junction marker ZO-1.

**Results:** GSH uptake in LEGSKO blocked GSH biosynthesis was more than 5x increased supporting the existence of GSH uptake system in lens. For the monolayer culture system, the TEER showed significant elevation starting at day 5 and reached relative stable value (~60ohm) at day 9. The 1-hour LY rejection value reached to 95% at day 12 from 35% at day 5. The tight junction marker ZO-1 was highly expressed at day 12 based on immunocytochemistry stain using ZO-1 antibody. GSH transport assay by applying H3-GSH to apical chamber showed 5% of GSH penetrated through HLE-B3 monolayer at 30min, and reached to 15% at 1 hour. The results indicated that monolayer has dynamic regulation in GSH transporting through the epithelial cells.

**Conclusions:** The successful establishment of HLE-B3 cells into monolayers is expected the greatly facilitate the identification and characterization of specific GSH transporters into the lens.

**Commercial Relationships:** Xingjun Fan, None; Jeremy Whitson, None; Vincent M. Monnier, None; Ulrich Hopfer, None
Support: EY 07099 and the VSRC grant P30EY-11373

Program Number: 1215 Poster Board Number: C0303
Presentation Time: 3:15 PM–5:00 PM
Broccoli consumption alleviates protein aggregation in cataract

**Annie Abraham**, **Sivapriya S. Girija**. Department of Biochemistry, University of Kerala, Kariavattom Campus, Thiruvananthapuram, India.

**Purpose:** Cataract the opacification in the lens is the leading cause of vision impairment. Biochemical evidences suggest that oxidative damage of the lens proteins is involved in the genesis of cataract. Usually this eye disorder is treated surgically. Besides possible complications and charge of costs, an artificial lens does not have the optical properties of the normal lens. Delaying the onset of cataract by pharmacological means may bridge the gap between the high incidence of cataract blindness and the provision of surgical treatment. Broccoli (Brassica oleracea var italica) offers many health-promoting properties owing to its content of antioxidants. We conducted the study to illustrate the efficacy of broccoli flavonoid fraction (FFB) on rat-lens crystallins in selenite-induced cataract in vivo.

**Methods:** Rat pups, eight-day-old Sprague–Dawley were grouped as control (G I), experimental (G II) and FFB treated (G III). The rat pups in G II, and G III received a single subcutaneous injection of sodium selenite (4 μg/g bodyweight). The treatment groups (G III) were administered with FFB (2.5 mg /kg body weight) respectively from the 8th to 15th day. Cataract was visualized from the 16th day. Size exclusion chromatography was done for isolation of lens crystallins (α, β, and γ). The chaperone activity of α crystallins was measured by heat, DTT, and oxidation-induced aggregation and refolding assays. Concentration of total proteins (soluble and insoluble) and SDS–PAGE analysis of soluble proteins were also done.

**Results:** Alterations in protein profile and insolubilization of soluble proteins have been considered to be the ultimate factor in lens opacification. This study shows that treatment with FFB prevented the loss of α crystallin chaperone activity, and prevent subsequent protein insolubilization prevailed during selenite-induced cataract.

**Conclusions:** These results suggest the protective effects of FFB-rich in antioxidants- in modulating the chaperone activity of lens crystallins in experimental cataract. Synergism between bioactive components of a plant may result in unexpected metabolic outcomes within the plant and within the animal that consumes it.

**Commercial Relationships:** Annie Abraham, None; Sivapriya S. Girija, None

Program Number: 1216 Poster Board Number: C0304
Presentation Time: 3:15 PM–5:00 PM
Properties of Membranes Derived from the Total Lipids Extracted from the Clear and Cataractous Human Lenses of 61- to 70-Year-Old Donors

**Laxman Mainali**, **Marija Raguz** 1, 2, **William J. O’Brien** 1, **Witold K. Subczynski** 1, 2
1Biophysics, Medical College of Wisconsin, Milwaukee, WI; 2Medical Physics and Biophysics, University of Split, Split, Croatia.

**Purpose:** To compare the domain structure and properties of human lens lipid membranes prepared from the total lipids extracted from the clear and cataractous lens cortex and nucleus of 61- to 70-year-old donors. This research is important for further studies of intact human lens membranes, especially for the elucidation of the effect of membrane proteins on the lateral organization of the lipid bilayer portion of these membranes.

**Methods:** Human lens lipid membranes were prepared using a rapid solvent exchange method. Their properties and organization were investigated using electron paramagnetic resonance (EPR) spin-labeling methods. Formation of cholesterol crystals was detected using the differential scanning calorimetry (DSC). Measured membrane properties included: the phospholipid alkyl chain order, alkyl chain fluidity, oxygen transport parameter within the membranes, and hydrophobicity of membrane interior.

**Results:** The measured cholesterol-to-phospholipid molar ratio in human lens membranes was 1.8 and 4.4, respectively, for cortex and nucleus of clear lenses and 1.14 and 1.45 for cataractous lenses. In
the nucleus of clear lenses cholesterol crystals, formed presumably outside membranes. However, in the nucleus of cataractous lenses cholesterol crystals were not formed. In all investigated membranes cholesterol existed in two distinguished environments: (1) cholesterol dispersed in phospholipid bilayer and (2) cholesterol in non crystalline cholesterol domains (cholesterol bilayer domains, CBDs). Only in the preparation from clear lens nuclei was the third environment detected, namely cholesterol crystals.

**Conclusions:** All profiles of membrane properties were very similar to those reported for lens lipid membranes of 41- to 60-year-old donors. This confirms that saturation with cholesterol determines properties of the phospholipid bilayer of lens lipid membranes. The presence of the CBD ensures that the phospholipid bilayer in all investigated membranes was saturated with cholesterol. Formation of cholesterol crystals in aged fiber cells cannot be treated as precursors or signs of age-related cataract formation.

**Commercial Relationships:** Laxman Mainali, None; Marija Raguz, None; William J. O'Brien, None; Witold K. Subczynski, None

**Support:** NIH Grant EY015526, EB002052, EB001980, and EY01931

**Program Number:** 1217 **Poster Board Number:** C0305

**Presentation Time:** 3:15 PM–5:00 PM

A Chinese pedigree with retinitis pigmentosa accompanied with characteristic complicated anterior subcapsular cataract

Mingxing Wu1, Min Hou1, Rong Wen1. 1Cataract, Zhongshan Ophthalmic Center, Guangzhou, China; 2Bascom Palmer Eye Institute, Miami, FL

**Purpose:** Posterior subcapsular cataract in patients suffering from retinitis pigmentosa are frequently observed and studied for it’s common to appear in the lenses. Occasional a three-generation Chinese family undergoing retinitis pigmentosa was discovered to improve anterior subcapsular cataract as well as posterior subcapsular cataract. The purpose of this study is to investigate the clinical features in the three generations, especially the anterior subcapsular cataract.

**Methods:** Family history was recorded. Detailed examinations were performed. All participated were evaluated by slit-lamp biomicroscopy and ophthalmoscopy. Color photography of anterior segment and fundus were taken.

**Results:** Four members from two generations affected by retinitis pigmentosa, who suffered from nyctalopia and visual loss from childhood. Fundus of all the four provided typical RP manifestations of waxy pallor of the optic disk, attenuation of retinal vessels and pigmented deposits resembling bone spicules. All the four cases showed bilateral posterior subcapsular complicated cataracts with nuclear lens opacity in different extent. Among them, the elder two shows typical bilateral anterior subcapsular cataracts in the center of the pupil area. The rest members of the family showed no symptoms or morphological changes of fundus conformed to retinitis pigmentosa. The lens did not present subcapsular opacity either. One of them had phacoemulsification and intraocular lens implantation and the visual acuity was rehabilitated successfully.

**Conclusions:** Complicated anterior subcapsular lens opacity (ASC) appears on the way of retinal degeneration progressing. Cataract surgery proved to ameliorate the visual loss of such patients to date, and interference with ASC formation might be a novel treatment strategy to preserve residual visual acuity in the future.

**Commercial Relationships:** Mingxing Wu, None; Min Hou, None; Rong Wen, None

**Support:** National Natural Science Foundation of China, 81270982

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**Program Number:** 1218 **Poster Board Number:** C0306

**Presentation Time:** 3:15 PM–5:00 PM

**Development of Pseudophakic Model in Young Non-Human Primates**

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**Purpose:** To develop a model of the pseudophakic eye with and without posterior capsulorrhesis in young non-human primates.

**Methods:** Thirteen male 2-3 year old cynomolgus monkeys underwent intraocular lens (IOL) removal via a 25g vitrector and IOLs were placed in the right eyes. Posterior capsulorrhesis was performed in the right eyes of six monkeys at the time of surgery. Eyes were assessed via ophthalmic exams and histopathology and immunochemistry.

**Results:** Immediate postoperative findings included significant anterior segment inflammation characterized by varying degrees of hyphema, fibrin formation, varying degrees of aqueous cell and flare, a degraded view of the fundus due to fibrin and anterior chamber inflammation, and synechias formation starting few days post surgery. The severity of inflammation in the first animals to undergo surgery necessitated administration of intracameral tissue plasminogen activator and modification of the peri- and postoperative treatment regimen to include systemic steroids, increased duration of topical steroids, addition of topical atropine starting on the day of surgery, and placement of an anterior chamber air bubble at the end of surgery. Inflammation gradually decreased and by 4 weeks post-surgery all pseudophakic eyes had varying degrees of posterior synechias, capsular fibrosis, and peripheral anterior synechias. Other variable findings included incomplete pupil dilation, vitreous to the incisions, vitreous to the endothelium, and small anterior synechias. Due to post surgery complications two animals were euthanized approximately 1 month following surgery. One animal developed phthisis bulbi and the other animal developed complete posterior synechia resulting in elevated intraocular pressure. Histopathology revealed capsular and extra-capsular lens epithelial cell (LEC) proliferation associated with posterior synchias and pigment dispersion in the trabecular meshwork membrane. Proliferating LECs showed epithelial to mesenchymal transition including expression of smooth muscle actin.

**Conclusions:** A pseudophakic model in young NHP was successfully developed. Aggressive peri- and post-operative anti-inflammatory therapy was necessary for successful lens removal and IOL placement. The clinical observation of posterior synechia correlated with LEC proliferation.

**Commercial Relationships:** Ewa Budzynski, Covance Laboratories (E); Vladimir Bantseev, Genentech Inc. (E); Chris Schuetz, Genentech Inc. (E); Fiona Zhong, Genentech Inc. (E); Cindy Farman, Genentech (E); Leandro B. Teixeira, OSOD (C); Richard R. Dubielzig, OSOD (C); Michael Struck, OSOD (C); Elliot Bentley, OSOD (C); Evan Thackaberry, Genentech Inc. (E)
The Jagged/Notch pathway is involved in TGFβ2-mediated epithelial-mesenchymal transition of human lens epithelial cells and retard anterior subcapsular cataract

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Purpose: The epithelial-mesenchymal transition (EMT) of lens epithelium cells (LECs) plays a key role in anterior subcapsular cataract (ASC) and posterior capsule opacification (PCO), which are important reasons of visual impairment. The Jagged/Notch pathway has been reported to be essential in EMT during embryonic development, fibrotic diseases and cancer metastasis. However, the function of Jagged/Notch signaling in LEC EMT is unknown. We hypothesized that a crosstalk between Notch and TGFβ2 signaling could induce EMT in LECs, which subsequently contributes to ASC and PCO.

Methods: The human lens epithelial cell line SRA01/04 was cultured in the presence of TGFβ2 alone or with DAPT for 48 hours. The levels of mRNA and protein expression were measured by real-time quantitative PCR and immunoblotting. The expression of EMT markers were also determined by immunofluorescence. The lenses from adult rats were cultured with 5 ng/ml of TGFβ2 for 7 days to induce ASC. Cryo-section and immunofluorescent staining were used to examine the subcapsular clumpy opacities and EMT markers expression.

Results: Simulation of LECs with TGFβ2 for 48 h increased the expression of Jagged-1, Notch-1, Notch-2, Notch-3 and Notch target genes Hes-1 and Hey-1, and induced typical morphological changes of EMT. In contrast, blockade of Notch pathway with DAPT (a specific inhibitor of Notch receptor cleavage), inhibited TGFβ2-induced the up-regulation of collagen type IV, fibronectin, and N-cadherin expression and cytoskeletal changes. Besides canonical Smad signaling, noncanonical PI3K/Akt and MAPK pathways also contribute to TGFβ2 induced activation of Notch pathway in LECs. In addition, treatment of LECs with DAPT attenuated the TGFβ2-induced phosphorylation of Smad2/3. When the lenses were cultured with TGFβ2 for 7 days, lenses developed obvious clumpy anterior opacities beneath the lens capsule, whereas the lenses cultured with DAPT remained transparent, retained normal lens morphology, and did not have accumulation of fibronectin, type IV collagen, α-SMA and vimentin.

Conclusions: Our data suggest that the Jagged/Notch signaling pathway plays a critical role in TGFβ2-induced EMT in human LECs and may contribute to the development of ASC and PCO. Inhibition of the Jagged/Notch signaling therefore may have therapeutic value in the prevention and treatment of ASC and PCO.

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Conclusions: Erlotinib and Gefitinib induced a long-term attenuation of cellular growth in the human capsular bag model after being added for a short time period after preparation. Both inhibitors might become of clinical relevance in PCO prophylaxis. The potential role of EGFR-inhibition in PCO prophylaxis was highlighted.

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Tropomyosin Regulation of Stress Fiber Formation and EMT in Lens Epithelial Cells

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Purpose: The epithelial-mesenchymal transition (EMT) of lens epithelial cells (LECs) is a leading cause of posterior capsule opacification (PCO) after cataract surgery. Transforming growth factor (TGF)-β2 and fibroblast growth factor (FGF)-2 are frequently added for a short time period after preparation. Both inhibitors might become of clinical relevance in PCO prophylaxis. The potential role of EGFR-inhibition in PCO prophylaxis was highlighted.

Methods: Primary cultures of lens epithelial cells obtained from human anterior lens capsule were established and seeded in triplicates on the surface of IOLs consisting of a copolymer of hydroxyethyl methacrylate, ethyl methacrylate and methyl methacrylate (Loflex®, Mediphacos, Belo Horizonte, Brazil) treated or not (controls) with polyethylene glycol. After 26 h, the lenses were washed and trypsinized and the number of viable adherent cells was counted in a hemocytometer. To guarantee the reliability of the results, remnant cells in the residual medium of each triplicate were also counted.

Results: Significantly lower cell adhesion was observed for treated IOLs compared to untreated lenses (p=0.05). Two proliferation patterns were observed in the cultures: round or dendritic cells. In samples of round cells, no significant difference was observed, but adhesion tended to be higher for untreated IOLs (p=0.421). There was also no significant difference for dendritic cells; however, an expressive tendency towards greater adhesion was observed for untreated IOLs (p=0.095).

Conclusions: Coating hydrophilic acrylic IOLs with polyethylene glycol was effective in inhibiting cell adhesion (p=0.05) and this treatment may play a role in the posterior capsule opacification prevention.

Study of hydrophilic intraocular lenses coated with polyethylene glycol using human lens epithelial cells

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Purpose: To evaluate the adhesion of human lens epithelial cells to hydrophilic acrylic intraocular lenses (IOLs) coated with polyethylene glycol.

Methods: Primary cultures of lens epithelial cells obtained from human anterior lens capsule were established and seeded in triplicates on the surface of IOLs consisting of a copolymer of hydroxyethyl methacrylate, ethyl methacrylate and methyl methacrylate (Loflex®, Mediphacos, Belo Horizonte, Brazil) treated or not (controls) with polyethylene glycol. After 26 h, the lenses were washed and trypsinized and the number of viable adherent cells was counted in a hemocytometer. To guarantee the reliability of the results, remnant cells in the residual medium of each triplicate were also counted.

Results: Significantly lower cell adhesion was observed for treated IOLs compared to untreated lenses (p=0.05). Two proliferation patterns were observed in the cultures: round or dendritic cells. In samples of round cells, no significant difference was observed, but adhesion tended to be higher for untreated IOLs (p=0.421). There was also no significant difference for dendritic cells; however, an expressive tendency towards greater adhesion was observed for untreated IOLs (p=0.095).

Conclusions: Coating hydrophilic acrylic IOLs with polyethylene glycol was effective in inhibiting cell adhesion (p=0.05) and this treatment may play a role in the posterior capsule opacification prevention.
Figure 2: dendritic cells

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