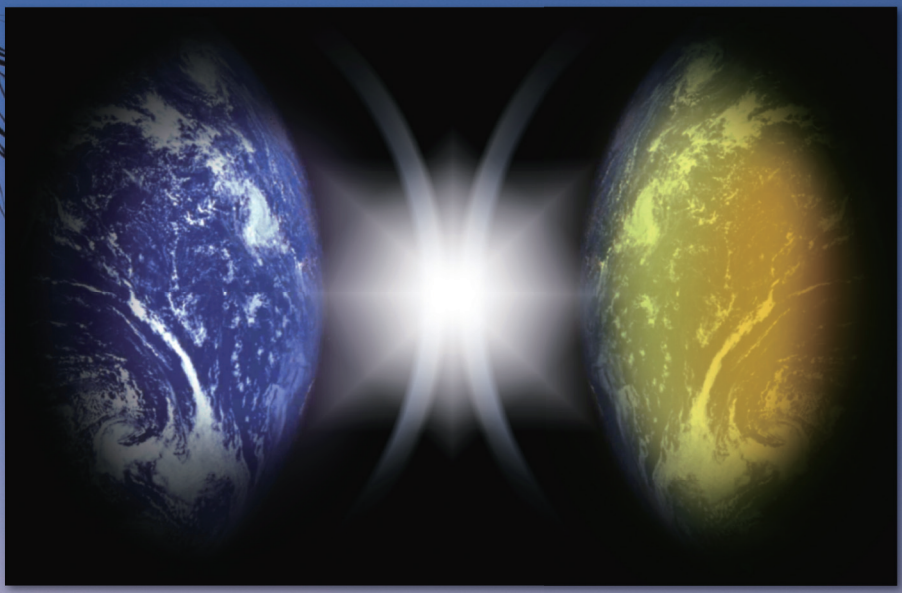
The 3rd International Conference on the Lens 2015



A small interactive meeting focused on the biology of the normal lens and lens-related diseases

December 6 -11, 2015
Kona, Hawaii

The 3rd International Conference on the Lens 2015

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Program and Abstracts

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Program Committee

Dorairajan Balasubramanian
Steven Bassnett
John I. Clark
Paul J. Donaldson
Paul G. Fitzgerald
Velia M. Fowler
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Frank J. Giblin
Xiaohua Gong
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Richard T. Mathias
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Vincent M. Monnier
Mikako Oka
Shizuya Saika
Hiroshi Sasaki
Dhirendra P. Singh
Judith A. West-Mays
Thomas W. White
I. Michael Wormstone
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Acknowledgements

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A special thanks goes to Shelley Golard for her expert organization and production of the Conference brochure.

In addition, a special thanks goes to people in the alumni association of Department of Ophthalmology, Kanazawa Medical University, and its related hospitals in Japan for their generous donations.

The NFER Announces the Following Awards for 2015

Kinoshita Lectureship

Professor Robert C. Augusteyn, Ph.D.

The Vision Cooperative Research Centre, Ivanhoe, Victoria, Australia has been named the 2015 Kinoshita Lecturer. He will present a special lecture on his work on December 10, 2015.

Henry Fukui Award

Kirsten J. Lampi, Ph.D.

Oregon Health and Science University, Portland, Oregon, USA

Fredrick Bettelheim Award

Salil A. Lachke, Ph.D.

University of Delaware, Newark, Delaware, USA

G. Robison Award

Gabriel Rodrigues Cavalheiro

Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil

Alvira Reddy Award

Noriaki Nagai, Ph.D.

Kinki University, Higashi-Osaka, Osaka, Japan

NFER Young Travel Awards

Kumarasamy Anbarasu, Ph.D.
Bharathidasan University, Tamil Nadu, India

Catherine Cheng, Ph.D.
The Scripps Research Institute, La Jolla, California, USA

Bhavana Chhunchha, Ph.D.
University of Nebraska Medical Center, Omaha, Nebraska, USA

Xingjun Fan, Ph.D.
Case Western Reserve University, Cleveland, Ohio, USA

James B. Fields
University of California, Irvine, California, USA

Caitlin M. Logan, Ph.D.
Thomas Jefferson University, Philadelphia, Pennsylvania, USA

Renita Martis
The University of Auckland, Auckland, New Zealand

Mitchell Nye-Wood
The University of Auckland, Auckland, New Zealand

Timothy F. Plageman, Ph.D.
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University of California, Berkeley, California, USA

Irene Vorontsova, Ph.D.
University of California, Irvine, California, USA

Janice L. Walker
Thomas Jefferson University, Philadelphia, Pennsylvania, USA

Investigator Travel Award

Viviana M. Berthoud, Ph.D.
University of Chicago, Chicago, Illinois, USA

Kanazawa Medical University Young Investigator Travel Awards

Zhiying (Jessy) Kong
The University of Auckland, Auckland, New Zealand

Yoshiki Miyata, Ph.D.
Teikyo University, Tokyo, Japan

Koichiro Mukai, MD.
Dokkyo Medical University, Mibu, Tochigi, Japan

Yosuke Nakazawa, Ph.D.
Keio University, Tokyo, Japan

Michael O'Connor, Ph.D.
Western Sydney University, Sydney, Australia

Andrew J. O. Smith, Ph.D.
University of East Anglia, Norwich, England

Yuki Sugiyama, Ph.D.
The University of Sydney, Sydney, Australia

Takumi Takata, Ph.D.
Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan

Presentation Guidelines

Instructions for platform presenters

- Sessions will be 120 minutes (for 6 presentations) or 100 minutes (for 5 presentations) with the time for each presentation being evenly divided within the time allocated.
- At least 3 minutes of the time slot should be left for questions/discussion.
- Presentations should be prepared in PowerPoint.

Instructions for poster presenters

- Poster boards are 36 inches high x 48 inches wide.
- Posters should be put up either Sunday evening or Monday morning.
- Posters should be taken down Thursday evening.
- Each presenting author (first author) should prepare one PowerPoint slide summarizing the major conclusion from the work presented in the poster.
- Each first author should prepare a 3-minute oral summary to go with the slide.
- On Tuesday, 4:10-6:20 pm, each first author will give a 3-minute 1 slide platform summary of the poster.
- Poster viewings will be held as follows;

  6:20-6:50 pm on Dec. 8 (Tue) with refreshments service
  8:00-8:30 am on Dec. 9 (Wed)

ICL2015 At-A-Glance

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Program

December 6 (Sun)

4:00-6:30 pm	Registration
6:00-8:00 pm	Welcome Reception

December 7 (Mon)

Day 1

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Robert C. Augusteyn

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12:00-12:10 pm **Closing Comments by Peter F. Kador and Hiroshi Sasaki**

12:10- pm Lunch

Depart

Abstracts (Oral)

Session 1-1

Actin cytoskeleton in lens architecture and mechanics

Velia M. Fowler, Catherine Cheng

Department of Cell and Molecular Biology, The Scripps Research Institute, La Jolla, CA, USA

Purpose: Lens shape and integrity relies upon highly patterned morphogenetic differentiation and maturation of fiber cell layers. Previous studies indicated that deletion of the F-actin capping protein, Tmod1, led to loss of lens γ-tropomyosin (γTM) and disruption of the spectrin-F-actin membrane skeleton, with perturbations in fiber cell morphology and compromised lens stiffness in outermost fiber cell layers. Like other TMs, γTM associates with F-actin and stabilizes filaments from depolymerization. To test the hypothesis that effects of Tmod1 deletion may be due to loss of γTM and consequent F-actin instability, we studied γTM deletion in the mouse lens.

Methods: *γTM-/-* mice were obtained from Dr. Peter Gunning at University of New South Wales, Australia, and loss of γTM in lenses confirmed by western blotting. γTM localization was investigated by immunostaining of cryosections and confocal microscopy. Lens transparency was assessed by light microscopy, and lens stiffness by axial compression using sequential application of discrete loads and digital light microscopy measurements.

Results: Dissected lenses from 1 month-old *γTM-/-* mice showed subtle increases in lens opacity at the anterior, with older animals developing obvious cataracts. Immunostaining of wild-type lens equatorial sections revealed γTM localized in a punctate pattern on broad and short sides of differentiating fiber cells, persisting after nuclear loss, when it became distributed more uniformly along the membranes. Mechanical compression tests revealed that loss of γTM had little effect at low loads (~10% strain) but significantly reduced lens stiffness at higher loads (~30% strain), unlike *Tmod1-/-* lenses, which had reduced stiffness at low but not high loads.

Conclusions: We conclude that γTM expression is coordinated with fiber cell differentiation and maturation, and promotes lens transparency and integrity under mechanical load. In addition, Tmod1 may act independently of γTM to control the F-actin cytoskeleton, fiber cell architecture and lens mechanical properties.

Session 1-2

Fiber cell membrane cytoskeletal scaffolding mechanisms and lens cytoarchitecture, biomechanics and transparency

P. Vasantha Rao

Departments of Ophthalmology and Pharmacology & Cancer Biology, Duke University School of Medicine, Durham. NC, USA

Purpose: While the membrane cytoskeletal network is considered to play a crucial role in maintaining lens fiber cell hexagonal geometry, packing, deformability and membrane organization, the identity and role of scaffolding proteins which play key roles in these crucial cellular processes is poorly known. Here we explored a possible interaction of periaxin; a PDZ domain protein expressed preferentially in myelinating Schwann cells and lens fibers with ankyrin-B and their coordinated role(s) in lens fiber cell membrane scaffolding, cytoarchitecture and stiffness.

Methods: Periaxin interaction with ankyrin-B and their coordinated role in lens fiber cell hexagonal shape, adhesion, membrane cytoskeletal organization and stiffness were determined using the periaxin null and ankyrin-B haploinsuffient mouse lenses, high resolution confocal microscopy, microstrain analyzer and biochemical analyses.

Results: Our studies revealed that ankyrin-B, a well characterized adaptor protein involved in linking the spectrin-actin cytoskeleton to integral membrane proteins, is required for membrane association of periaxin in lens fibers and colocalizes with periaxin at hexagonal fiber cell vertices and long arms. Significantly, under ankyrin-B haploinsufficiency, periaxin accumulates in the soluble fraction with a concomitant loss from the membrane-enriched fraction of mouse lenses. Moreover, ankyrin-B haploinsufficiency-induced age-dependent disruptions in fiber cell hexagonal geometry and radial alignment, and decreased compressive stiffness in mouse lenses parallel the changes observed in periaxin null mouse lens. Both ankyrin-B and periaxin deficient mice exhibit disruptions in membrane organization of the spectrin-actin network and the dystrophin-glycoprotein complex in lens fiber cells.

Conclusions: Taken together, these observations reveal that ankyrin-B is required for Periaxin membrane anchoring and for maintenance of lens fiber cell hexagonal geometry, membrane skeleton organization and biomechanics.

Session 1-3

A critical role for N-cadherin in lens fiber cell elongation and tissue morphogenesis

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Purpose: Tissue development and regeneration involve high-ordered morphogenetic processes that are governed by elements of the cytoskeleton in conjunction with cell adhesion molecules. Such processes are particularly important in the lens whose structure dictates its function. Previous work from our lab has demonstrated a role for N-cadherin junctions in lens fiber cell elongation and actin organization within the lens, and recent work has begun to unveil the importance of N-cadherin in the regulation of developmental migration. Here we investigated the role of N-cadherin in lens morphogenesis.

Methods: A lens-specific N-cadherin conditional knockout mouse (*mlr10* N-cadherin cKO) was generated so as to examine effects of loss of N-cadherin on lens morphogenesis. Analysis was performed on cryosections from the above lenses labeled for ZO-1, phospho-myosin and/or F-actin and imaged by confocal microscopy.

Results: By E13.5, *mlr10* N-cadherin cKO lenses demonstrated loss of fiber cell directionality. This loss of directionality led to an increased clustering of actin at newly differentiating fiber cell apical tips at the transition zone. By E16.5, actin had reorganized from its usual distribution along lateral cell-cell interfaces to become concentrated at cell vertices. This was accompanied by severe morphogenetic defects and failure of elongation. Lens fiber cells maintained connections along their apical interfaces by a persistence of ZO-1. *mlr10* N-cadherin cKO lenses also demonstrated increased activation of myosin II in fiber cells. Postnatal lens demonstrated complete loss of lens structure. These results demonstrate the necessity of N-cadherin in lens fiber cell elongation, lens morphogenesis, and the maintenance of lens cytoarchitecture.

Conclusions: Lens fiber cell elongation and morphogenesis depends on N-cadherin.

Session 1-4

Is directed migration of the lens fibre cells led by guidance signals?

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Purpose: Directed migration of the apical and the basal tips of the elongating lens fibre cells to the anterior and the posterior poles, respectively, underlies spheroidal lens formation. It is not clear whether their highly coordinated movement is driven by cell autonomous mechanisms or led by external guidance signals. We recently found that each fibre cell has a cilium on the apical surface that locates asymmetrically to the anterior pole side. This polarity is uniform across the elongating fibres so that in global view, lens fibres exhibit planar cell polarity (PCP). Given the role of primary cilia as antennae to detect extracellular signals and also their involvement in PCP formation, we hypothesised that the primary cilia on the fibre cells detect a putative guidance signal to promote directed fibre migration. We also showed that overexpression of Sfrp2, a modulator of Wnt ligands and Frizzled receptor binding, in lens disrupted directed migration of the fibre cells, suggesting Wnt is the guidance molecule to orchestrate fibre cell alignment.

Methods: To test these hypotheses formation of cilia was blocked in lens by conditional knocking-out of IFT88, a key component of the intraflagellar transport (IFT) complex, using MLR10- and LR-Cre lines. Exogenous Wnt5a expression was conducted by crossing Cre-inducible Wnt5a line with MLR10 line.

Results: Irrespective of which Cre-line was adopted, in IFT88-depleted cells the ciliary axoneme was absent or substantially shortened, confirming the disruption of primary cilia formation. However, the lens fibre cells maintained normal alignment without any indication of abnormality. The lenses with ectopic Wnt5a expression formed normal Y-sutures at the anterior pole without any irregularity of fibre alignment.

Conclusions: The primary cilia do not play an essential role in the precise cellular alignment of fibre cells. The overexpression experiment did not support the role of Wnt5a as the guidance signal to direct fibre migration.

Session 1-5

Mapping the subcellular distribution of AQP0 and AQP5: implications for the water permeability of lens fiber cells

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Purpose: To investigate the relative contributions of AQP0 and AQP5 to the water permeability of fiber cells in different regions of the lens.

Methods: Fresh lenses or lenses pre-incubated in Artificial Aqueous Humor of varying osmolarity (270-405mOsm) were either fixed for immunohistochemistry or separated into epithelial cells and fiber cell vesicles preparations. Confocal imaging of immunolabelled lens cryosections and membrane vesicles was used to determine changes in the subcellular distribution of AQP5 under different incubation conditions in different lens regions. Isolated epithelial cells or fiber cell vesicles were loaded with the fluorescent dye Calcein AM and the rate of change in fluorescence intensity in response to an applied osmotic gradient used to calculate an apparent PH2O. Experiments were performed in the presence or absence of HgCl2 that preferentially blocks AQP5, but not AQP0.

Results: A change in the subcellular distribution of AQP5 from the cytoplasm to the cell membrane was observed in the outer cortex of the rat lenses, but was restricted to only superficial layers of fiber cells in the mouse lens. Within these zones of cytoplasmic AQP5 labelling the organ culturing of lenses in the presence of an osmotic challenge resulted in an increased insertion of AQP5 into fiber cell membranes. PH2O measured in mouse fiber cell membrane vesicles was significantly decreased by the presence of HgCl2, while vesicles from the rat lens were not, results that are consistent with the observed sub-cellular location of AQP5 in the cytoplasm.

Conclusions: AQP5 exists in the lens as two pools of water channels: an inactive cytoplasmic pool and an active membrane pool. External stimuli such as osmotic or mechanical stress can increase the recruitment of AQP5 to the plasma membrane to dynamically up regulate the PH2O of the lens.

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Session 2-1

Lens proliferation and survival factors

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Purpose: The developing lens is enclosed by a capsule and patterns of proliferation migration and differentiation occur in defined regions. Unlike other cellular systems there is no turnover of differentiated fibre cells and levels of epithelial cell death are low, suggesting cell death is tightly regulated. However, disruption of cell signalling pathways, by us and others, commonly leads to cell death, highlighting the sensitivity of the lens to death pathway activation. This paper reviews some of the studies that have demonstrated a 'survival' role for various factors in lens.

Methods: Cre-mediated recombination using LeCre or MLR10 lines was used to study the role of various genes (*Ilk, Smo, β-catenin)* in lens development. Phenotypes of mutant lenses were examined by TUNEL and immunodetection of signalling intermediates, cell cycle and differentiation markers of.

Results: Deletion of *Ilk* from E13.5 resulted in altered epithelial cell proliferation (S-phase defect) and epithelial cell death from E17.5, which correlated with decreased activation of Shp2, Akt and ERK, suggesting an FGFR-dependent mechanism in ILK-mediated cell survival. This is consistent with studies demonstrating integrin augmentation of receptor tyrosine kinase signalling during lens survival. Inhibition of hedgehog signalling pathway by *Smo* deletion affected lens development only if deletion occurred prior to E13.5, indicating a discrete window of Hh activity. The phenotype is characterised by an M-phase defect and loss of epithelial cells from E14.5. By contrast, while loss of Wnt signals by conditional *Catnnb* deletion from E13.5 affected epithelial cell proliferation (S-phase defect) it did not have an immediate effect on cell survival. Similar findings were demonstrated when the Wnt target gene, myc, was conditionally deleted in lens.

Conclusion: While the FGF, integrin/ILK, Shh and Wnt/β-catenin pathways all impact lens cell proliferation only the FGF, integrin/ILK, Shh pathways appear to mediate survival signals directly during lens differentiation.

Session 2-2

Cell division and cadherin-mediated adhesion regulate lens epithelial cell movement in zebrafish

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Purpose: Lens consists of anterior lens epithelium and posterior lens fiber core. During development, lens epithelial cells proliferate, move posteriorly, and differentiate into lens fiber cells at the lens equator. However, spatial regulation of lens epithelial cell movement is not fully understood. Here, we conducted confocal time-lapse imaging of cell proliferation, cell division, and cell movement in zebrafish lens epithelium, and examined the role of cell division and adhesion in lens epithelial cell movement.

Methods: To visualize cell proliferation and movement, we used a zebrafish transgenic line expressing GFP-tagged Histone and mCherry-tagged Geminin, which mark chromatin and the S/G2 phase, respectively. To examine the role of cell adhesion in lens epithelial cell movement, we inhibited E-cadherin and N-cadherin, using a zebrafish *e-cadherin* mutant and morpholino antisense against N-cadherin. To measure lens epithelial tension, we applied single cell laser ablation method combined with confocal time-lapse scanning.

Results: Cell movement was spatially patterned in zebrafish lens epithelium: longitudinally biased in the anterior region and circumferentially shifted in the peripheral region, which generates a spiral-like movement pattern. Furthermore, almost cell intercalations were triggered by cell division in the lens epithelium. Speed of lens epithelial cell movement was increased in E-cadherin mutant, but decreased in N-cadherin morphant. Cell ablation experiment showed that a pulling tension is prominent in the lens epithelium, which is balanced by E- and N-cadherin.

Conclusion: Our data suggest that cell division is a major driving force for lens epithelial cell movement. E-cadherin suppresses cell movement by packing lens epithelium, whereas N-cadherin enhances cell movement by promoting lens fiber growth, which subsequently antagonizes E-cadherin-mediated epithelial packing. We propose that cell division and cadherin-mediated adhesion cooperate to regulate lens epithelial cell movement through the modulation of lens epithelial tension.

Session 2-3

Glutaredoxin2 (Grx2) and aging

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Purpose: Glutaredoxin2 (Grx2) is a mitochondrial enzyme with disulfide reductase and peroxidase activities that regulate protein thiol/disulfide balance in cells. Deletion of Grx2 in mouse (Grx2 KO) induced early cataract formation during aging. The association of Grx2 with lens aging and opacity was examined in this study.

Methods: Lens light scattering in 1-22 m old Grx2 KO and age-matched wild-type (WT) mice was examined using slit lamp. Total lens proteins from 2, 6, 16, 22 m old were quantified for protein-thiol mixed disulfides of P-SSglutathione (PSSG) and P-SS-cysteine (PSSC) by a Dionex system. Glutathionylated proteins were identified by Western blot, immunoprecipitation and mass spectrometry. ATP, GSH levels, and complex I, IV, DNase IIβ enzyme activities were assayed. Lens differentiation was examined in new born with confocal immunohistological images.

Results: Lenses from Grx2 null mice showed similar growth pattern as the WT mice. KO mice developed nuclear opacity 3 m sooner than that of the age-matched controls, and the cataract was progressed much faster and more severe. PSSG and PSSC were accumulated in these lenses with high glutathionylation in structural proteins of actin, filensin, CP49 and αA crystallin. Comparing with the age-matched controls, the lenses of Grx2 KO mouse had only 50% activity in mitochondrial complex I and complex IV; less than 50% GSH and 10% ATP pools. The newborn KO mouse displayed a higher nuclei density at the germinating zone, and a partial de-nucleation deep into the inner fibers. The de-nucleation enzyme, DNase IIβ, was glutathionylated and inactive.

Conclusion: Grx2 gene deletion causes lens structural protein/enzyme dysfunction and suppressed energy production. It also alters lens differentiation by partial de-nucleation in the fiber cells. These changes may associate with the premature and accelerated age-related cataract development. Grx2 KO mouse can be a good model to study human senile cataract.

Session 2-4

Lens fiber cell denucleation co-opts the Cdk1 mitotic machinery

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Denucelation of lens fiber cells is an essential event in formation of a clear lens, as failure to remove lens fiber cell nuclei is associated with various congenital cataracts. Degradation of chromatin occurs via the lysosomal enzyme DNaseIIβ. Over the last few years we demonstrated that the unidirectional process of lens fiber cell denucleation (LFCD) is driven by the mitotic cyclin-dependent kinase Cdk1, which is indispensable for progression through cell cycle. Cdk1 is in turn regulated by several activators and inhibitors.

We demonstrated that expression of the ubiquitin mutant K6W-Ub in the lens in two strains of mice, including WT C57BL/6, causes delayed development and attenuated LFCD. These lenses do not show an unfolded protein response (UPR). Recently, we observed deficient LFCD in three different models mouse models, all of which are characterized by increased endoplasmic reticulum (ER) stress leading to an unfolded protein response (UPR). A common feature off all four models is the accumulation of p27, a potent Cdk1 inhibitor. In the K6W-Ub model, we showed that p27 was stabilized by impaired ubiquitin-mediated proteolysis.

Cdk1 kinase activity leads to phosphorylation of lamin A/C, along with other targets, which leads to nuclear envelope breakdown. Using immunofluorescent approaches and ultra-high resolution focused ion beam electron microscopy, we have observed lysosomes bearing DNaseIIβ in nuclei in denucleating LFCs. Conversely, LFCs that cannot undergo LFCD, either due to expression of K6W-Ub or deletion of Cdk1, do not undergo nuclear envelope breakdown and lysosomes are sequestered around the nuclear envelope. Organelle breakdown appears to be a separate program from LFCD, as Cdk1-deficient lenses still show loss of ER and mitochondria.

Thus, it appears that the lens has appropriated many controls of cell cycle regulation for unidirectional LFCD. Divergence of these events occurs prior to entry of lysosomes into the nucleus.

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Session 2-5

N-myc regulates mouse lens growth and differentiation *in vivo*

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Purpose: Myc transcription factors (c-myc, N-myc and L-myc) regulate cell cycle dynamics, cell growth and survival in various developing tissues. These cellular events must be precisely coordinated during formation of a functional lens, raising the question of whether Myc genes participate in lens development. We have shown that c-myc is crucial for promoting cell proliferation in the embryonic lens epithelium. Possible mechanisms include repression of p27Kip1 and Prox1 as well as activation of Foxe3 in lens progenitors. Here, we investigate the roles of N-myc in the developing lens.

Methods: To study N-myc function during lens development, we used Le-Cre, Mlr10-Cre and Mlr39-Cre mouse lineages to genetically inactivate N-myc in the surface ectoderm (N-mycLe-Cre), lens vesicle (N-mycMlr10-Cre) or post-mitotic fiber cells (N-mycMlr39-Cre).

Results: We found that N-myc is highly expressed in the lens during early embryonic (E12.5) and newborn stages. N-myc genetic inactivation (cKO) in N-mycLe-Cre and N-mycMlr10 mice resulted in congenital microphakia/microphtalmia which persisted through adulthood (P90). Optomotor response tests failed to reveal differences in visual acuity between N-mycMlr10 and control mice, while N-mycLe-Cre mice had impaired visual response. We did not detect cell proliferation defects in either N-mycLe-Cre or N-mycMlr10 embryonic (E14.5) lenses, contrary to what was observed for lens-specific c-myc inactivation. N-mycLe-Cre mice presented congenital cataracts, associated with defects in nuclei removal in the fiber cells. Interestingly, c-myc-null lenses did not exhibit significant alterations in nuclei degradation during embryonic stages. Remarkably, lens-specific double-inactivation of N-myc and c-myc led to extremely severe microphakia/microphtalmia as compared to single cKO (N-myc- or c-myc-null) lenses.

Conclusions: We found that N-myc is required for proper lens growth. Our findings support the hypothesis that Myc transcription factors regulate multiple cellular events during lens formation *in vivo*. These findings are the first description of the physiological roles played by Myc genes during lens development.

Session 2-6

Hyperglycemia enhances the production of amyloid β_{1-42} in the lenses of Otsuka Long-Evans Tokushima Fatty rats, a model of human type 2 diabetes

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Purpose: Several studies have reported that the accumulation of amyloid β (Aβ) in brain was enhanced by the onset of diabetic mellitus. In this study, we investigate the effect of hyperglycemia on $A\beta_{1-42}$ accumulation in the lens of Otsuka Long-Evans Tokushima Fatty (OLETF) rat, which is a model of human diabetes mellitus developed *via* a metabolic syndrome.

Methods: Male OLETF rats and human lens epithelial cell line SRA 01/04 (HLE cell) were used in this study. The mRNA expression levels were determined by the quantitative real-time RT-PCR method, and $A\beta_{1-42}$ levels were measured using the ELISA method.

Results: The OLETF rats over 20 weeks of age developed the diabetes mellitus with hyperglycemia. The $A\beta_{1-42}$ levels and amyloid precursor protein (APP), β-(BACE1), or γ-secretase (PS) mRNA were also elevated in the lenses of OLETF rats with aging, and the high $A\beta_{1-42}$ levels in OLETF rats were observed in lens capsule-epithelium and cortex. Moreover, the enhanced gene expression levels of APP, BACE1 and PS in the lenses of OLETF rats were prevented by the food restriction (25 g/day/rat). We also investigated the effects of glucose levels on the production of $A\beta_{1-42}$ using HLE cell. The APP, BACE1, PS mRNA and $A\beta_{1-42}$ levels in HLE cells under the high glucose condition (glucose content, 20 mM) were significantly higher than that in normal condition (glucose content, 5.6 mM).

Conclusions: We hypothesize that the high glucose increase the expression of levels of genes related to Aβ production, resulting in the accumulation of Aβ in the lens. These findings provide significant information that can be used to design further studies aimed at reducing the lens opacification in diabetic mellitus patients.

Session 3-1

Fibre differentiation – where we have been and where we are going

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Over the years *in vivo* and *in vitro* studies have provided compelling evidence that fiber differentiation is triggered by vitreous-derived FGF. Other growth factors appear to contribute, but FGF-induced signalling has been shown to be a necessary ingredient for any mix of factors that activate the fiber differentiation process. Whilst much progress has been made in understanding regulation of fiber differentiation, outstanding questions remain around the issues of how the behaviour of individual fibers is coordinated so that they become organised to generate the precise spheroidal arrangement that delivers normal lens function. In addition, how the balance between fiber generation and maintenance of the epithelial progenitor pool is achieved and maintained, is little understood. Epithelial cells and fiber cells populate spatially distinct compartments, and the size of each must be tightly regulated and modulated so that the lens achieves and maintains appropriate dimensions during growth and ageing. Recent research indicates that reciprocal inductive interactions mediated by Wnt-Frizzled and Notch-Jagged signaling pathways are important for maintaining and organizing these compartments. The Hippo-Yap pathway has also been implicated in maintaining the epithelial progenitor compartment and regulating growth processes. Thus, whilst some regulatory pathways have been identified, an important area for future research will be to gain a better understanding of how these pathways interact to determine and modulate lens size and shape from cradle to grave.

Session 3-2

Isolation of stem cells from lens epithelial cells and its environment for differentiation

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Purpose: To better understand the environment for fiber cell differentiation.

Method: Murine lens epithelial cells were isolated and stained with Hoechst 33342. A side population (SP) of cells were sorted using fluorescence activated cell sorting (FACS). Gene expression was analyzed by semi-quantitative real time PCR. Cells were cultured in DMEM and 10% FBS. To localize SP cells in the lens, whole lens were incubated in DMEM supplemented with Hoechst, and wholemounts of the epithelium were observed using fluorescence microscopy.

Results: SP cells in the mouse lens epithelial cells distinctly expressed higher levels of the stem cell markers ATP-binding cassette transporter G2 (ABCG2), p75 neurotrophin receptor (p75NTR), nestin (Nes), B-cell lymphoma 2 (Bcl2), and cell surface antigen Sca-1mRNA compared to the main epithelial population. The SP cells were smaller in size than non-SP cells and their number in lens epithelia decreased with age of mouse. These results suggest that SP cells contain a high proportion of stem cells. Histological analysis showed SP cells localized near the equatorial region of the lens epithelium. SP cells cultured in DMEM and 10% FBS did not proliferate, but did so when the media was supplemented with FGF2. These SP cells could not to differentiate into fiber cells with FGF. When Porcine SP cells were cultured with vitreous they demonstrated higher proliferation rates to that induced by FGF2. Vitreous also induced these porcine SP cells to form lentoid bodies.

Conclusion: The SP cell population in the lens epithelium contains stem cells, and these stem cells are localized near the germinative zone in situ. Vitreous is suggested to induce these lens stem cells to undergo lens fiber differentiation.

Session 3-3

Connexin23 is dispensable for lens transparency

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Purpose: Two connexins (Cx46 and Cx50) are crucial for maintaining lens transparency and Cx50 is important for lens growth, but the contributions of a third lens fiber connexin (Cx23) are poorly understood. Therefore, we studied the consequences of absence of Cx23 in mouse lenses.

Methods: Cx23-null mice were generated by homologous Cre recombination. Cx23 expression was assessed by RT-qPCR. Lenses were examined by dark-field microscopy and laser scanning. Levels and distribution of connexins were determined by immunoblotting and immunofluorescence.

Results: Deletion of Cx23 did not affect the viability, size, gross appearance, or activity of the mice. Cx23-null lenses did not express Cx23 transcripts. The transparency, gross anatomy, refractive properties and optical quality of Cx23-null lenses were similar to those of wild type mice. Deletion of Cx23 did not affect Cx46 or Cx50 localization, but it did decrease their levels (by 22-38%).

Conclusions: These results demonstrate that although Cx23 expression influences levels of the co-expressed connexins, Cx46 and Cx50, the lack of mouse Cx23 does not affect lens transparency or refractive properties. Thus, Cx23 appears dispensable for lens function.

Session 3-4

Conserved RNA binding proteins in lens fiber differentiation

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Purpose: Although the human genome encodes over fifteen hundred RNA binding proteins (RBPs), few RBP genes have been investigated in eye development. Here, we report on the detailed characterization of three RBPs that exhibit highly enriched expression in lens fiber cells and perform specific functions in their differentiation program.

Methods: The bioinformatics-based eye gene discovery tool *iSyTE* (integrated Systems Tool for Eye gene discovery, http://bioinformatics.udel.edu/Research/iSyTE) was applied to identify RBP genes that exhibit enriched expression in mouse lens development. Mouse mutants carrying targeted deletion alleles of the RBP-encoding genes *Celf1*, *Caprin2*, and *Tdrd7* were investigated for characterization of lens defects.

Results: The enriched expression pattern of Celf1, Caprin2 and Tdrd7 in differentiating fiber cells is conserved in multiple vertebrate species. Further, homozygous null mouse mutants for all three RBP genes exhibit distinct defects in fiber cell differentiation. *Tdrd7* deficiency in mice results in down-regulation of the heat shock protein Hspb1 and the alpha actinin protein Actn2, causing F-actin defects in fiber cells, which eventually culminate into severe cataracts by the third week of birth. *Caprin2* deficiency in mice causes an abnormally compacted central lens region of terminally differentiated fiber cells. Finally, *Celf1* deficiency in mouse, frog or fish causes severe lens defects including cataracts. *Celf1* null mouse lenses show abnormal retention of nuclei in centrally located fiber cells, indicative of defective differentiation. RNA-immunoprecipitation assays indicate that different RBPs bind to specific lens-expressed mRNAs and control their stability or translation.

Conclusions: These findings suggest that distinct RNA binding proteins have evolved to control conserved gene regulatory networks on the post-transcriptional level in vertebrate lens development. Further, they suggest that like transcription factors, RBPs have critical regulatory function in fiber differentiation. This research is supported by NIH R01 EY021505 and The Pew Charitable Trust Scholars Program in Biomedical Sciences.

Session 3-5

Regulation of growth factor-induced lens fibre differentiation

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Purpose: The ability of vitreous humour to induce and regulate lens fibre differentiation, is primarily due to its growth factor constituents, none more so than FGF that is well established as a primary inducer of this process. Other ocular growth factors, such as BMPs, Wnts, IGF, EGF and PDGF, have also been implicated to be involved in this process, but their exact contribution independent of FGF is not well understood. Here we further examine the role of some of these factors and how lens fibre differentiation signalling is regulated.

Methods: Rat lens epithelial cells from lens explants were induced to differentiate into fibre cells using different growth factors (bovine vitreous, FGF2, IGF, EGF) or a combination of these, for up to 5 days. Fibre differentiation was determined by cell elongation, together with Prox1 and/or ß-crystallin accumulation. FGFR-signalling was compromised using SU5402 or a dominant negative FGFR to determine its role in fibre differentiation.

Results: While IGF or EGF could both stimulate lens cell proliferation; together, they induced a fibre differentiation response, resulting in multicellular lentoid bodies comprised of elongating cells expressing β-crystallin and Prox1, very similar to vitreous- or FGF-induced lens fibre differentiation. Blocking FGFR signalling prevented this IGF/EGF-mediated fibre differentiation.

Conclusions: While IGF1 and EGF are primarily mitogens for lens epithelial cells, together, they act synergistically to mediate lens fibre differentiation, and this process is was shown to be dependent on FGFR-signalling. This study highlights a putative novel mechanism for the activation of FGFR-signalling via other independent ocular growth factors (such as IGF and EGF), and supports the key requirement for FGFR-signalling in lens fibre differentiation.

Session 4-1

Aging and the human lens

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Purpose: We now have a reasonable picture of cataract etiology, at least with respect to human nuclear cataract. Lens proteins do not turnover and over time they undergo massive changes. This protein degradation is due predominantly to spontaneous processes and these can be classified into three broad categories: a) racemisation/deamidation, b) truncation and c) cross-linking.

The processes and time courses for racemisation/deamidation and truncation of crystallins in the human lens have now been documented, however the mechanism of protein-protein cross-linking is still poorly characterised.

Methods: Mass spectrometry of proteins from aged lenses provides the data. The basic processes can then be characterised by incubation of peptides.

Results: One important covalent cross-linking process in the lens involves the spontaneous formation of dehydroalanine (DHA). Once DHA forms, it becomes a site for nucleophilic attack by Lys and Cys residues leading to covalent cross-linking. Beta elimination of phosphoserine residues is one source of DHA in proteins [1]. Recently in collaboration with Kevin Schey's team, we have been investigating another source of DHA: Cys residues. The results of this investigation will be presented.

Conclusions: Characterising the spontaneous, non-enzymatic processes of protein degradation should enable us to understand the basis of age-related changes to the lens such as presbyopia and cataract. The ramifications of this work are significantly wider however, since long-lived proteins that occur in other parts of the human body also breakdown. Such proteins are widely distributed in the body e.g. brain, heart, joints. Understanding the mechanisms behind these degradative processes will provide a window into the fundamental biochemical events that underpin human aging and longevity.

Wang, Lyons, Truscott and Schey. Human protein aging: modification and crosslinking through dehydroalanine and dehydrobutyrine intermediates. Aging Cell (2014) 13, 226–234.

Session 4-2

Role of the cystine/glutamate antiporter in maintaining glutathione levels in ocular tissues

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Purpose: Oxidative stress plays a major role in the onset of age-related eye disease such as cataract, glaucoma and macular degeneration. Antioxidants such as glutathione (GSH) are essential in minimising oxidative damage to the tissues thus delaying the onset of ocular pathologies. Cysteine is the rate limiting precursor for GSH synthesis, and intracellular accumulation of cysteine is mediated by the cystine/glutamate antiporter (xCT). Using a xCT knockout mouse (KO), we have investigated the role played by xCT in providing GSH to the tissues of the eye and minimising oxidative stress.

Methods: Corneas, lenses, and retinas were collected from 3-6 week old C57BL/6 wild type (WT) and KO mice. GSH concentrations were measured using a biochemical assay and the oxidative stress marker 4-Hydroxynonenal (4-HNE) visualised using immunohistochemistry. Ocular structures were examined using biomicroscopy.

Results: Although GSH concentrations in the lens and retina were similar between the two phenotypes, the GSH levels in the cornea were significantly lower in the KO mouse. Similarly, there were no differences in the 4-HNE levels in the lens and retina between the two phenotypes. However, the cornea showed higher levels of 4-HNE in the KO relative to the WT mouse. No ocular pathologies were evident in the WT or KO mice.

Conclusions: The decreased GSH levels in the cornea of the KO mouse suggest that xCT is vital in providing intracellular cysteine for GSH synthesis in this tissue. In addition, the increase levels of oxidative stress suggests that with increasing age, these mice may be more susceptible to the earlier onset of oxidative stress induced corneal disease. In contrast, xCT in the lens and retina may not be critical for GSH synthesis. It is likely that other amino acid transporters exist that compensates for the loss of xCT to maintain intracellular GSH levels.

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Session 4-3

Nrf2 promotes lens epithelial cell death by enhancing oxidative stress through activation of Klf9-mediated repression of Prdx6

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Purpose: Dysfunction of antioxidant defense system is prominent in development of diseases related to oxidative stress and age. We investigated the regulation and contribution of Peroxiredoxin (Prdx) 6, to cellular resistance against oxidative stress. We unveiled that when oxidative load increased beyond a threshold, Nrf2 activated transcription Kruppel-like factor (Klf9), leading to further Klf9-mediated increase of oxidative load, Prdx6 repression and subsequent cell death.

Methods: Lens epithelial cells (LECs) treated with variable concentrations of H_2O_2 (0 – 300μM) were used to examine expression of Prdx6, Nrf2, Sp1 and Klf9 by real-time PCR and Western analyses. Actinomycin D treatment determined modulation in Nrf2, Sp1 and Klf9 mRNA levels. Nrf2 activation of Klf9, and Klf9-dependent Prdx6 repression was examined by transfecting LECs with shRNA (sh) Nrf2 and shKlf9, respectively. Competitive CAT assay with Human Prdx6 promoter-CAT (-1589/+109nts) was performed with Klf9 and Sp1 for Prdx6 promoter activity. LECs overexpressing GFP-Nrf2 or Klf9, and underexpressing Klf9 were used to examine effects on cell viability and ROS by using MTS and H2DCFH-DA dye, respectively.

Results: LECs facing oxidative stress (>100μM H_2O_2) had increased expression of Nrf2, Klf9, and ROS with decreased Prdx6 mRNA and protein. With actinomycin D treatment, increased Nrf2 and Klf9 expression were linked to activation of their transcription. shRNA experiments showed Nrf2 activation of Klf9 and Klf9-mediated Prdx6 repression. Sp1 enhanced Prdx6 transcription, while Klf9 repressed it. Cells overexpressing Klf9 showed increased ROS expression and reduced Prdx6 expression and were more susceptible to cell death. shKlf9 reversed the process, suggesting Klf9 independently caused the ROS-driven cell death.

Conclusions: Protective activities of both Prdx6 and Nrf2 are attenuated due to Nrf2 activation of Klf9, which in turn leads to Klf9-mediated repression of Prdx6, enhanced ROS production and cell death. Because loss of Klf9 suppresses oxidative stress, this should be a therapeutic target in oxidative stress-associated blinding disorders.

Session 4-4

Small molecules to treat age-related eye diseases: a clue from activators of Nrf2-HO-1 antioxidant axis

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Purpose: The nuclear factor erythroid derived 2-related factor 2 (Nrf2) and the antioxidant protein heme oxygenase-1 (HO-1) are crucial components of the cellular stress response. These two systems work together to combat oxidative stress and inflammation and are attractive pharmacological targets for counteracting different age-related eye diseases such as age-related macular degeneration (AMD) and age-related cataract (ARC). The purpose of the present study was to identify the most effective Nrf2/HO-1 activators useful to manage age-related ocular diseases.

Methods: we searched the literature and selected 56 compounds reported to activate Nrf2 or HO-1 and analyzed them for HO-1 induction at 6 and 24 h and cytotoxicity in BV2 microglial cells *in vitro*.

Results: Approximately 20 compounds up-regulated HO-1 at the concentrations tested (5–20μM) with carnosol, supercurcumin, cobalt protoporphyrin-IX and dimethyl fumarate exhibiting the best induction/low cytotoxicity profile. Up-regulation of HO-1 by some compounds resulted in increased cellular bilirubin levels but did not augment the expression of proteins involved in heme synthesis (ALAS 1) or biliverdin reductase. Bilirubin production by HO-1 inducers correlated with their potency in inhibiting nitrite production after challenge with interferon-γ (INF-γ) or lipopolysaccharide (LPS). The compounds down-regulated the inflammatory response (TNF-α, PGE_2 and nitrite) more strongly in cells challenged with INF-γ than LPS, and silencing HO-1 or Nrf2 with shRNA differentially affected the levels of inflammatory markers.

Conclusions: These findings indicate that some small molecule activators of Nrf2/HO-1 antioxidant axis are potent modulators of oxidative stress and inflammation suggesting their use to manage age-related ocular diseases.

Session 4-5

Oxidative stress induced changes to the optical properties of the bovine lens

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Purpose: To determine the effects of oxidative stress on the optical properties of the lens.

Methods: Bovine lenses were exposed for up to 15 hours to hyperbaric oxygen (HBO) to induce oxidative stress, or hyperbaric nitrogen (HBN) that served as a control. The gradient in refractive index (GRIN) and surface curvatures were measured by T2-weighted MRI and inputted into an optical model of the bovine eye to measure the optical properties of the lens and overall vision quality. To determine how the GRIN (i.e. water-to-protein ratio) is affected by oxidative stress, T1 weighted MRI to map free water content, and MALDI imaging mass spectrometry to map the major soluble proteins and peptides in cultured bovine lenses was performed. Proteins were identified using LC-MS/MS with electron transfer dissociation.

Results: Exposure of bovine lenses to HBO, but not HBN for 15 hours caused a decrease in the GRIN in the core of the lens, but did not significantly affect the surface curvatures of the lens. This decrease in GRIN induced a hyperopic shift in the optical properties of the bovine eye. The decrease in GRIN was shown to be due to an increase in free water in the lens core by T1 imaging, with minimal changes to crystallin spatial distributions. No significant changes to α-crystallin were observed, although specific modifications to γ- and β-crystallin were detected in HBO-treated lenses.

Conclusions: Oxidative stress due to hyperbaric oxygen exposure induces an increase in the free water content in the core of lens that decreases the GRIN and results in a hyperopic shift in the optical properties of the lens. Since a similar hyperopic shift is seen in the aging human eye our results provide a link between oxidative stress and age-related changes to the optical properties of the lens.

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Session 4-6

Biphasic PARP-1 repair of DNA in human lens epithelial cells exposed to UVB light

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Purpose: Long-term exposure to solar UVB radiation is a risk factor for cortical cataract. DNA present in the human lens epithelium is a target for UVB-induced damage. The nuclear enzyme poly (ADP-ribose) polymerase-1 (PARP-1) aids in the repair of DNA strand breaks by producing polymers of ADP-ribose units (PAR) from NAD^+. Here, we investigate PARP-1 and PAR repair of damaged DNA in UVB-exposed cultured human SRA01/04 lens epithelial cells (LECs).

Methods: Cells were exposed to UVB light for 2.5 min (280-380 nm wavelength, 312 nm peak, room temperature, air, 0.9 mW/cm^2, 0.14 J/cm^2), and then cultured normally. At various times from 1 to 180 min after UVB-exposure, the following assays were conducted: cell viability (MTT assay), single-cell gel electrophoresis (the Comet assay) to detect DNA single-strand breaks, reactive oxygen species (ROS) detection using CellROX® reagents, and fluorescence immunocytochemistry using antibodies to PARP-1 and PAR.

Results: Exposure of LECs to UVB light resulted in the death of approximately 25% of the cells over the 3 hr experimental period. The level of PARP-1 fluorescence in the cell nucleus remained constant in control and UVB-treated cells at all time periods. DNA strand-breaks were evident at 1 min, and again at 90 min, after exposure, but not at 30 min. Similar results were obtained for PAR, i.e. a strong PAR fluorescence at 5 min and 90 min post UVB-exposure, but not at 30 and 60 min. Interestingly, ROS were detected only at the 90 min time period.

Conclusion: Biphasic damage and repair of DNA in UVB-exposed human LECs were observed to occur at 1 min and 90 min post irradiation. Possible causes for this effect are being investigated. The results indicate that PARP-1 and PAR play active roles in protecting the human lens against UVB light.

Session 5-1

Lens ER-stress response in MIP-mutant mice

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Purpose: Major intrinsic protein (MIP/AQP0) is a member of the aquaporin family of water-channels that plays a key structural role in establishing lens fiber cell architecture. Genetic variants of MIP have been associated with cataract and several mutant forms of MIP have been shown to accumulate in the endoplasmic-reticulum (ER) like membranes of lens fiber cells.

However, beyond MIP loss-of-function at the plasma membrane the associated pathogenetic mechanisms are poorly understood. Here we have used global gene expression profiling to detect lens transcriptome changes during cataract formation in MIP-mutant mice.

Methods: Lens mRNA transcripts were quantified using microarray hybridization analysis and validated by RT-PCR. Confocal microscopy imaging of mRNA transcripts, proteins, and DNA-fragmentation in lens sections was achieved by *in situ* hybridization, immunofluorescence labeling and TUNEL assay, respectively.

Results: In the embryonic MIP-mutant lens (E14.5) no genes were differentially regulated ≥2-fold ($p = \leq 0.05$) when compared with age-matched wild-type lenses. However, in the postnatal MIP-mutant lens (P7) 100 genes were up-regulated and 75 were down-regulated compared with wild-type. The most up-regulated genes (>4-fold) included *Chac1* > *Ddit3* > *Atf3* > *Trib3* > *Xbp1* and the most down-regulated genes (>5-fold) included *Hspb1* > *Hmox1*. Whereas *Ddit3* transcripts were localized to anterior epithelial cells and equatorial fiber cells, *Ddit3* immunofluorescence was localized to cell nuclei within the core of MIP-mutant but not wild-type lenses. TUNEL positive cell nuclei were also detected within the core of MIP-mutant but not wild-type lenses.

Conclusions: These data suggest that MIP-mutant lenses undergo proteotoxic ER-stress associated with strong down-regulation of cytoprotective genes, including those for a molecular chaperone (*Hspb1*) and an anti-oxidant enzyme (*Hmox1*), coupled with dramatic up-regulation of pro-apoptotic genes, including those for a DNA damage-inducible transcription factor (*Ddit3*) and a γ-glutamyl cyclotransferase (*Chac1*), which act downstream of the *Eif2ak3/Perk-Atf4* branch of the unfolded protein response.

Session 5-2

Live imaging and molecular dissection of organelle degradation in the lens

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Purpose: The eye lens is composed of fiber cells that undergo programmed degradation of all organelles during terminal differentiation. However, its underlying mechanism remains largely unknown.

Method: We have addressed the above question by gene-targeting and live-imaging techniques using mice and zebrafish.

Results: We have tested whether this process depends on macroautophagy, which is mediated by the formation of autophagosomes. We generated mice with lens-specific deletion of either *ATG5*, *PIK3C3/VPS34*, or *FIP200/RB1CC1*, all of which are essential for macroautophagy, and found that the lens organelle degradation was not affected. Thus, we concluded that macroautophagy is not involved in this process. To better understand its mechanism, we performed live-imaging analysis of the organelle degradation in mouse and zebrafish lenses. In the mouse lens organ culture, we observed that lysosomes associate with other organelles such as nuclei and mitochondria immediately before their degradation. The organelle degradation was inhibited by treatment with an inhibitor of the lysosomal acidification. As we essentially observed similar processes in zebrafish lens in vivo, we screened candidate genes responsible for this process using zebrafish. We found that DNase1l3l, a member of DNase1 family, localizes to the lysosome and is essential for nuclear DNA degradation. We also found that several SNARE proteins, which are involved in membrane fusion events, are essential for this process.

Conclusion: These findings suggest that the organelle degradation in the lens is achieved by a novel type of autophagy, in which lysosomes directly fuse with target organelles in a SNARE-dependent manner, but not by conventional macroautophagy.

Session 5-3

Requirements for cellular folate during lens development

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Purpose: Dietary folate deficiency is linked to morphogenetic birth defects, however the cellular role folate plays during development is unknown. Cataract formation has also been linked reduced dietary folate. To determine what role folate intake has on lens development, conditional ablation of the folate receptor gene, Folr1 was performed in the embryonic lens.

Methods: Immunofluorescent staining was performed on histological sections of embryonic and adult *Le-cre; Folr1*$^{flox/flox}$ and on transfected epithelial cells.

Results: While lens development appears to progress normally until embryonic development day 15, E16.5 stage embryos exhibit smaller lenses and aberrant cellular organization of the lens fibers. Furthermore, adult mutant eyes are microphthalmic, have pronounced eye recession, and cataracts. *In vitro* experiments have indicated that Folr1 increases the phosphorylation of non-muscle myosin in a Rock-independent manner specifically through the activation of myosin light chain kinase. Ongoing efforts are focused at determining whether myosin activation is disrupted by the absence of Folr1 in embryonic lenses.

Conclusions: Folate is a necessary metabolite for the development of the lens and requires intake through the Folr1 receptor. Because the absence of Folr1 causes severe lens fiber disorganization, it is possible that the lens maintains cellular organization through folate-dependent stimulation of actomyosin filaments.

Session 5-4

Modeling lens growth in the mouse

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Purpose: The lens is an unusual system in that it grows steadily throughout life. Although the germinative zone of the epithelium is the source of cells required for lens growth, the relationship between proliferative behavior of the cells in this region and overall growth of the lens is not well understood. We therefore developed a mathematical model to gain insights into the growth process.

Methods: Biological growth parameters (distribution of proliferative cells, cell sizes, rates of radial growth, cell population kinetics, etc.) were measured in lenses E12 to 3.5 years of age. Data were interpreted using a stochastic growth model (*J. Theor Biol*. 376:15).

Results: Simulations obtained using the growth model were in good agreement with empirical measurements. Modeling suggested that the explosive increase in the number of cells during embryonic development may reflect significantly shorter cell cycle times at early stages. In aged animals, the relationship between radial growth and cell deposition rate held only when the model was modified to include a cellular compaction function. Epithelial cells at all positions increase significantly in size (area) over time with the exception of cells in the central zone of the adult lens. This phenomenon may serve to prevent light-exposed cells from eventually entering the fiber cell population.

Conclusions: Using the stochastic growth model, a lens of appropriate size and cell number could be generated by varying relatively few parameters. Of note, the size of individual epithelial cells and their proliferative activity seemed to be particularly important. These model parameters may be the mathematical analogs of TOR and Hippo, two biochemical pathways recently implicated in the control of organ growth.

Session 5-5

Investigation of zebrafish *occhiolino* as a model for human Alports syndrome

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Purpose: Characterization of visual deficients in the zebrafish *occhiolino (occ)* mutant.

Methods: *Occ*, identified in an N-ethyl-N-nitrosourea screen, has a small pupil and abnormal lens. *Occ* variation from wild type (WT) was visualized with H&E staining and time-lapse imaging. Whole genome sequencing and SNP-mapping identified a mutation in the *collagen4a5* gene.

Results: Normal eye development was observed up to 3 days post fertilization (dpf). At 4-5 dpf, thigmotropism (orientation along the wall of a container) was observed when fish were placed in a 10 cm petri plate. Using histological sections and H&E staining the retina appeared normal and the lens abnormal. Live multiphoton imaging determined similar eye development in *occ* and WT embryos to 3 dpf. Subsequently, a second lens cell mass formed adjacent to the original lens in *occ*. At 4 dpf, the *occ* lens basement membrane was a disorganized layer on the external surface of the lens. By 4.5 dpf, the developing capsule of the *occ* lens was distributed irregularly around the lens mass allowing epithelial cells to form a second cell mass by 5 dpf. Sequencing identified a T>G mutation in the intron 41 splice-donor site of the collagen4a5 gene resulting in the inclusion of intron 41 and a premature stop codon.

Conclusions: The *collagen4a5* mutation accounted for the abnormal capsule leading to the formation of a second lens. Similar genetic mutations in human Alport's patients' lead to end-stage kidney disease, eye abnormalities and hearing loss. We hypothesize that *occ* zebrafish is a potential model for the human Alport's syndrome.

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Session 6-1

Evolutionary origins of Pax6 control of crystallin genes

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Purpose: The birth of novel genes, including their cell-specific transcriptional control, is a major source of evolutionary innovation. The lens-preferred proteins, crystallins (vertebrates: α- and β/γ-crystallins), provide a gateway to study eye evolution. Diversity of crystallins was thought to originate from convergent evolution through multiple, independent formation of Pax6/PaxB-binding sites within the promoters of genes able to act as crystallins.

Methods: Multiple sequence alignments with DNA and protein sequences were conducted using ClustalW and similar programs. The phylogenetic tree analysis was conducted with and without distance correction. DNA alignments of regulatory regions were also generated using CONREAL package. Expression of Cryaa, Cryab, Hspb1, Hspb2 and Hspb6 in mouse newborn heart and E16.5 lens was tested by qRT-PCR. Six copies of candidate Pax6-binding sites identified in B. floridae (WT1 and WT2), their mutants (M1 and M2), and "optimized" sites (O1 and O2), were synthesized by GeneScript and cloned into a E4 TATA-luc minimal promoter followed by transient co-transfection experiments in mouse lens epithelial cell line αTN4-1 and embryonic carcinoma cells P19.

Results: We propose that αB-crystallin arose from a duplication of small heat shock protein (Hspb1-like) gene accompanied by Pax6-site formation from heat shock element (HSE) followed by another duplication to generate the αA-crystallin gene in which HSE was converted into another Pax6-binding site. The founding β/γ-crystallin gene arose from the ancestral Hspb1-like gene promoter inserted into a Ca2+-binding protein coding region, early in the cephalochordate/tunicate lineage. Likewise, several invertebrate crystallin promoters evolved from an ancestral aldehyde dehydrogenase (Aldh) gene.

Conclusions: The present data reconstruct the evolution of diverse crystallin gene families, identify stress response cis-regulatory sites as seed sequences for formation of novel Pax6-binding sites, and propose why Hspb- and Aldh-like proteins evolved into lens crystallins.

Session 6-2

The role of adaptor protein Crk and CrkL in lens development

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Purpose: Crk and CrkL are SH2- and SH3-containing proteins that transduce signals from upstream tyrosine phosphorylated protein to downstream effectors, including Ras, Rac1 and Rap1, which are important for cell proliferation, adhesion and migration. Underlying their diverse function, these two adaptor proteins have been implicated in receptor tyrosine kinase signaling and focal adhesion assembly. Here, we investigated the functional requirement of Crk and CrkL in FGF signaling-dependent lens development.

Methods: We employed Cre/LoxP system to generate a lens specific knockout of Crk/CrkL. The mutants are analyzed by histology and immunohistochemistry.

Results: Deletion of Crk/CrkL led to a sharp reduction in lens size, rotation of the anterior epithelium, and disorganization of the lens fiber cells. By immunohistochemistry and western blot, we observed significant loss of an important downstream effector of FGF signaling pathway, phospho-ERK. Deletion of Crk and CrkL in the lens also mitigated the gain-of-function phenotype caused by overexpression of FGF3, indicating an epistatic relationship between Crk/CrkL and FGF signaling. Crk/CrkL mutants showed a dramatic decrease in laminin and β-Dystroglycan in lens capsule, suggesting that Crk/CrkL is required for formation of extracellular matrix. Consistent with the cell adhesion defects, active β1-integrin and actin nucleator Arp 2/3 were reduced in developing lens.

Conclusions: These findings suggest that Crk and CrkL play an important role in integrating FGF signaling and cell adhesion during lens development.

Session 6-3

For Want of a Nail: How the mutation R48H in human γC-crystallin hardly affects structure or stability, but is associated with pathology

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Purpose: Mammalian lens crystallins have exra-lenticular roles, e.g., in the development of the eye. We have been studying human γ-crystallins in this connection and found that 3 Indian children with monocular cataract plus micro-cornea and persistent fetal vasculature (PFV) carry the mutant R48H of human γC-crystallin (HGCC). We have attempted to compare the molecular structural features of this mutant protein with those of the wild type HGCC, in order to look for any structural changes that might be related to the pathology.

Methods: Wild type (wt) HGCC and its R48H mutant (obtained through site-directed mutagenesis), were cloned, isolated, purified and verified using standard procedures. Their solution state structural properties were compared using circular dichroism and fluorescence methods using probes such as bis-ANS, Nile Red, and Thioflavin T. Their structural stabilities (thermal, and also upon the addition of denaturants) were compared using changes in intrinsic fluorescence. Tendency to form light scattering particles was monitored using spectral methods.

Results: Very little difference was observed between the wt and the mutant molecule in any of the properties, excepting pI value (6.88 vs 6.51). The two have identical secondary structures and essentially the same structural stability. R48H has slightly more exposed aromatic residues, and a slightly more compact overall surface. A comparison of molecular models obtained from molecular dynamics simulations suggested no major differences in topology, but the mutant displays changes in the positive surface charge around the mutation locale.

Conclusions: Given that the mutation does not affect the overall structural features of the molecule, and that the residue R48 is conserved in evolution, one wonders whether loss of any specific interactions of the R48 residue with a putative 'receptor' (or external partner molecule) upon mutation is the factor related to the pathology.

Session 6-4

Genetic variances of periaxin and CP49 manifest lens transparency and stiffness by affecting fiber cell morphogenesis

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Purpose: To identify and characterize genetic variances in common mouse strains that influence the severity of nuclear cataracts in Gja3 knockout (-/-) mice. To test a hypothesis that gap junctions and cytoskeletons synergistically control lens homeostasis and surface interlocking structures of fiber cells for its transparency and stiffness.

Methods: Mouse lens phenotypes were evaluated by slit-lamp examination and quantified by measuring lens light scattering. Linkage markers were used for mapping mouse genetic variances. Cell morphogenesis was characterized by histology, immunostaining and confocal imaging. Lens proteins were examined with biochemical and cellular assays.

Results: We have identified that periaxin (Prx) gene variances, showing substitution of four amino acid residues in the proteins, between C57BL/6J (B6) and 129SvJae and 129SvJ (129) strains. The 129-Prx proteins were correlated with severe nuclear cataracts and extensively associated with the membrane/F-actin network of both peripheral and interior fiber cells while the B6-Prx proteins restrictively appeared in peripheral differentiating fibers. The 129 Prx/F-actin complex impaired surface protrusions of hexagonal shaped fibers needed for cell organization. The129-Prx was colocalized with many other membrane/cytoskeletal proteins. Both wild-type CP49 and B6-Prx influence the severity of nuclear cataracts and stiffness of Gja3-/- lenses.

Conclusions: The 129-Prx gene variances and a CP49 deletion promote the formation of severe cataracts. The 129-Prx is a gain-of-function scaffold protein that alters F-actin network, which subsequently impairs protrusions. Gap junctions serve dual functions: one provides a pathway for lens homeostasis and the other serves as adhesion sites for the arrangement of interlocking structures required for fiber cell organization. Dysfunctional gap junctions and cytoskeleton/scaffold proteins synergistically impair lens homeostasis and the interlocking structures of maturing fibers, which trigger fiber cell degeneration mediated by calcium-dependent protease, ultimately leading to dense nuclear cataracts.

Session 6-5

Effect of interaction between aquaporin 0 and filensin for functions of aquaporin 0

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Purpose: Aquaporin 0 (AQP0) is a member of the aquaporin family that is water channels and has very limited water permeability compared to other aquaporin families. It has been suggested that AQP0 is a component of cell adhesion, but it is still obscure. In this report, the contribution of AQP0 to the cell-adhesion and the effect of the interaction between AQP0 and filensin were investigated.

Methods: Stable L-cell lines expressing AQP0 (L-AQP0) and expressing AQP1 (L-AQP1) were established. Expression vectors of filensin rod or tail were transfected to L-AQP0 and L-AQP1 transiently. Each cell lines were divided into two groups and were stained with CellTracker dyes, one group was cultured on a dish until becoming confluent monolayer, and then, the other group was seeded over the monolayer. To study the cell adhesion stoichiometry, the number of under layer and upper layer cells were measured. To study the permeability, L-AQP0 and L-AQP1 were cultured in the medium containing ascorbic acid and the intracellular ascorbic acid concentration was measured by HPLC.

Results: There were more upper cells that adhered to under cells in L-AQP0 / L-AQP0 homotypic pair than other pairs such as L-AQP1 homotypic pair or L-AQP0 / L-AQP1 heterotypic pair. Although filensin tail or rod region did not affect the cell adhesion function of AQP0, filensin tail region could affect the AQP0 ascrobic acid permeability.

Conclusions: In this report, it was clearly that the cell adhesion occurred between L-AQP0 / L-AQP0 homotypic pair. It was suggested that the filensin did not affect the cell adhesion ability of AQP0, but it regulated as AsA permeability of AQP0 via tail region.

Session 7-1

Growth factor restriction impedes progression of wound healing following cataract surgery; identification of VEGF as a putative therapeutic target

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Purpose: Posterior capsule opacification (PCO) causes secondary visual loss in a significant number of patients following cataract surgery. New open bag IOL designs separate the anterior capsule (AC) and posterior capsule (PC) and these devices appear to further reduce PCO incidence relative to traditional IOL closed bag designs. One possible mechanism that affords this benefit is reduction in growth factor/cytokine availability due to improved irrigation. We therefore explored the role of growth factor restriction on PCO using human cell and tissue culture models.

Methods: Match-paired human capsular bags were prepared by simulated surgery on donor eyes. Cover of the central PC was imaged and quantified. Bioplex suspended bead array analysis determined cytokine levels. The myofibroblast marker αSMA was visualised using fluorescence microscopy. The effect of cytokines on human lens epithelial cell (FHL124) growth was evaluated using Coomassie blue staining, which was extracted from cells and absorbance measured at 550nm.

Results: Cytokine dilution by increasing culture media volume (from 1.5 to 6ml) significantly reduced cell cover of the central PC of capsular bags. Decreased αSMA expression was observed in cells on the central PC at end-point (Day 28) in 6ml cultures. Bioplex analysis of 1.5ml versus 6ml closed bag cultures established significant reductions in 9 of the 27 factors assessed. IL-10, IL-12, IL-15, IL-1ra, IP-10, MCP-1 or MIP1β significantly increased cell proliferation of FHL124 cells. Inhibition of VEGF receptors (10μM Axitinib) reduced cell survival, migration, growth and αSMA expression in both the cell line and capsular bags.

Conclusions: Reducing cytokine levels decreases cell growth and myofibroblast formation within the capsular bag. Separating anterior and posterior capsules following surgery could limit cytokine availability and therefore reduce PCO formation. VEGF provides a therapeutic target to further manage PCO development in conjunction with open bag IOL designs.

Session 7-2

Gene expression profiling of lens epithelial cells in posterior capsular opacity and the role of decorin

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Purpose: Epithelial–mesenchymal transition (EMT) is crucial in wounded tissue repair. Using an *in vivo* rat posterior capsular opacification (PCO) as a model, here we investigated gene expression changes during early and late stage PCO, using a microarray-based approach and showed the contribution of decorin.

Methods: Experiments accorded with National Institutes of Health Guide for the Care and Use of Laboratory Animals. Extracapsular lens extraction (ECLE) was performed in nine, 12 weeks-old Sprague Dawley rats to generate the PCO model. To compare gene expressions during PCO, LECs were isolated at Day0 (control: 6 lenses), 1week (W) (6 lenses), and 2W (6 lenses) following ECLE. Total RNA was extracted from the lenses and labeled cDNA was hybridized to Agilent SurePrint G3 Rat 8x60K microarray. Changes in gene expression were analyzed. Real-time PCR and western-blotting were used to validate microarray results. Expression and role of decorin gene was determined using cells transfection experiments.

Result: PCO was present at 1W and 2W following ECLE. Expression of genes including decorin, a member of the small leucine-rich proteoglycan family, collagen (type 1, 3, 5, 6) and fibronectin were upregulated (3-6 fold) in rat LECs compared to control. LECs also showed increased expression of TGFβ- inducible gene h3 and Tpm2 mRNA and protein compared to control (Day 0). Expressions of γ-crystalline and filensin were higher in LECs isolated at both 2W and 1W after ECLE. Addition of recombinant decorin protein reduced the expression of TGFβ- inducible gene h3.

Conclusion: Decorin may act as a negative regulator of TGFβ and regulate EMT in LECs. The findings revealed stage specific gene expression patterns during PCO formation. Determining the regulation of genes involved in EMT or fiber differentiation in rat PCO models may inform development of stage-specific therapeutic agents that may slow or halt progression of PCO.

Session 7-3

Elucidating the role of αV integrin in TGFβ1 mediated fibrotic responses: implications for PCO

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Purpose: Posterior capsule opacification **(**PCO), the major complication following cataract surgery, results from fibrotic changes. Transforming growth factor beta (TGFβ) signalling is heavily implicated in PCO. αV integrin is also linked to fibrosis and is believed to mediate TGFβ1 activation and myofibroblast formation. We, therefore, investigated the relationship between αV integrin and TGFβ in PCO.

Methods: The human lens epithelial cell line, FHL124, and an in vitro human capsular bag model were used. Cells were exposed to latent TGFβ1 in the presence or absence of the synthetic small molecule RGD peptidomimetic αV integrin antagonist, CWHM-12. The expression of myofibroblast marker α-SMA, was assessed by western blot and immunocytochemistry. FHL124 cell contraction was measured by patch assay. Cell growth and modification of capsular bags was observed by phase-contrast microscopy and images subjected to image analysis.

Results: Latent TGFβ1 added to FHL124 cells and capsular bags resulted in increased matrix contraction and α-SMA levels at end-point. Pre-treatment of FHL124 cells with CWHM-12 before TGFβ1 treatment resulted in reduced α-SMA expression relative to controls. In terms of contraction, CWHM-12 alone initiated contraction without a significant loss of cells. This response was further enhanced when CWHM-12 was added with latent TGFβ1 treatment. Capsular bags treated with CWHM-12 in the presence of latent TGFβ1 did not reveal differences in α-SMA expression at 28 days compared to controls, but a clear increase in matrix contraction was observed.

Conclusions: αV integrin does not appear to be essential for activation of latent TGFβ1 in human lens epithelial cells. αV integrin appears to play an important role in promoting TGFβ1 induced transdifferentiation, but conversely has an inhibitory function with matrix contraction.

Session 7-4

αB-crystallin is essential for the TGF-β2 mediated EMT of lens epithelial cells

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Purpose: PCO remains a major cause of visual impairment and it occurs when leftover epithelial cells after cataract surgery undergo epithelial-to-mesenchymal transition (EMT) and subsequent fibrosis. αB-Crystallin is a major protein in lens epithelial cells and its role in EMT remains unknown. Here we show the role of αB-crystallin in TGF-β2 induced EMT of lens epithelial cells.

Methods: Bovine lens epithelial explants, epithelial cells from WT and αB-crystallin -/- mice and human lens epithelial cell (FHL124) were used in the study. They were treated with TGF-β2 (20 ng/ml) for 24–48 h to induce the EMT response. To study the role of αB-crystallin, some cells were treated with 100 nM of siRNA for αB-crystallin for 24 h prior to TGF-β2 treatment. Expressions of various EMT markers were analyzed by qPCR and western blotting. Lensectomy (surgical removal of the fiber cell mass) was carried out in WT and αB-Crystallin -/- mice to determine effects on subsequent EMT.

Results: TGF-β2 treatment resulted in the up/downregulation of EMT markers at the mRNA and protein levels. This was accompanied by increase in the phosphorylation of p44/42 MAPK, p38MAPK, Akt and phosphorylation and translocation of Smad2, 3, 4 and Snail1 to the nucleus. However, all these changes were significantly reduced in αB-crystallin depleted or knockedout lens epithelial cells. In FHL124 we found binding of αB-crystallin with Smads and Snail1, which was reduced by the downregulation of αB-crystallin. The removal of the fiber cell mass from the lens of the WT mice resulted in the upregulation of EMT markers in the capsule-adherent epithelial cells, which was reduced in the αB-crystallin -/- mice.

Conclusion: Our results suggest that αB-crystallin plays an important role in the TGF-β2 induced EMT of lens epithelial cells and could be targeted to prevent PCO.

Session 8-1

Matricelilar protein family modulation of TGFβ/Smad signaling in EMT

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Post-cataract surgery fibrosis in lens capsule (PCO), and anterior subcapsular cataract (ASC), are both accompanied with epithelial-mesechymal transition (EMT) of lens epithelium. Prominent features of EMT-related fibrotic lesion in PCO or ASC is the presence of myofibroblasts and accumulation of extracellular matrix (ECM) secreted by the cells. ECM in PCO or ASC contains major fibrous components, i.e., collagen types I and III as well as a number of minor components, i.e., osteopontin, tenascin family members (tanasin C and tanasin X) or lumican. Transforming growth factor β (TGFβ) -activated Smad signaling plays a critical role in mediating EMT-inducing processes among TGFβ signaling cascades. Here we will present the data that indicate TGFβ/Smad signal is further modulated by osteopontin, tenascin family members or lumican. In a mouse model of lens injury loss of each of these components attenuated Smad3 activation and delayed the process of lens epithelial cell EMT and formation of fibrotic lesion in the injured lens *in vivo*. Although TGFβlSmad signal is the main cascade that mediates lens epithelium EMT, crosstalks between Smad and ECM-related signalings are also to be candidates of targets for prevention of PCO and ASC. The data also provides insights to understanding the pathobiology of EMT-related diseases in other non-ocular tissues.

Session 8-2

Structure activity relationship study of novel synthesized flavones as matrix metalloproteinase inhibitors in lens epithelial cells

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Purpose: Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that have been shown to participate in fibrotic cataract, such as anterior subcapsular cataract (ASC) or posterior capsular opacification (PCO). MMPs are produced by lens epithelial cells (LECs) in lens organ and the proliferation and migration of LECs also play an important role in formation of ASC or PCO. This study investigated the effect of novel flavonoids on proMMPs production and proliferative activity in LECs. In addition, we examined the structure-activity relationships related to regulation of proMMPs production or cell proliferation by flavonoids.

Methods: We synthesized metabolites and derivatives of nobiletin (3',4',5,6,7,8-hexamethoxyflavone) isolated from citrus fruits. We investigated the effect of these flavonoids on the production of proMMPs in human lens epithelial cell line SRA01/04. Gelatin zymography method was employed to evaluate the expression of proMMP-9 in conditioned medium. AlamarBlue analysis was performed to evaluate the effect of flavonoids on cell proliferation.

Results: Nobiletin was found to inhibit the production of proMMP-9. The IC_{50} values of nobiletin in PMA or TNF-α-stimulated SRA01/04 cells are 20.9±6.5 μM or 17.0±1.6 μM, respectively. Nobiletin showed dose-dependently growth inhibitory effects in SRA01/04 cells. Among newly-synthesized flavonoids, 2'-hydroxylated flavone showed markedly potent inhibitory activity against proMMP-9 production in PMA-stimulated SRA01/04 cells (IC_{50}: 0.4 μM) with extremely high selectivity (IC_{50} against TNF-α-stimulated cells: 68.0 μM). 4'-hydroxylated flavones showed the stronger inhibitory action against proMMP-9 production compared to 4'-methoxylated flavones. On the other hand, 4'-methoxylated flavones showed greater anti-proliferative activity than 4'-hydroxylated flavones in SRA01/04 cells.

Conclusion: Our results suggested that nobiletin or newly-synthesized flavones may exert the anti-cataract action, especially against fibrotic cataract through suppressing proMMP-9 production and cell proliferation in LECs. Ongoing studies to elucidate the mechanism of action are currently underway.

Session 8-3

Development of pseudophakic model in young non-human primates

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The development of suspensions for intravitreal (ITV) injection carries a risk for particle movement within the eye, leading to visual disturbances or blockage of Schlem's canal and potentially increasing intraocular pressure (IOP). The risk of such particle movement may be increased in patients who have undergone cataract surgery, specifically patients with artificial intraocular lenses (IOLs), termed pseudophakic patients, and pseudophakic patients who have undergone posterior capsulotomies.

To directly evaluate particle movement in a nonclinical setting, a pseudophakic non-human primate (NHP) monkey model was created to mimic clinical procedures for surgical intervention for cataracts. This model was then validated using Triesence®, an approved ITV suspension with a history of successful use in pseudophakic patients.

Thirteen male 2-3 year old cynomolgus monkeys underwent lens removal and IOLs were placed in the right eyes. Posterior capsulotomies were performed in the right eyes of six monkeys at the time of surgery. Eyes were assessed via ophthalmic exams and histopathology. Two animals were euthanized about 4 weeks following surgery due to post surgery complications and histopathology confirmed posterior synechia and proliferating lens epithelial cells in the stroma of the iris. Following 4-6 weeks of post-surgical recovery, 11 animals were included in the study and were dosed bilaterally with ITV Triesence®. Following ITV Triesence® dosing, no significant particle movement was observed in the control (non-surgery) eyes or the pseudophakic eyes. A small amount of test article moved into the anterior chamber of 2/5 pseudophakic + capsulotomy eyes only. Movement of Triesence® did not result in increased IOP or any other clinical complications. These results are in line with the clinical performance of Triesence® in these patient populations, and suggest that this pseudophakic NHP model may be appropriate for the assessment of particle movement with novel ITV suspensions.

Session 8-4

Mechanisms of fibrosis in a mock cataract surgery wound repair model

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Purpose: These studies examine initiating signals that promote myofibroblast emergence, the cellular culprit of the lens fibrotic disease Posterior Capsule Opacification (PCO). The aim was to identify the mechanotransduction signaling events by which a rigid extracellular matrix environment induces the myofibroblast phenotype.

Methods: In a clinically relevant ex vivo mock cataract surgery model for PCO, in which we have studied repair of the denuded lens capsule, lens epithelial cells and CD44+ mesenchymal repair cells can be induced to move from the explant onto the ExtraCapsular Zone (ECZ) of the tissue culture substrate. Emergence of myofibroblasts (αSMA+) in this rigid microenvironment relative to production of Extracellular Matrix (ECM) proteins associated with fibrosis was determined by immunofluorescence analysis. Live labeling with CD44+ was used to track cell differentiation to myofibroblasts. Function of the mechanosensor Focal Adhesion Kinase (FAK) in myofibroblast differentiation was examined using the FAK inhibitor PF573228.

Results: Myofibroblasts emerged at the leading edge of the ECZ region by day 3 in culture. CD44+ mesenchymal cells located to the leading edge prior to myofibroblast differentiation were shown to be precursors of αSMA-expressing myofibroblasts. Since ECM landscape can directly impact mechanical signaling, we examined the spatiotemporal distribution of fibrosis-inducing ECM molecules in relation to myofibroblast differentiation. Tenascin-C and Fibronectin-EDA were deposited along the substrate prior to myofibroblast differentiation; Collagen I was expressed concurrently. Inhibition of FAK activation, a mechanosensor for transducing ECM/integrin signals to the cell prevented αSMA expression.

Conclusions: The cells that migrate onto a rigid tissue culture substrate in response to an ex vivo mock cataract surgery provide an optimal reductionist model in which to study the signals responsible for inducing cells to become myofibroblasts. Using this model the mechanosensor FAK was identified as an essential mechanotransducer of the initiating signal for myofibroblast differentiation from a rigid extracellular matrix microenvironment.

Session 9-1

Corneal and conjunctival damages during cataract surgery

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Purpose: To evaluate that the healing effect of dry-eye ophthalmic solution in cataract surgery-induced corneal and conjunctival damage.

Methods: 3% diquafosol tetrasodium (Santen, Japan) (D group) and 2% rebamipide (Otsuka, Japan) (R group) were instilled to Japanese albino rabbits 4 times a day for 2 weeks preoperatively. And no pre medicated control group was also prepared (C group). Under general anesthesia, rabbit eyes were dried by lid retractor for 1 hour with or without topical anesthesia (4% lidocaine). The ocular surface damages were evaluated by lisamine green staining score. Periodic acid Schiff reagent (PAS)-positive cell count (cells / 0.25mm^2) in the bullar conjunctiva was measured by impression cytology.

Results: The lissamine green staining score, in C group (1.8±0.5 points) was higher than in R group (1.0±0.8 points) and D group (0.8±0.5 points) without topical anesthesia ($P<0.05$, Tukey-Kramar). The number of PAS-positive cells in C group (158±29 cells) was lower than in R group (231±35 cells) and D group (273±62 cells) without topical anesthesia ($P<0.01$, Tukey-Kramar). The topical anesthesia exacerbated lissamine green staining score and number of PAS-positive cells significantly.

Conclusions: Corneal and conjunctival epithelium was damaged by xerosis by lid retractor and topical anesthesia. Preoperative medication of dry-eye ophthalmic solutions is effective to prevent in cataract surgery-induced corneal and conjunctival damage.

Session 9-2

Posterior capsular opacification and anterior capsule contraction

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Purpose: Cataract surgery is developed and it produce good visual functions; however, posterior capsular opacification (PCO) and anterior capsule contraction (ACC) are still remained as postoperative complications. We evaluate these complications about resent hydrophobic intraocular lenses (IOLs).

Methods: ZCB00V (AMO), XY1 or FY-60AD (HOYA) were randomly selected and implanted after phacoemulsification. Anterior images were photographed using slit lamp and EAS1000 (NIDEK) at 1 weeks, 1 month, 3 months and 6 months postoperatively. Posterior capsular opacification and capsulorhexis opening area were analyzed. Fibrosis of anterior capsule were also measured using area analysis software (Area Q, S-Tech).

Results: The densities of posterior capsules of ZCB00V, XY1 and FY-60AD were 8.21±5.64, 7.09±4.27, 11.72±4.42CCT respectively. Reduction rate of capsulorhexis opening area were 0.25±2.36% (ZCB00V), 1.05±2.22% (XY-1) and 5.44±10.15% (FY-60AD) after 6 months postoperatively. There were statistically significance ($P<0.01$). The area of fibrosis in anterior capsule were 16.90±8.34% (ZCB00V), 43.21±15.97% (XY-1) and 62.24±21.32% (FY-60AD). There were statistically significance, too ($P<0.01$).

Conclusions: The resent hydrophobic acrylic IOLs reduce ratios of PCO and ACC postoperatively.

Session 9-3

Tass and infectious endophthalmitis

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Acute postoperative endophthalmitis (POE) is the most serious complications of cataract surgery and often results in severe visual impairment. Toxic anterior segment syndrome (TASS), is a sterile postoperative inflammatory reaction caused by a noninfectious substance, was often needed to differentiate with POE. The purpose of this presentation was to summarize clinical topics associated with acute POE and TASS.

In 2015, the prospective study for POE in Japan was ended. The latest incident rate of POE was 0.02%, 1/5000. That rate was decreased year by year. The most commonly preventive measure for POE is the administration of antibiotics drop three days before the surgical day. In recent years, intraoperative surface irrigation with the povidone-iodine in balanced salt solution plus was also often used. On the other hand, intracameral antibiotics injection was not often used.

TASS is a rarely postoperative complication, almost not reported in Japan. However, the first report of an outbreak of late-onset TASS after implantation of a particular type of IOL was published. The cause of the outbreak strongly suggested that toxicity of used IOL contaminated with the aluminum.

In our research, infiltrated bacteria into the eye may spread to the vitreous cavity and anterior chamber passing through the lens capsule in endophthalmitis. To get the better understanding of the pathophysiology of POE, it will be necessary to further study for the relationship among the lens capsule, bacteria and the IOL.

Session 9-4

Differences in the behavior of various OVDs in torsional phacoemulsification

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Purpose: To evaluate the behavior of various ophthalmic surgical devices (OVDs) during torsional phacoemulsification (PEA).

Methods: Provisc® (sodium hyalronate 1%), Viscoat® (sodium hyalronate 3% - chondroitin sulfate 4%), DisCoVisc® (sodium hyalronate 3%-chondroitin sulfate 4%), and Healon V® (sodium hyalronate 2.3%) were stained with fluorescein sodium. A corneal tunnel incision was created with a 2.0mm angled slit knife in porcine eye, and one of the above OVDs was injected into the anterior chamber. Balanced® tip (Centurion®, Alcon) positioned just anterior to the lens capsule at the center of the pupil. Simulated torsional PEA was performed for 1minute. To determine the residual volume of the OVDs after torsional PEA, the distances between the corneal endthelium and the anterior capsule of lens (residual thickness) were measured using Pentacam HR (OCULUS).

Results: There was a difference in behavior of OVDs in torsional PEA. Provisc® was removed easily. DisCoVisc and Viscoat were removed as small fragments and Healon 5 was as moderate fragments. The residual thickness of Provisc® was less than those of others ($p<0.01$). The residual thickness with 70% power of Torsional PEA was less than that of 40% power.

Conculusion: Provisc® is removed easily. Viscoat®, Healon 5®, and DisCoVisc were remained during simulated torsional PEA. Torsional power may affect residual volume of OVDs.

Session 9-5

Evaluation of accommodation at hydrogel lens refilling

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Purpose: We developed nanocomposite gels which are based on a thermosensitive hydrophobically modified poly (ethylene glycol). It exhibits a reversible sol-gel phasetransition near the body temperature. Nanocomposite gels was confirmed following conditions in vitro: 1) no cytotoxity, 2) good transparency, 3) favorable stability and operationality. Further, refractive index over 1.40 is obtained. Therefore, we, evaluate refraction and accommodation before and after gel lens refilling by using cynomolgus monkey.

Methods: Two cynomolgus monkeys, ages nine and eight years respectively were used.

With forceps, about 2.0 mm diameter circular capsulorrhexis was completed in the lens periphery. After hydrodissection, lens substance was removed by phacoemulsification system, INFINITI Vision System (Alcon Laboratories, Inc., Fort Worth, Texas). After removal of the lens substance, the empty capsular bag was filled with the nanocomposite gels through the capsulorrhexis and self-sealed.

Accommodation was stimulated with 4% pilocarpine eye drop and was measured with a hand held refractometer (Retinomax K-plus, Righton)at regular times during a follow-up period of 12 weeks. Lens thickness and anterior chamber depth changes were measured with a scheimpflug camera (EAS-1000, Nidek) The monkeys had previously undergone bilateral, complete iridectomies and underwent refilling of the lens capsular bag with nanocomposite gel at OD.

Results: Scheimpflug image shows nanocomposite gel keeps original shape of lens capsule and no leakage from lens capsule. The maximum accommodative amplitude of the surgically treated eyes ware 2.3D and 2.5D, respectively. The maximum anterior chamber depth changes ware 0.1 mm and 0.2mm respectively. A certain level of accommodation can be restored after lens refilling in adolescent cynomolgus monkey.

Conclusions: Although it is necessary for long-term observation, nanocomposite gel is a good candidate for lens refilling material.

Session 10-1

Identification of genetic mutation of the transcription factor encoding genes for lens development in mice

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Purpose: The transcription factors expressed in the lens regulate the proliferation of lens epithelial cells and the differentiation of fiber cells in vertebrates. Several mutations of these genes cause ocular developmental disorder such as microphthalmia and cataracts in human. To identify causative genes for ocular disorders, the establishment of mouse models may be highly effective. Here, we describe about the identification of responsible mutations of transcription factor encoding genes for lens defects in novel model mice by approaches of forward genetics in our previous publications.

Methods: To identify the responsible mutation of these mutant mice, we performed linkage analysis using polymorphic microsatellite markers. The expression analysis of genes and proteins were conducted by qRT-PCR, *in situ* hybridization or immunohistochemistry.

Results: Rinshoken cataract (*rct*) mouse, which shows anterior polar opacity in the lens, was isolated spontaneously from SJL/J strain. Based upon linkage analysis, we identified the mutation of *Foxe3* that underlies the onset of mild microphthalmia and cataracts in *rct* mice. This mutation is a 22-bp deletion in a putative *cis*-acting element of the *Foxe3* gene. Moreover, we demonstrated that the *rct* mutant has reduced expression of *Foxe3* in the lens during development. On the other hand, we established a novel spontaneous microphthalmia and aphakia (*miak*) mouse in a KOR1/Stm strain colony. We also identified that a novel nonsense mutation (p.147Tyr.X) located outside the homeodomain in *Pitx3* causes microphthalmia and aphakia in *miak* mice. Moreover, we found that truncated PITX3 caused by this nonsense mutation leads to overexpression of own mRNA.

Conclusion: We identified that hypomorphic mutation of *Foxe3* which has been known as causative gene for various eye defects leads to congenital anterior polar and cortical cataracts, and also that truncated PITX3 expression on developing lens which is attributed to nonsense mutation causes mirophthalmia and apakia in mice.

Session 10-2

A critical role of an exon junction complex (EJC) factor in regulation of embryonic eye development

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Nonsense-mediated mRNA decay (NMD) is an RNA surveillance mechanism that degrade mRNAs carrying premature termination codons (PTCs). This mechanism requires several key EJC factors to distinguish PTC from the normal stop codon. But how this mechanism modulates the embryonic neurodevelopment and behaviors is largely unknown. In this study, we demonstrated that RBM8a plays a key role in neural progenitor proliferation and differentiation. First, RBM8a is highly expressed in the subventricular zone of early embryonic cortex, suggesting that RBM8a may play a role in regulating neural progenitor cells (NPCs). To test this hypothesis, we used *in utero* electroporation to overexpress or knock down RBM8a in mouse brain at E14. RBM8a stimulates embryonic neural progenitor proliferation and suppresses neuronal differentiation, indicating that RBM8a positively regulates NPC proliferation. Conversely, knockdown of RBM8a in the neocortex reduces NPC proliferation and promote premature neuronal differentiation. Consistently, *Nes-cre; RBM8a*$^{fl/+}$ mice show severe developmental defects including microcephaly and postnatal lethality. The cortex of *Nes-Cre;RBM8a*$^{fl/+}$ mice is very thin, and the two hemispheres fail to meet on the midline. Interestingly, these mice also have disorganized corticies. Cux1, a marker for cortical layers 2/3 is present in cells all throughout the cortex of *Nes-Cre;RBM8a*$^{fl/+}$ mice, with the greatest density in layer 4. Foxp2, a marker for cortical layer 5/6, is also present in layer 4. This indicates either deficits in cell fate specification, or deficits in migration. Progenitors in the eye also fail to proliferate and differentiate properly. To uncover the underlying mechanisms, genome-wide RNAseq identifies potential substrates of RBM8a in the brain, which have been implicated in neurogenesis and plasticity. Interestingly, autism- and schizophrenia-risk genes are highly representative in RBM8a-associate transcripts. Taken together, we identify a novel role of RBM8a in regulation of neurodevelopment and behaviors. Our studies provide a deeper insight on causes of neurological illnesses and will facilitate the development of new therapeutic strategies for neurodevelopmental illnesses.

Session 10-3

Lens fiber regeneration after cataract surgery

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Lens from rabbits, monkeys and humans remain the capacity of fiber regeneration after surgery removal of lens fibers. In this work we characterized the lens fiber regeneration process using rabbit as model animals. We found that intact lens epithelium is essential for lens fiber regeneration. Small capsule opening during surgery removal of lens fibers by phacoemulsification resulted in a faster and more ordered regeneration of lens fibers as compared to large capsule opening. Damage of lens epithelial during surgery resulted in epithelial-mesenchymal transition and disordered lens regeneration. As indicated by Brdu incorporation, removal of lens fibers resulted rapid lens epithelium proliferation. Lens epithelial cells rapidly migrated toward to posterior capsule. By the 7^{th} day after removal of lens fibers, epithelial cells covered the whole capsule. The fiber differentiation began from equatorial region as soon as 3 days after cataract surgery, whereas the epithelial cells that migrated to the central posterior capsule differentiated last. Thus, the regenerated lenses at early stages (up to 1 month after surgery) were donut-shaped. The regeneration process was almost completed by 2 month after surgery. Intravitreous supplementation of FGF-2 promotes the regeneration process. The arrangements of regenerated lens fibers were similar to that of normal lens. Protein expression profiles of the regenerated lenses were also similar to that of normal lenses. However, fiber nuclei retained in the center of the regenerated lenses and the regenerated lenses were semi-transparency. This work demonstrates the lens fiber regeneration process recapitulated the embryonic lens development process and lens regeneration in rabbit is an ideal model to investigate lens fiber differentiation regulation.

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Session 10-4

Regulation of lens differentiation by the tumor suppressor, p53

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Purpose: The tumor suppressor is an important regulator in the ocular lens. Inactivation of p53 by viral gene expression or knockout, and its overexpression both lead to cataractogenesis. In this study, we summarize the known mechanisms by which p53 regulates lens differentiation.

Methods: Human lens epithelial cells, αTN4-1 mouse lens epithelial cells, wild type and p53 knockout mice were used as testing systems. Immunocyto-chemistry, EMSA, reverse transcription polymerase chain reaction, western-blot analysis and reporter gene activity assays were used to study mechanisms by which p53 regulates lens cell differentiation.

Results: During mouse lens development, p53 can exerts control on lens differentiation through following aspects: 1) p53 directly control lens transcription factors such as c-Maf and Prox-1; 2) p53 directly regulates lens crystalline genes including those coding for αA-, βA3/A1- and γA-crystallins; 3) p53 directly regulates Bak to mediate developmental apoptosis. Finally, p53 interacts with HSF4 and co-regulates downstream target genes.

Conclusion: The tumor suppressor, p53 plays important roles during lens development and cataractogenesis.

Session 10-5

AlphaA-crystallin negatively regulates p53-dependent apoptosis through modulation of ATM/ATR kinases

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Purpose: αA- and αB-crystallins are distinct antiapoptotic regulators. Regarding the antiapoptotic mechanisms, we have recently demonstrated that αB-crystallin interacts with the procaspase-3 and partially processed procaspase-3 to repress caspase-3 activation. They also prevent staurosporine-induced apoptosis through interactions with members of the Bcl-2 family. In this study, we present evidence to show that αA-crystallin can regulate p53-dependent apoptotic pathway by modulating the function of ATM/ATR kinases

Methods: Human lens epithelial cells were used as a testing system. Stable transfection, RT-PCR, Co-IP and western-blot analysis were used to study the functions of αA-crystallin in regulating p53-dependent apoptosis.

Results: αA-crystallin is found to prevent stress-induced apoptosis, which is p53-dependent. The regulation p53 expression and activity by αA-crystallin is dependent on its modulation on ATM and ATR kinases.

Conclusion: αA-crystallin regulates ATM/ATR kinases to suppress p53-dependent apoptosis.

Session 10-6

Insights into lens biology, anti-cataract drug screening and lens regeneration using a new, large-scale source of human lens cells

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Purpose: We established a simple and reproducible method of generating purified human lens epithelial cells (hLECs) from pluripotent cells. We hypothesized these hLECs will i) help define molecular mechanisms of human lens and cataract development, ii) provide a large-scale hLEC source for anti-cataract drug screening, and iii) enable investigation of lens regeneration as a cell-based therapy for cataract and presbyopia.

Methods: Purified hLECs obtained by magnetic cell separation from differentiating pluripotent cells were analyzed via microscopy, flow cytometry, RNA-seq and PCR. Bioinformatic and proteomic analyses were used to investigate gene expression networks. Cell culture was used for combinatorial growth factor screening, and to establish anti-cataract drug screening conditions. Transplantation of purified hLECs into NZ white rabbits was used to assess a potential cell-based therapy for lens regeneration.

Results: Extensive characterization of hLECs purified from multiple pluripotent cell lines showed the hLECs to be phenotypically and transcriptionally highly similar to primary hLECs. This includes expression of over 100 defining hLEC genes but not key pluripotency genes. Bioinformatic predictions of novel lens transcription factors were confirmed via PCR and Western blotting in the purified hLECs. Combinatorial growth factor screening identified improved proliferative conditions that permit serial hLEC passaging. These conditions also permitted hLEC seeding in 96 well-plates and establishment of a drug screening assay for candidate anti-posterior capsule opacification agents. MRI and histology demonstrated imperfectly regenerated lens material 3 months after a pilot hLEC transplantation into NZ white rabbits, with more lens material associated with hLEC transplantation than control.

Conclusions: Our data indicate access to large numbers of normal or diseased purified hLECs will provide valuable new knowledge of the molecular mechanisms underpinning human lens, cataract and presbyopia development. These hLECs will also facilitate anti-cataract drug screening, as well as regenerative approaches to treating paediatric and/or adult cataract and presbyopia.

Session 11-1

Structural and functional studies of lens aquaporins

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Purpose: Lens protein modifications play key roles in lens fiber cell development and differentiation as well as in lens pathology. The purpose of this work is to measure structural changes to aquaporins in lens fiber cells as a function of fiber cell and lens age. An additional goal is to determine the functional consequences of age-related modifications.

Methods: Spatially-resolved proteomics methods, using microdissection/LC-MS/MS analysis and imaging mass spectrometry, were used to identify and quantify modifications to lens aquaporins throughout the lens as a function of age. Functional studies were carried in CHO cells expressing native and modified aquaporins and in vesicles prepared from mouse and rat lens fiber cells.

Results: In addition to previously reported truncation, phosphorylation, and lipid acylation sites, recent proteomics analyses revealed modifications to lens aquaporins including new deamidation sites and palmitoylation sites. Imaging mass spectrometry revealed zones of AQP0 deamidation and lipidation that started in the youngest human lenses examined. Analysis of the fiber cell lipid raft proteome revealed distributions of AQPs in raft and non-raft fractions and confirmed the presence of lipidated AQP0 in the lipid raft domains. Expression of AQP0 in CHO cells induced filopodia formation that was not observed in the S235A mutant or in wild type AQP0 expressing cells after treatment with a PKA inhibitor; indications that water permeability plays a role in this phenomenon. Swelling assays in vesicles from mouse and rat lenses revealed contributions from both AQP0 and AQP5.

Conclusions: Aquaporin modifications identified to date play important roles in AQP trafficking, distribution in the fiber cell membrane, and water permeability; functions that impart key fiber cell properties in the developing lens. Further, age-related modifications are predicted to alter protein-protein interactions. The global impact of lens AQP modification on fiber cell function and lens homeostasis will be discussed.

Session 11-2

Phosphorylation of aquaporin zero and the calmodulin interaction

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Purpose: To understand how phosphorylation of aquaporin zero (AQP0) modifies the effect of calcium, acting through calmodulin (CaM), on AQP0 osmotic water permeability (Pf). The motivation for this investigation arises in part from the observation that the phosphorylation state of AQP0 varies from cortex to nucleus in the lens.

Methods: We employed a combination of Brownian and molecular dynamics simulations to identify the critical features of the AQP0-CaM interaction. Brownian dynamics (BD) was used to assess the binding affinity of CaM for AQP0 as a function of its charge state. Molecular dynamics (MD) was used to analyze the structure-function relationship of AQP0 phosphorylation on the AQP0-CaM complex and gating mechanism.

Results: BD simulations suggest that serine phosphorylation of AQP0 does not significantly reduce CaM-binding to the whole AQP0 protein, in contrast to the experimental observation that phosphorylation does significantly reduce binding to Cterminus AQP0 peptides. This result may explain the observation that many charge mutants of AQP0 retain CaM-mediated calcium-sensitivity. Comparative MD simulation studies show that AQP0 phosphorylation changes contacts between AQP0 and CaM, particularly at a small arginine-rich loop on the AQP0 cytosolic face. This charged loop allosterically couples CaM to the second constriction site residues of AQP0 through an interaction with R156. Additionally, we observe that R153 increases the size of the pore opening through an interaction with the Y149 hydroxyl group, which is necessary for maintaining high permeability states of AQP0.

Conclusions: CaM controls the dynamics of the pore-gating tyrosine Y149 of AQP0 through an interaction with a previously uninvestigated arginine-rich loop on its cytosolic face. Experimental and simulation data support the notion that serine phosphorylation of AQP0 changes the calcium sensitivity of Pf by modifying the AQP0-CaM interaction interface rather than by inhibiting CaM-binding.

Cx50D47A causes lens ER stress

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Purpose: Lenses from *No2* mice have nuclear cataracts and compromised differentiation caused by a mutant Cx50 (Cx50D47A) with impaired trafficking. Homozygous *No2* lenses also contain increased levels of αB-crystallins, which are responsive to several types of stress. Therefore, we determined whether expression of Cx50D47A led to ER stress in the lens.

Methods: Changes in mRNA expression were determined by quantitative RT-PCR. Levels and distributions of ER stress-related proteins were determined by immunoblotting and immunofluorescence.

Results: ER stress can be transduced by three different pathways: IRE1α, ATF6 and PERK. No increase in the spliced/total *Xbp1* ratio or in the levels of cleaved ATF6 was detected in *No2* mice compared with wild type animals, implying that neither the IRE1α nor the ATF6 pathway was activated. In contrast, heterozygous and homozygous *No2* mice showed an increase in phosphorylated PERK and in the phosphorylated/total EIF2α ratio (2.4- and 3.3-fold, respectively) compared with wild type. These changes were associated with elevated levels of ATF4 in homozygotes (390%), leading to changes in expression of downstream targets including CHOP.

Conclusions: These results suggest that expression of Cx50D47A induces ER stress, triggering PERK pathway-dependent unfolded protein response, which contributes to the pathophysiology of cataracts and differentiation defects.

Session 11-4

Cx46 hemichannels contribute to the sodium leak conductance in lens fiber cells

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Purpose: We used dissociated fiber cells isolated from transgenic mouse lenses to investigate the hypothesis that Cx46 hemichannels may mediate the basal permeability of fiber cells to sodium observed in the intact lens.

Methods: Differentiating fiber cells were isolated from transgenic mouse lenses using collagenase. Membrane currents were recorded in fiber cells using whole cell patch clamping.

Results: KOCx50 fiber cells exhibited a large, nonselective cation current that was absent in fiber cells isolated from double KO (Cx50(-/-)Cx46(-/-)) mouse lenses confirming that it was due to Cx46 hemichannels. This current could be reversibly blocked by external calcium and magnesium in a concentration- and voltage-dependent manner. Cx46 hemichannels were largely closed at a resting voltage of -60 mV in the presence of physiological divalent cation concentrations (1 mM Ca2+, 1 mM Mg2+). However, even though the vast majority of these channels were closed at -60 mV, a small, persistent, nonselective cation current could still be detected using the whole cell patch clamp technique. This current could be mostly blocked by exposure to 1 mM La3+ and was not observed in fiber cells isolated from double KO mouse lenses suggesting that it was due to Cx46 hemichannels. In addition, KOCx50 fiber cells showed increased open channel noise and a depolarized resting potential as compared with double KO fiber cells.

Conclusions: Our results suggest that Cx46 hemichannels may serve as an important player in the lens internal circulation system by allowing the entry of sodium from the extracellular space into the lens fiber cells.

Session 11-5

Cx43 and Cx50 channels have differential permeability to cAMP, IP_3 and Ca^{2+}

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Purpose: Gap junction channels exhibit connexin dependent biophysical properties, including selective intercellular passage of larger solutes, such as second messengers, which could in turn influence events like cell division and differentiation.

Methods: We determined the cAMP, IP_3 and Ca^{2+} permeability through channels composed of Cx43 or Cx50 using simultaneous measurements of junctional conductance and intercellular transfer. For cAMP detection the recipient cells were transfected with a reporter gene, the cyclic nucleotide-modulated channel from sea urchin sperm (SpIH). cAMP was introduced via patch pipette into the cell of the pair that did not express SpIH. SpIH-derived currents were recorded from the other cell of a pair that expressed SpIH. For IP_3 and Ca^{2+} detection, cells were loaded with the Ca^{2+} binding dye Fluo-8. IP_3, or Ca^{2+}, were introduced via patch pipette into one cell of a pair and changes in fluorescence intensity were recorded.

Results: cAMP and IP_3 transfer was observed for homotypic Cx43 channels over a wide range of junctional conductance. In contrast, homotypic Cx50 channels exhibited no permeability to cAMP or IP_3 when compared to Cx43. Both Cx43 and Cx50 were highly permeable to Ca^{2+}.

Conclusions: These data suggest that Cx43 permeability to second messengers results in a rapid delivery of cAMP, IP_3 and Ca^{2+} from cell to cell in sufficient quantity to trigger relevant intracellular responses. The data also suggest that the reduced cAMP and IP_3 permeability of Cx50 may play a role in regulating cell division and differentiation in the lens.

Session 11-6

AQP0 and N-cadherin/adherens junctions play a cooperative role for the integrity of interlocking domains and transparency of the lens

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Purpose: We have shown that complete loss of AQP0 specifically causes severe disruption of interlocking protrusions which lead to fiber cell separation and cataract formation in the $AQP0^{-/-}$ lenses. Here, we use the $AQP0^{+/-}$ lenses to compare the changes of membrane structures and N-cadherin/adherens junctions during a slower cataract formation process.

Methods: $AQP0^{-/-}$, $AQP0^{+/-}$ and WT lenses were used for immunolabeling of AQP0, N-cadherin and β-catenin. Changes in protrusions and adherens junctions were examined with SEM and TEM.

Results: Nuclear cataracts were observed early in $AQP0^{-/-}$ lenses at age 1 week, and gradually extended to superficial cortex at 4 weeks and older. However, nuclear cataracts were first found in $AQP0^{+/-}$ lenses at 8 weeks, and were not extended to superficial cortex at 12 weeks studied. Although protrusions were regularly disrupted in cortical fibers in $AQP0^{-/-}$ cataracts, only minor alterations were seen in cortical fibers (~240 μm deep) in $AQP0^{+/-}$ lenses. Importantly, while many enlarged extracellular spaces were regularly distributed in superficial cortex in $AQP0^{-/-}$ cataracts at 4-8 weeks, they were rarely observed in $AQP0^{+/-}$ lenses at ages 8-12 weeks studied. Many elongated, deformed protrusions were dispersed inside the enlarged extracellular spaces. Furthermore, many spotty adherens junctions were regularly associated with protrusions in cortical fibers in WT and $AQP0^{+/-}$ lenses. Immunolabeling confirmed that N-cadherin/β-catenin complexes were specifically localized in protrusions. Also, the immunoreactivity of N-cadherin was significantly decreased in the cortical fibers in the $AQP0^{-/-}$ cataracts, but not in the $AQP0^{+/-}$ and WT lenses.

Conclusions: The presence of the reduced amount of AQP0 is able to delay disruption of protrusions and formation of enlarged extracellular spaces in cortical fibers in $AQP0^{+/-}$ mice. The concomitant distribution of N-cadherin/adherens junctions in protrusions may play a cooperative adhesion function in maintaining the integrity of protrusions in WT and $AQP0^{+/-}$ lenses to delay cataractogenesis.

Session 12-1

Role of lanosterol in reducing protein aggregation in cataracts

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Purpose: Cataracts are the most common cause of blindness worldwide, affecting tens of millions of people1, and currently the only treatment is surgical removal of cataractous lenses. The precise mechanisms by which lens proteins both prevent aggregation and maintain lens transparency are largely unknown. Lanosterol is an amphipathic molecule enriched in the lens. It is synthesized by lanosterol synthase (LSS) in a key cyclization reaction of a cholesterol synthesis pathway. Here we examine the effect of lanosterol on cataract formation.

Results: We identify two distinct homozygous LSS missense mutations (W581R and G588S) in two families with extensive congenital cataracts. Both of these mutations affect highly conserved amino acid residues and impair key catalytic functions of LSS. Engineered expression ofwildtype, but notmutant,LSS prevents intracellular protein aggregation of various cataract-causing mutant crystallins. Treatment by lanosterol, but not cholesterol, significantly decreased preformed protein aggregates both in vitro and in cell-transfection experiments. We further show that lanosterol treatment could reduce cataract severity and increase transparency in dissected rabbit cataractous lenses in vitro and cataract severity in vivo in dogs.

Conclusion: Our study identifies lanosterol as a key molecule in the prevention of lens protein aggregation and points to a novel strategy for cataract prevention and treatment.

Session 12-2

Role of autophagy in formation of the lens organelle free zone

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Purpose: A fundamental property of vertebrate lenses is the regulated loss of membranous organelles in fiber cells to produce an organelle free zone (OFZ). Different pathways have been implicated, including autophagy. The goal here is to examine ultrastructural features of nuclear degradation in a chick embryonic model, searching for autophagic vesicles containing nuclear components.

Methods: Chick embryo lenses were examined from days 10 through 15 (D10-D15). Freshly dissected lenses were fixed in 10% formalin for 24 hr followed by 4% paraformaldehyde for 48 hr. Fixed lenses were processed for transmission electron microscopy. Thin sections near the equatorial plane were examined for different stages of nuclear breakdown.

Results: Images of the capsule and annular pad epithelium showed normal morphology and good preservation. D15 lenses showed all stages of nuclear degradation and were examined extensively. Just beneath the annular pad, young fiber cells had large nuclei with dispersed chromatin and clear nucleoli typical of active protein synthesis. At 300µm depth, nuclei showed distinctive signs of degradation with prominent indentations that sometimes revealed a new macromolecular aggregate attached to the nuclear envelope. A double membrane structure was visible in some aggregates but was not consistent with an autophagic vesicle. This aggregate appeared to degrade the outer nuclear membrane followed by the inner nuclear membrane at about 500µm depth. Remnants of the nuclear envelope appeared as multilamellar structures with 5 nm spacing consistent with pure lipid bilayers. The denuded nucleoplasm then degraded by fragmentation of small nuclear components at 600-700µm depths. None of these stages displayed autophagic vesicles.

Conclusions: A molecular complex is formed to degrade the nuclear envelope prior to degradation of the nucleoplasm to form the OFZ. Although autophagic vesicles are reported to participate in organelle degradation in general, they do not appear to be involved in lens fiber cell nuclear degradation.

Session 12-3

Lens epithelial cell mitochondrial translocation and protection by αB-crystallin prevents protein oxidation and apoptosis resulting from lens oxidative stress and UV-light exposures

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Purpose: Exposure of lens epithelial cells to oxidative and UV-light stress results in oxidation and dysfunction of essential lens epithelial cell proteins, apoptosis of lens epithelial cells and cataract formation. Mitochondria are important players in these processes since their dysfunction results in the production of damaging reactive species (ROS) that inactivate lens epithelial cell proteins and in the release of cytochrome c that can initiate apoptosis of lens epithelial cells. In this study, we sought to establish a potential role for αB-crystallin in protection of lens epithelial cell mitochondrial against oxidative and UV-light damage.

Methods: Lens epithelial cells or *ex vivo* cultured lenses were exposed to UV-light or H2O2-oxidative insults and mitochondrial function examined by monitoring of mitochondrial membrane potential, ROS release, cytochrome c oxidation and cytochrome c release. Interactions between αB-crystallin and cytochrome c were monitored by immunofluorescent co-localization and FRET analysis. Levels of lens epithelial cell apoptosis and viability were correlated with altered mitochondrial functions. The requirements for αB-crystallin chaperone activity were monitored by comparing mitochondrial protective abilities of chaperone-defective mutant forms of αB-crystallin.

Results: Exposures of lens epithelial cells to cataract-associated insults results in translocation αB-crystallin to lens epithelial cell mitochondria. Mitochondrial αB-crystallin specifically interacts with cytochrome c, preserves mitochondrial electron transport, prevents release of mitochondrial ROS and prevents oxidation of cytochrome c and its subsequent release from the mitochondria. Mitochondrial protection by αB-crystallin appears to be independent of its chaperone function.

Conclusions: These results provide evidence that lens epithelial cell mitochondrial translocation and protection by αB-crystallin prevents protein oxidation and apoptosis resulting from oxidative and UV-light exposures that are associated with cataract formation.

Session 12-4

Kinostat™ prevents cataracts in diabetic dogs

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Purpose: A majority of dogs develop blinding bilateral cataracts within 6 months after diagnosis of diabetes mellitus (DM). Here, we present an interim analysis of a randomized masked placebo controlled clinical trial (1/3 placebo) of the topical aldose reductase inhibitor Kinostat™ that is being conducted at 11 centers across the United States.

Methods: 135 dogs are being evaluated by board certified veterinary ophthalmologists at the time of enrollment and then at 1, 2, 3, 6 and 9 months. The dog's owners administer the topical formulations TID. Dogs not developing cortical cataracts during the 9-month period are then given Kinostat™ with ophthalmic evaluations required at 6-month intervals.

Results: Newly diabetic dogs of all sizes, breeds, and sex with only equatorial vacuoles of less than 360° present and no other ocular disease were recruited. The results, to date, confirm the initial proof of concept study (Vet. Ophthlamol. 13:363-8, 2010) that daily administration of Kinostat™ to diabetic dogs significantly prevents cataract formation for up to 6-years. A required toxicology study found that daily application of Kinostat™ at doses of up to 5x the recommended doses did not induce any direct local or systemic toxic effects in any of the tissues examined.

Conclusion: Kinostat™ is the first drug to prevent the clinical development of diabetic cataracts and reduce the need for cataract surgery. Because Kinostat™ meets an unmet medical need, the FDA has granted Kinostat™ a fast-track Minimum Use in a Major Animal Species designation.

Drs. Kador, Wyman and Paulos have financial interest in Therapeutic Vision, Inc.

Session 12-5

Protective effect of caffeine against UVR induced oxidative stress in the lens

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Purpose: To determine the protection of topically applied caffeine against UVR-300 nm induced oxidative stress in the lens.

Methods: 6 weeks old Albino Sprague-Dawley rats were topically applied with caffeine in methylcellulose and exposed *in vivo* to UVR-300 nm. Induced forward light scattering was measured 1 w after exposure to UVR. Active caspase-3 was observed immunohistochemically 7 h after exposure. GSH was measured colorimetrically 1 week after exposure. The protection factor for topically applied caffeine was estimated as threshold dose ($MTD_{2.3:16}$) with caffeine/threshold dose without caffeine. Caffeine concentration in the lens and blood was measured with HPLC as a function of time 30-120 min after topical application of caffeine. Caffeine in the lens and blood was measured at 30 min after topical application as a function of for topically applied caffeine in the concentration interval 0.72-70 mM. Pupil diameter was measured 0-60 min. after topical application of 72 mM caffeine.

Results: Topically applied caffeine, 72 mM, 55 min prior to *in vivo* exposure to 8 $kJ \cdot m^2$ UVR-300 nm protected against development of forward light scattering as measured 1 w after exposure. There was qualitative signs of active caspase-3 signal 7 h after exposure. There was no difference in GSH-concentration at one week after exposure. The protection factor for topically applied caffeine was 1.2. In the lens, the concentration of caffeine decayed exponentially. In the blood, the concentration of caffeine increased linearly. Lens concentration increased linearly with concentration of topically applied caffeine. Blood concentration increased linearly. The pupil diameter dropped exponentially towards a minimum diameter asymptote but the miosis was reversible with tropicamide.

Conclusions: Topically applied caffeine protects against oxidative stress from *in vivo* UVR exposure, penetrates into the lens and blood and induces miosis in the anesthetized animal. The miosis is reversible with tropicamide.

Session 12-6

In vitro lens culture studies can identify potential cataractogenic mechanisms and identify anticataract drugs

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Purpose: The appearance of lens opacities during the toxicological phase of systemic drug development in long-term rat or dog studies or in preclinical or clinical human studies can quickly result in the death of a promising drug project. The specific adverse biochemical mechanism(s) of action of a candidate drug can quickly be identified by *in vitro* lens culture studies. These lens culture studies can also be used to evaluate the anticataract potential of candidate drugs.

Methods: Intact lenses, dissected using a posterior approach, were cultured *in vitro* using TC-199 - bicarbonate medium containing 30 mM fructose. Basic biochemical analyses included the evaluation of choline, aminoisobutyric acid (AIB), and rubidium uptake, as well as GSH and ATP levels and the activities of GR, GPx, G3PD, G6PDH, LDH, Catalase, and ER stress.

Results: Lenses from mouse, rat, rabbit, dog or human can be successfully cultured *in vitro* using TC-199 - bicarbonate medium containing 30 mM fructose. Lenticular changes leading to cataract formation were identified within 3 days of culture while the lenses still appeared clear. As a result, culture studies can not only elucidate the experimental mechanism(s) of cataract formation, but also identify the toxicological mechanism(s) of action of how a drug can alter lens biochemistry and clarity. In vitro lens cultures have also been used to identify and evaluate the anticataract effect of potential drugs.

Conclusion: Lens culture studies are a powerful research tool that can be used rapidly to identify the specific beneficial or adverse mechanism(s) of action of a drug candidate.

Session 13-1

In vivo assessment of molecular aging by quasi-elastic light scattering in human lens

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Purpose: Crystallin proteins comprise ~90% of lens protein in mature lens fiber cells, do not undergo turnover, and thus remain extant throughout life. Crystallins undergo various posttranslational modifications during aging that disrupt the normal functioning of the proteins, facilitating aggregation and insolubilization. We expand on previous work by others and hypothesize that these cumulative alterations in the lens may constitute an *in vivo* biomarker of molecular aging for the human body.

Methods: Quasi-elastic light scattering (QLS) has previously been used to study the aggregation of lens proteins. QLS tracks time-dependent changes in hydrodynamic radius, polydispersity, and supramolecular order of proteins. Here we use QLS to measure changes in human lens proteins as a function of time *in vitro* and aging *in vivo*.

Results: QLS tracked time-dependent changes in human lens protein during long-term incubation (~1 yr) and in response to oxidation *in vitro*. Time-dependent QLS signal changes detected *in vitro* were consistent with observed age-dependent QLS signal changes in human subjects *in vivo* across a wide chronological age range (5 to 61 years).

Conclusions: QLS assessment of the human lens may provide a practical technique and metrics for noninvasive evaluation of molecular aging.

Session 13-2

Aggregation of lens homogenates by the deamidation mutant βB2 Q70E/Q162E

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Purpose: We have identified deamidation as a major modification of lens crystallins during aging and cataracts. In 15 deamidated mimics of β-crystallins generated in our laboratory, most were less stable to thermal and chemical unfolding, had a greater propensity to aggregate, and were not completely rescued by α-crystallin. Deamidation at critical interfaces within β-crystallin dimers created solvent accessible pockets that increased susceptibility to proteolysis and UV-B damage. Deamidation on surfaces altered interactions between β-crystallins. Our findings suggest that a threshold is reached where deamidation accumulates enough to unfold a protein initiating a cascade of aggregation and precipitation.

Methods and Results: To test our hypothesis, we first determined if a deamidated βB2 caused aggregation of lens homogenates. A deamidation mimic, βB2 Q70E/Q162E, was incubated with lens homogenates and preheated at 50 °C with no change in turbidity. Upon placing at 60°C, turbidity increased rapidly for the mutant, but not for the wild type nor for homogenates alone from the 3 month or 91 year old donor lenses. When the deamidated mutant was heated with the lens homogenate from the 3 month donor, aggregation was suppressed. In contrast, incubating with lens homogenate from a 91 year old donor only initially suppressed aggregation. When the samples were heated at 60 °C without pre-warming, the lens homogenate from the 3 month donor no longer suppressed aggregation of the deamidated βB2, suggesting the more rapid denaturation prevented the protection from the younger lens proteins.

Conclusion: We conclude that deamidation at the critical interface in the βB2 dimer, not only increases the propensity of βB2, but also of lens homogenates, to aggregate. These results support our model that an accumulation of deamidation contributes to light scattering in the lens. βB2Q70E/Q162E is being overexpressed in the zebrafish to determine the longterm effects on lens transparency.

Session 13-3

βA3/A1-crystallin: Possible function in the RPE

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Purpose: To investigate the physiological function of βA3/A1-crystallin in the retinal pigmented epithelium (RPE).

Methods: Studies have been performed by standard methods on tissue derived from genetically engineered mice in which *Cryba1* (gene encoding βA3/A1-crystallin) has been deleted, either globally or conditionally in the RPE, and on primary RPE cell cultures derived from these mice and from floxed/control mice.

Results: We have shown that βA3/A1-crystallin, an abundant lens protein, is also expressed in RPE cells and ocular astrocytes, where it is localized to lysosomes. Our mouse models, the RPE-cKO (conditional knockout), where *Cryba1* is specifically deleted from the RPE, and the complete KO are characterized by RPE atrophy, accumulation of autophagosomes, basal laminar deposits, retinal degeneration, impaired visual function, and immune cell infiltration. These effects have all been associated with age-related macular degeneration (AMD), the leading cause of blindness in developed countries. By inducing autophagy, both in vivo in our cKO and KO mice, and in cultured RPE cells, we have provided novel evidence that βA3/A1-crystallin, acting through V-ATPase (vacuolar-type H^+-ATPase)/mTORC1 signaling, is essential for normal autophagy-mediated clearance in RPE. Interestingly, V-ATPase-mTORC1 signaling has been implicated as an essential mechanism for maintenance of lysosomal homeostasis in other systems; dysfunction of the lysosomal degradation pathway in the brain is believed to contribute to the onset and progression of various neurodegenerative diseases.

Conclusion: We have developed a unique model system in which to study the mechanisms whereby lysosomal homeostasis is maintained in RPE cells. We believe that βA3/A1-crystallin represents a potential avenue of therapeutically targeting the autophagic-lysosomal process in RPE and that rescuing or maintaining normal lysosomal function in RPE may offer new and safer modes of treatment for AMD.

Session 13-4

Mass spectrometry based accurate quantitation of crystallins in human lens aggregates using recombinant crystallin standards

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Purpose: The composition of protein aggregates in human lenses remains largely unknown due to heterogeneity caused age-related modifications of crystallins. The aim of this study was to develop an analytically rigorous method to measure the stoichiometry of all major crystallin subunits in lens aggregates using internal standards of human crystallins and mass spectrometry.

Methods: N-terminal 6xHis SUMO tagged human crystallins were expressed in E. coli, purified, and their concentrations determined using synthetic heavy labeled SUMO peptide standards. Ten tryptic digests were then prepared from the water-soluble and water-insoluble protein of nuclear regions of 4 human lenses ranging from 78-85 years of age, whole soluble protein from a 5-day-old human lens, and synthetic crystallin internal standards. These 10 digests were then labeled with tandem mass tagging reagents (10-plex TMT), samples mixed, and the abundance of individual crystallins determined by reference to the internal crystallin standards of known concentration.

Results: The major crystallins in the nuclear region of aged human lenses were αA, βB1, and γS. These crystallins were in at least 3-fold higher molar abundance than other crystallins and comprised the greatest proportion of the water-insoluble fraction. αA was also the least soluble of all nuclear crystallins, with a stoichiometric ratio 3 fold higher in the water-insoluble fraction compared to the water-soluble one.

Conclusions: Aggregated water-insoluble protein of the aged human lens is predominately composed of αA, βB1, and γS-crystallins, with γS-crystallin becoming the major insoluble protein in the oldest nuclear regions. Knowledge of the stoichiometric relationships between crystallins in the water-insoluble fraction of the lens nucleus will help elucidate the structure of light scattering aggregates in cataract.

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Session 13-5

Subunit kinetics in α-crystallins

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"*Is α-Crystallin static?*" This question is raised from uncertainness of number of subunits in this aggregated protein: several researches reported different aggregation numbers for α-Crystallin, ranging between 20 and 30. Under this circumstance, we supposed that ***the aggregates of α-Crystallins could exchange their subunits*** and, in the exchange process, the aggregation number is dispersed.

To prove this hypothesis, we employed deuteration-assisted small-angle neutron scattering (DA-SANS) method. In neutron scattering, there is large difference in scattering length between proton (-3.74fm) and deuteron (+6.64fm). When all hydrogens in α-Crystallin are exchanged with deuteriums, its scattering length density (SLD) is remarkably larger than the SLD of not-deuterated one. A scattering intensity from a protein in a solution is proportional to $(\rho_p-\rho_s)^2$: ρ_p and ρ_s are SLDs of protein and solvent, respectively. Because the SLD of 81% D_2O solvent has an intermediate value between deuterated and not-deuterated α-Crystallin, in this solution the deuterated α-Crystallin and not-deuterated one have a same scattering intensity but the α-Crystallins including both deuterated and not-deuterated subunits have lower scattering intensities depending upon their ratios. Accordingly, when the deuterated α-Crystallins and not-deuterated ones are mixed in 81% D_2O, the scattering intensity should decrease corresponding to the subunits exchange ratio.

We examined the subunit exchange with αB-Crystallin. The DA-SANS clearly showed the subunits of αB-Crystallins are exchanged. Interestingly, the exchange speed depends upon temperature; at 10C no subunit exchanged, at 25C, 20% subunits exchanged in 12 h and at 37C all subunits exchanged in 12 h. Furthermore, at 47C, where the chaperone activity is highly activated, all subunits are exchanged in 15 min but the size of aggregates becomes larger.

In the presentation, we would like to discuss the mechanism and the relation to abnormal aggregation of this subunit kinetics with the results of DA-SANS and mass spectroscopy.

Session 13-6

Ageing variations in the refractive index of human lenses

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Purpose: As the lens ages its optical properties change. These changes may not be the same in all eyes, given the differences in refractive power and in the extent to which the lens may be used in accommodation. This will depend on visual demand and be linked to lifestyle. The purpose of this study was to determine how the refractive index varies in lenses of different ages and the extent to which individual variations, based on differences in refractive power and in physiology could mask age-related changes.

Methods: Sixty-six human lenses aged between 16 and 91 years donated to the Bristol Eye Bank were used in this study. Measurements were conducted at the SPring-8 synchrotron facility in Japan using the X-ray Talbot interferometer constructed on beamline BL20B2 using X-rays of 25 keV. Lenses were set in physiologically balanced media and measurements took around 50 minutes. Repeat scans were conducted on selected samples.

Results: Along the optic axis, the profiles of refractive index showed distinct micro-fluctuations in the nuclear region with steep cortical gradients. There were no age-related trends in refractive index along the optic axis. Decentration of refractive index contours in the sagittal plane were found in some older lenses but these could not be considered a general ageing trend as they were masked by individual variations. In the equatorial aspect, refractive index profiles did show variations with age that were statistically significant particularly for lenses that were in the seventh decade and older. Maximum refractive index in the lens centre showed a slight decrease with age. Repeat scans showed no variations within increments of 0.005 refractive index units.

Conclusions: Age-related trends were evident in the equatorial but not in the sagittal plane. Individual variations in refractive index along the optic axis are relevant to the design of implant lenses.

Session 14-1

Lysine acylation alters structure and function of human lens αB-crystallin

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Purpose: To determine acylation of lysine residues in aging and cataractous human lens αB-crystallin and evaluate effects on functions.

Methods: Succinylated and acetylated αB-crystallin in aging and cataractous human lenses were detected by western blotting using monoclonal antibodies to succinyllysine and acetyllysine. Acylated lysine residues in human lens αB-crystallin were detected by LC/MS-MS and western blotting. Effects of acetylation on the structure of αB-crystallin was evaluated by CD-spectroscopy, protein refolding ability and DLS− MALS analysis, and the effect on αB-crystallin's function was evaluated by chaperone and anti-apoptotic assays.

Results: Both acetylation and succinylation were detected in several proteins, including crystallins. Age of the lens and cataract has little effect on these modifications. LC/MS-MS analyses human lens αB-crystallin revealed lysine acetylation at K92 and K166 and succinylation at K150. We introduced an acetylation mimicking modification (AcK) at K92 by site directed mutagenesis of lysine to cysteine followed by conversion of cysteine to methylthiocarbonyl-aziridine to obtain AcK. The mono-acetylated protein had slightly altered secondary and tertiary structures and exhibited higher surface hydrophobicity. The acetyl protein had more client protein binding per subunit of the protein and higher binding affinity relative to that of the native protein and showed 25− 55% higher chaperone activity than the native protein. In addition, the acetyl protein was ~20% more effective in inhibiting chemically induced apoptosis than the native protein. Similar to acetylation, succinylation also improved the chaperone activity of αB-crystallin.

Conclusion: Lysine acylation occurs in human lens proteins, and in αB-crystallin it alters structure and enhances biological functions.

Session 14-2

Molecular basis for lens crystallin denaturation and precipitation by vitamin C oxidation products

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Purpose: The human lens is exposed to high levels of vitamin C which to protect it from photoxidation. The lens is maintained in reduced form primarily by glutathione whose levels decrease with age and during cataract formation. As a consequence, vitamin C forms dehydroascorbate, which decomposes into C4 and C5 sugars such as erythrulose and xylosone. We hypothesized that these highly reactive ketoaldehydes modify lysine and arginine residues thereby altering the charge and aggregation state of the protein, leading to protein unfolding, exposure of sulfhydryl groups and denaturation of the protein.

Methods: In order to investigate these hypotheses we carried in vitro incubations with calf lens crystallin homogenate that was separated into α-, βH-,βL- and γ-crystallin rich fractions by Sephadex G-200 gel filtration chromatography, and fractions were incubated with 20mM ascorbic acid until precipitation occurred. Proteomic analysis with mass spectrometry was used to reveal and identify protein modification sites that are selectively present in precipitated vs. soluble proteins, focusing on the AGE hydroimidazolones from xylosone (XYH), methylglyoxal (MG-H1), CML, CEL, and tryptophan oxidation to N-formyl kynurenine (NFK) and kynurenine(KYN).

Results: Broad formation of AGEs and oxidation was observed in both precipitated and soluble crystallins. However, modification of selective lysine (K) and arginine (R) residues, and oxidation of embedded tryptophan (W) residues occurred in precipitated but not soluble fractions rich in CRYBB1, CRYBB2, CRYBA4 and CRYBA1 crystallins: Major sites include CRYBB1 (K61CML, K119CML, R93XYH, W102NFK), CRYBB2(K68CML, R98XYH, K101CML, K168CML) CRYBA1 (K15CML, K114CML, R78XYH, R194XYH,W79NFK, W181NFK) and CRYBA4 (W31, R39, R40, R59XYH, K62CML). Surprisingly, although CRYGS (B,C, and Z) were detected and CRYGS was oxidized at W46 and W137, no relationship with precipitation was found. Interestingly, the CRYB A4 and CRYB A1 crystallins became completely insoluble and were heavily modified by XYH, CML and NFK. Molecular computer simulation (specifically in regard to molecular interactions) is now needed to understand how each of these modifications unfold and destabilize in particular the beta crystallins.

Conclusions: The above data provide strong support for a role of the Maillard reaction by Vitamin C in the age-related browning and destabilization of beta crystallins during aging. The identification of specific residues modified by ascorbylation could be critical for understanding protein denaturation during cataract development. Investigations with proteins from human cataracts are now needed to validate these in vitro findings. This research points to the need to develop drugs that can scavenge the reactive carbonyls resulting from Vitamin C oxidation and other oxidative insults to crystallins in order to delay the formation of age-related cataracts.

Session 14-3

Isomerization of Asp residues is different between monomer and hetero-polymer of alpha-crystallin in aged human lens

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Purpose: The lensα-crystallin is natively composed of two subunits (~ 20 kDa) αA- and αB-crystallin (αA-Crys and αB-Crys), which form a hetero-polymeric complex of ~ 800 kDa in the vertebrate lenses. Lens protein water-insoluble fraction, which is isolated from senile cataracts, involves a lot of modified α-crystallins containing site specifically isomerized aspartic residues (D-Asp). Recently, abnormal Asp isomerization (D-Asp96) has been reported in non-native αB-Crys monomeric fraction (Sakaue et al. 2015). On the other hand, it has never known the distribution of Asp isomers in non-native αA-Crys monomeric fraction from aged lens soluble fraction. Therefore, in order to investigate distribution of Asp isomers in monomeric αA-Crys, aged lens soluble fractions were separated into polymeric and monomeric fractions, then individually determined the ratio of Asp isomerization.

Methods: Lens of four different ages were homogenized and centrifuged, and the soluble fraction was applied to size-exclusion chromatography. The hetero-polymeric α-crystallin and monomeric α-crystallin fractions were obtained and concentrated independently. After trypsin digestion, each fraction was applied to mass spectrometry equipped with nano-scale liquid chromatography to extract each of αA-Crys -derived peptide containing Asp isomers. The isomerization ratio was determined by the comparison of peak area from four Asp isomer containing peptide.

Results: The αA-Crys was identified as monomeric state in lens soluble fraction. The Asp 58, Asp 84 and Asp 151 of αA-Crys were highly isomerized in the monomeric fraction, but not isomerized to the same level in the hetero-polymeric fraction. Each of Asp isomerization, increased in age-dependent manner, was site-specific and similar to previous results of equally aged lens water-insoluble fractions.

Conclusions: Our results provided a model that Asp isomerization would lead to the dissociation of monomeric αA-Crys subunit from the hetero-polymeric state, reduce the chaperone function and induce insolubilization of crystallins, resulting in senile cataract formation.

Session 14-4

The spontaneous breaking of homochirality in lens crystallins from elderly donors

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Purpose: Homochirality is essential for the development and maintenance of life. However, we detected D-aspartyl (D-Asp) residues in lens α- and β-crystallins from elderly donors. D-Asp residues arise from non-enzymatic racemization of L-Asp in the protein during the life span of the individual. The appearance of the Asp isomer may be responsible for the change in the higher order structure and the loss of function of crystallins. However, the detection of the Asp isomers in the proteins was difficult because optical resolution is only achieved using complex methodology. In this presentation, we demonstrate a new method for rapidly identifying Asp isomers in proteins based on a combination of LC-MS/MS and isomer-specific enzymes.

Methods: Lens samples (60-80 year-old) were homogenized in a buffer and fractionated into water-insoluble and water-soluble fractions by centrifugation. The samples were digested with trypsin, trypsin plus endoprotease Asp-N, trypsin plus L-isoaspartyl methyltransferase (PIMT) or trypsin plus paenidase, and the resulting peptides were applied to LC–MS/MS. Asp-N, PIMT and paenidase specificallty recognizes Lα-, Lβ- and Dα-Asp residues respectively, therefore the isomers are identified by using these enzymes.

Results: We identified the isomeric Asp sites precisely, quickly at the fentomole level in lens crystallins. Asp 58, 76, 84 and 151 of αA-crystallin, and Asp 62 and 96 of αB-crystallin were highly converted to Lβ-, Dβ- and Dα-isomers. The amount of isomerization of Asp was greater in the insoluble fraction at all Asp sites in lens proteins. These results were consistent with that of the conventional analysis of optical isomers of amino acids in the protein which we reported previously.

Conclusions: The isomerization of these Asp residues affects the higher order structure of the proteins and contributes to the increase in aggregation, insolublization and disruption of function of proteins in the lens leading to the formation of cataracts.

Session 14-5

Mechanism of cataract induced by splice mutation in beta A3/A1-crystallin

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Purpose: To determine the effects of the splice donor site mutation in the exon 3-intron 3 junction of the beta A3/A1-crystallin gene *in vivo* using a transgenic mouse model.

Methods: The betaA3/A1-crystallin gene (CRYBA3) containing a splice mutation at the intron 3 +1G to A (c.215+1G>A) was amplified from patients genomic DNA and cloned into the transgenic expression vector (pACP2). The resulting 7.5 kb insert was used to construct transgenic mouse lines by pronuclear injection. Mutant betaA3/A1cDNA (c.97_357del) obtained by RT-PCR of RNA from lenses of transgenic mice was cloned into the pCBB1 vector and transgenic mice were generated. Lenses were studied by morphometric and histological analysis. RNA and protein from transgenic mouse lenses were studied by Real-Time PCR and Western blotting respectively. Apoptosis was evaluated using the TUNEL assay.

Results: The splice mutation at c.215+1G>A resulted in skipping of exons 3 and 4 upon transgenic expression of the mutant gene, leading to an unstable mutant βA3/A1-crystallin protein lacking exons 3 & 4 and having a deletion (c.97_357del; p.Ile33_Ala119del). Transgenic expression of mutant CRYBA3/ c.97_357del cDNA, resulted in abnormal lenses that were altered in size and shape as compared with controls, cataract, and rupture of the lens capsule and showed histological changes. Expression of mRNAs of c.97_357del CRYBA3 and various beta-crystallins declined after postnatal day 7 in the transgenic lenses. In addition, expression of mRNAs for members of the unfolded protein response (UPR) and apoptotic pathways increased in transgenic lenses.

Conclusions: The splice mutation in CRYBA1 c.215+1G>A splice mutant leads to skipping of exons 3 and 4. This results in expression of a 15 kDa p.Ile33_Ala119del mutant βA3-crystallin with an in-frame deletion that is unstable, toxic to the developing lens fiber cells, and activates the UPR and apoptosis in the early post-natal period.

Session 15-1

Molecular inflammation in contralateral eye after the first eye cataract surgery

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Purpose: To assess the inflammatory status of the aqueous humor in the fellow eye after uneventful cataract surgery in the first eye.

Methods: In the screening stage, aqueous humor samples from 15 first-eye and 15 second-eye cataract patients were collected just before cataract surgery and assayed with RayBio® Human Cytokine Antibody Array. The screened cytokines were then verified using a Bio-Plex Suspension Array System in aqueous humor samples obtained from 35 first-eye and 36 second-eye cataract patients.

Results: The cytokine antibody array revealed that interleukin-1 receptor antagonist (Il-1ra), macrophage inflammatory protein (MIP)-1a, and MIP-1b were expressed at high levels in first-eye patients and were lower in second-eye patients, while opposite trends were found for monocyte chemoattractant protein 1 (MCP-1) and regulated on activation, normal T expressed and secreted (RANTES) (all $P < 0.05$, Student's t test). However, only MCP-1 and IL-1ra were significantly different between the two groups after Bonferroni correction (both $P < 0.00125$). In the replication stage, the suspension cytokine array revealed that only MCP-1 expression was significantly greater in the aqueous humor of second-eye patients compared with first-eye patients ($P = 0.0067$, Student's t test).

Conclusions: This study revealed that the expression of MCP-1, a pain-related inflammatory chemokine, was significantly increased in the aqueous humor in the contralateral eye after the first-eye cataract surgery. This suggests there may be a sympathetic-ophthalmic-type uveitis in the contralateral eye after the first-eye cataract surgery, and may help to explain why the second eye phacoemulsification often more painful.

Session 15-2

Bio-chemo-mechanical model of lens aging

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Purpose: Presbyopia is governed by the ever-changing balance of forces between the lens and capsule: the lens grows while the lens and capsule become stiffer. The apparent stiffness of the lens increases dramatically with age. This stiffening has been correlated with biochemical changes. This study proposes a mechanistic model linking biochemical and mechanical changes in the lens nucleus.

Methods: A chemical reaction model was proposed in which families of antioxidants, soluble proteins, insoluble proteins, enzymes, and reactive oxygen species interacted. Proteins were considered to begin in their native state, then undergo modification with age. Modified proteins were then susceptible to aggregation and binding. A sol-gel antiplasticization model was developed to describe the relationship between age-related changes in soluble protein state (e.g. modified, aggregated, or bound) and the shear modulus of the lens. Manipulation of these parameters allowed investigation of the role of each chemical family in the overall pathogenesis of lens stiffening.

Results: Good agreement between model predictions and literature values for age-related protein concentrations and mechanical properties from the literature. Protein-protein disulfide bonds are a major driver of lens stiffening. Age-related loss of thioredoxin activity is a major contributor to lens stiffening. Maintaining elevated levels of antioxidants in the nucleus could significantly slow the rate of lens stiffening.

Conclusions: This study clarifies the role of each family of chemicals involved in the lens stiffening process. It appears that the precipitation of soluble proteins is a major contributor to lens stiffening, primarily via an antiplasticization effect in which binding of these proteins to the cytoskeleton serves as structural reinforcement. The model also provides a framework by which one can understand the contributions of various lens-specific proteins. For example, beaded filaments may thermodynamically stabilize crystallins to preserve lens flexibility. Future work will focus on refinement and experimental validation of the model.

Session 15-3

Non-invasive determination of the tissue properties of the eye lens by Brillouin spectroscopy

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Purpose: Brillouin spectroscopy is a non-invasive optical technology that enables the in vivo determination of biomechanical properties in the crystalline lens.

Methods: Spontaneous Brillouin scattering is an inelastic scattering process arising from inherent density fluctuations (or acoustic phonons) in the medium. It provides direct information on the phonon's properties that are closely related to the viscoelastic and biomechanical properties of samples. In order to separate Brillouin light from elastically scattered light, an in-house developed very narrow band filter, in the form of a gas cell, in combination with a confocal alignment was implemented in the Brillouin setup. In vivo measurements results on rabbit eyes are presented and evaluated concerning their refractive index distribution, protein concentration, density and rheological properties.

Results: The Brillouin frequency shift as well as the bulk modulus could be determined spatially resolved with an axial resolution of 100 microns. The measurement time was approximately 1.0 sec for a single measurement point using a laser-power of 12mW. There was found a direct relationship between Brillouin-shift and refractive index. The found rabbit data are in line with literature.

Conclusions: The presented technique has a high potential for biomechanical in vivo characterization of the lens. One measuring principle enables spatially resolved information regarding the refractive index, protein concentration, density and bulk modulus.

Session 15-4

High spatial resolution *in vivo* magnetic resonance imaging of the human eye at 7.0 Tesla

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Purpose: Imaging of the human eye *in vivo* at 7 Tesla is an advanced magnetic resonance imaging application and not yet part of routine clinical practice. However, ultra highfield magnetic resonance imaging of the human eye provides benefits for *in vivo* evaluation of anatomy and morphology in sub-millimeter spatial resolution for today's clinical science and for future clinical applications.

Methods: To this end the purpose of the study was to examine the applicability of a 6 channel transceiver radiofrequency coil array in conjunction with an optoacoustic triggering regime for imaging of the orbital and intracranial structures at 7 Tesla *in vivo*. Magnetic resonance imaging was performed in 7 healthy volunteers (3 female/4 male) with T1-weighted 3D fast low angle shot and 2D T2-weighted rapid acquisition with refocused echoes sequences.

Results: The six-channel coil array supports high spatial resolution imaging with an in plane resolution of 0.25 x 0.28 mm. This facilitates the depiction of anatomical details of the eye, the orbit, the optic nerve and the optical nerve sheath. Motion related artifacts could be eliminated using optoacoustic triggering regime.

Conclusions: Our results underline the benefits of multi-element transceiver RF coil array technology and trigger protocols tailored for MRI eye applications especially *in vivo* imaging of the crystalline lens.

Session 15-5

Myopia and related factors in Tanzanian adults

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Purpose: Myopia and related factors in African adults has not been investigated in detail. We examined the prevalence of myopia and related factors in Tanzania Eye Study.

Methods: Right eyes of 931 adults aged 40 years and over living in three rural villages in Tanzania were examined. Refraction was measured by binocular open-field autorefractometer (WAM-5500). Lens refractive power was calculated from corneal refractive power (KR-8900), axial length and anterior chamber depth (AL-scan). Myopia was defined as spherical equivalent $<$-0.5D, and its prevalence rate was compared by age. Cataract type and grade were determined from slit lamp images and their correlations with spherical equivalent were evaluated.

Results: Among 730 eyes (57.2±10.4 years old) which were measurable by binocular open-field autorefractometer, spherical equivalents and myopia rates were 0.0±1.3D (18%), -0.1±1.8D (22%), -1.1±2.4D (45%), and -1.9±2.9D (69%) in those aged 40s, 50s, 60s, and ≥70s, respectively. Myopia was significantly increased in eyes aged 60s and over compared to eyes aged 40s & 50s ($p < 0.001$). The average axial length and corneal refractive power in all ages were 23.0±0.9mm and 44.1±1.7D, with no significant difference in either by age. Factors related to spherical equivalent include lens refractive power ($r=-0.74$, $p < 0.001$), lenticular nuclear opacity ($r=-0.63$, $p < 0.001$) and presence of retrodots ($r=-0.52$, $p < 0.001$).

Conclusions: Myopia was significantly increased in inhabitants aged 60s and over of rural Tanzania. The main factor was lens refractive power increased by lenticular nuclear opacity and retrodots.

The human lens nucleus

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Purpose: To examine the contribution of the lens nucleus to human lens growth.

Method: Published equatorial and sagittal refractive index gradients were aligned to generate isoindicial contours which were used to calculate protein contents at various locations from the centre of 19 human lenses, aged 7 to 82 years. The central 32 mg of protein was taken to be the nucleus and its dimensions and volume were determined from the contours.

Results: The volume of the nucleus decreases by around 25% due to compaction. This is complete by around age 40 and the volume remains constant thereafter. At the same time, the nuclear equatorial diameter increases whereas the sagittal thickness decreases, indicative of remodeling. These changes are similar to those observed with the whole lens. The difference between the nucleus and the whole lens, the cortical thickness, is the same for the equatorial and sagittal dimensions at all ages and increases linearly with age.

Conclusions: The changes in lens shape during the first two decades of life are due to remodeling and compaction of the nucleus. Postnatal lens growth is due only to growth of the cortex.

Session 16-1

Lenticular GSH homeostasis and its therapeutic potential

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Purpose: Glutathione (GSH) is the most abundant anti-oxidative compound found in the lens. It fulfills numerous cellular functions, including protection against oxidative stress, control of cellular redox potential and detoxification of xenobiotics. In age-related nuclear cataract (ARNC), GSH levels are impaired and accompanied with increased oxidation, protein disulfide formation and protein cross-link. Generally, lens GSH, is believed, supplied by GSH *de novo* synthesis. However, our lens GSH biosynthesis knockout mouse (LEGSKO mouse) proved that this is not exactly the case, since LEGSKO lens is still maintaining ~50% of its GSH concentration relative to WT mouse despite no mRNA, protein and activity of the key GSH de novo synthesis enzyme, Gclc, are found present in LEGSKO lens. This raises the question, how does lens manage to up take GSH from surrounding ocular structures in order to maintain its GSH homeostasis.

Method: We tested GSH uptake in both LEGSKO and wild type lenses by providing isotopic labeled GSH, [C^{13}-glycine]GSH in either aqueous humor or vitreous humor via local injection. We also delivered [C^{13}-glycine]GSH into eye circulation system by vascular eye perfusion approach. The gamma-glutamyl transpeptidase inhibitor acivicin was used to block GSH gamma glutamyl-cycle. The [C^{13}-glycine]GSH concentration in aqueous humor, vitreous humor and lens was determined by LC/MS analysis. The GSH concentration in these ocular structures from various species was also determined, such as rat, cow and porcine.

Results: LEGSKO lens was able to take up GSH from aqueous humor three folds higher than WT mouse when GSH is in 200-500μM ranges, but relative low uptake was found when GSH concentration is lower than 200μM in 30min uptake experiments. Surprisingly, mouse vitreous humor has high concentration of GSH (~1mM), and similar results were found in rats, but not cow and porcine (<20uM), while cow and porcine tend to have higher GSH level in aqueous humor (~100uM). The isotopic labeled GSH can reach both aqueous humor and vitreous humor from vascular eye perfusion, and with time, the isotopic labeled GSH can also reach to the lens. The current results from mouse study support that vitreous humor might be the potential resource to maintain lens GSH concentration in LEGSKO lens, though more work is needed to decipher the GSH origin that supplies the vitreous GSH dynamics.

Conclusion: Our study implicates a compensatory GSH homeostasis pathway that may be important in maintaining lens GSH homeostasis and lens transparency. This finding opens the door for therapeutic potential to prevent/delay cataractogenesis form age-related, as well as eye surgical complications, i.e. cataract from vitrectomy procedures.

Session 16-2

TRPV4 ion channels are part of a remote control mechanism for tyrosine kinases and Na,K-ATPase in lens epithelium

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Purpose: Lens ion and water homeostasis relies on Na,K-ATPase activity in a small number of cells at the periphery of epithelium monolayer. Therefore, the function of the epithelium must be integrated with the needs of the fiber mass. We have evidence that TRPV4 ion channels are part of a mechanosensitive remote control mechanism that activates Src family tyrosine kinases and adjusts Na,K-ATPase activity in the epithelium. The mechanism is able to be activated by remote fiber cell damage at the posterior pole.

Methods: Intracellular hydrostatic pressure was measured in mouse lens using a microelectrode/manometer system. Western blot was used to examine Src family tyrosine kinase (SFK) activation in pig lens. Na,K-ATPase activity was detected by ATP hydrolysis.

Results: The pig lens was subjected to localized freeze-thaw damage at posterior pole then incubated for 1-10 min in Krebs solution. Transient SFK activation was detected in the epithelium and Na,K-ATPase activity doubled. Similar responses were observed in response to injection of 5µl hyperosmotic mannitol solution beneath the surface of the posterior pole to cause swelling. The responses were prevented by TRPV4 antagonist HC067047 even though TRPV4 was detected in the epithelium but not the posterior fibers that were damaged. In mouse lens, we found evidence that TRPV4 is activated by positive intracellular hydrostatic pressure and serves a critical role in pressure feedback regulation.

Conclusions: The findings point to a mechanosensitive TRPV4-dependent mechanism in both mouse and pig lens. It enables the epithelial cells to detect remote damage in the fiber mass and respond within minutes by activating SFKs and increasing Na,K-ATPase activity. Adjustable Na,K-ATPase activity appears critical for ion, water and hydrostatic pressure homeostasis.

Session 16-3

Real-time monitoring the physiological optics of the lens

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Purpose: To develop a laser ray-tracing (LRT) system to investigate how the cellular physiology of the lens modulates its optical properties in real time.

Methods: Images of bovine lenses organ cultured in either artificial aqueous humour (AAH), AAH + ouabain or high-K^+-AAH were taken using two Canon EOS 1100D cameras. Simultaneous images from different perspectives with large (>0.6) overlapping fields of view were obtained to ensure accurate post-processing three-dimensional reconstruction of the lens. Lenses were photographed continuously for four hours while illuminated with a helium-neon laser (532nm) to trace the light path through the lens. A custom-written MATLAB script was used to extract coordinates of the anterior and posterior lens surfaces and the trajectories of the incoming and outgoing laser beams in 3D space.

Results: Optimization of the angle between the stereo cameras (45^o) and object-camera distance (1300mm) produced a LRT system that when calibrated produced accurate measurements of surface geometries and laser beam trajectories (error < 3.23%). Organ culturing lenses in ouabain to inhibit the Na^+ pump, or high-K^+ to depolarise the lens potential produced rounder geometries that were indicative of lens swelling. Ouabain-induced swelling was a linear process and more pronounced than that observed with high-K^+ incubation which exhibited a degree of volume regulation. In both conditions laser beams appeared to refract more, indicating that blocking the cellular physiology of the lens increased the refractive power of the lens to produce a myopic shift.

Conclusions: A LRT system has been developed that allows the effects of changing the cellular physiology of the lens on its optical properties to be assessed in real time.

Acknowledgements: We wish to acknowledge the Maurice and Phyllis Paykel trust of New Zealand (MPPT), Royal Society Marsden fund of New Zealand, and New Zealand Optometry and Vision Research Fund (NZOVRF) for the funding of this project.

Session 16-4

Aqp0a and Aqp0b expression in the zebrafish lens

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Purpose: AQP0 is the most abundant lens fiber cell membrane protein and is essential for lens transparency and development. Mammalian AQP0 has multiple functions, rendering it difficult to study these functions individually. To overcome this problem we study Aqp0 in the zebrafish (*Danio rerio*) lens. The zebrafish genome underwent duplication millions of years ago resulting in two genes, Aqp0a and Aqp0b, which have evolved distinct functions. The purpose of this study was to characterize morphological changes that occur in response to Aqp0a and b morpholino (MO) knock down and to localize expression of both Aqp0s in the zebrafish lens.

Methods: Embryonic and adult wild type and Aqp0a- and Aqp0b-deficient zebrafish lenses were PFA-fixed and cryosectioned. Plasma membranes were labeled with wheat germ agglutinin (WGA). Purified antibodies directed against the C-termini of Aqp0a and b, respectively, as well as commercially available antibodies that do not distinguish between the two forms, were used for regional and cellular lens mapping and imaged using confocal microscopy.

Results: MO knock-down of both Aqp0a and b revealed loss of lens transparency and disruption to cell packing at 72 hours postfertilization. In Aqp0a-deficient lenses, cell swelling was localised at the core, while in Aqp0b-deficient lenses swelling was more pronounced in the cortex. As previously observed for mammalian AQP0, both zebrafish Aqp0a and b C-terminus labelling was restricted to the outer cortex in the adult lens, while in embryonic lenses, labelling was evident throughout the lens.

Conclusions: Aqp0a and b are important for lens development, however their roles appear to be functionally different. As in mammals, the zebrafish C-terminus becomes unavailable for labelling in the adult lens in the deeper cortex and core. This suggests that there may be sterical hindrance from interacting proteins, or post-translational modification such as C-terminus cleavage in zebrafish lenses as fiber cells age.

Session 16-5

An arginine rich loop plays a critical role in the modulation of AQP0 water permeability

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Purpose: To show that arginines in the cytoplasmic loop from 152 to 156 play a critical role in controlling the water permeability (P_f) of aquaporin zero (AQP0).

Methods: We used experimental measurements of P_f of AQP0 mutants, molecular dynamics (MD) simulations of calmodulin (CaM) interaction with AQP0 to investigate the mechanism of P_f regulation by Ca^{2+}/CaM

Results: Three arginines, 152, 153 and 156, are in close proximity to tyrosine 149 which we previously showed acts as a gate at constriction site II (CSII). Because these arginines may be connected allosterically to the binding site of calmodulin (CaM) we tested if mutations would alter the calcium response the P_f of AQP0. R152A responds to Ca^{2+} like wild type, R153A has P_f locked low. R156A has P_f locked high, and the double mutant R153A/S229D has a P_f which is essentially zero in 2 mM Ca^{2+} and increases in response to 0 mM Ca^{2+}.

Conclusions: Though regulation of AQP0 P_f is controlled in part by the gating action of tyrosine Y149 (as suggested by experiment and confirmed by MD simulations), R153 and R156 play critical roles in transferring the effect of CaM to Y149. Calcium regulation of AQP0 P_f is essential for lens transparency and development. Furthermore the double mutant, R153A/S229D, appears to position Y149 or other aspects of the AQP0 molecule so as to reduce the P_f to zero.

Memo

Abstracts (Poster)

Poster 1

Operation for blunt ocular trauma, traumatic lens dislocation, secondary high pressure of eye (CASE REPORT)

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The patient, Female, 42 years-old. Chief complaint: Decreased vision acuity of right eye injured by wood for 20 days. Previous history and family history are normal. After conservative therapy, the patient was diagnosed Blunt ocular trauma, Traumatic lens dislocation, secondary high pressure of right eye in Chongqing Aier Eye Hospital, when he was admitted, he complain pain and blurred vision of right eye. The best corrected vision of right eye, BCVA 0.04, IOP vary from 35 to 42 mmHg (NCT). He received phacoemulsification + IOL Implantation + trabeculectoy operation with vitreous body support on April 7, 2011. 4 year after the surgery, the best corrected vision, BCVA 0.4, IOP vary from 12 to 14 mmHg (NCT). Operation video and PowerPoint as follows;

Figure 1

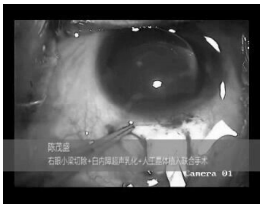


Figure 2

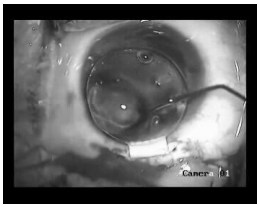


Figure 3

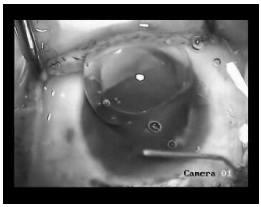


Figure 4

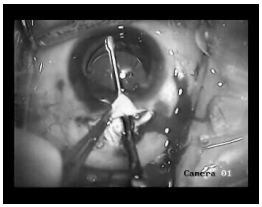


Figure 5

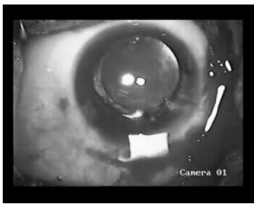
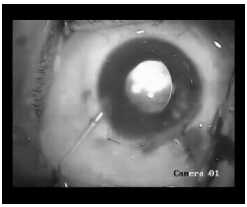


Figure 6



Poster 2

The investigation surgical timing traumatic cataract with capsula broken and clinical effect of secondary intraocular lens implantation

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Objective: The purpose of this study was to investigate the surgical timing of traumatic cataract with capsula broken and clinical effect of secondary intraocular lens implantation.

Methods: 72 patients (72 eyes) suffer traumatic cataract with capsula broken underwent primary and secondary cataract extraction and intraocular lens (IOL) implantation, according to the age, type and extent of trauma, degree of inflammation and accompanying injuries. In principle, Corneal wound need prompt surgical intervention. If cortex extruded into anterior chamber or secondary glaucoma, the surgery was done in emergency. For severe ocular inflammation, surgical intervention was done until inflammation was controlled. 72 eyes underwent subsequent IOL implantation in 14 days.

Results: Follow-up visit after operation was 3 to 6 months. Best-corrected visual acuity (BCVA) was 0.05-0.08 in 8 eyes (11.1%),0.1-0.2 in 11 eyes (15.3%), 0.3-0.4 in 22 eyes (30.6%), 0.5-0.8 in 27 eyes (37.5%) ,better than 1.0 in 4 eyes (5.6%)

Conclusion: The vision of patients with capsula broken was improved after surgical intervention. Appropriate surgical timing is important for patients suffer traumatic cataract with capsula broken to achieve final best-corrected visual acuity and decreases the postoperative complications.

Key words: traumatic cataract; broken capsula; surgical timing; intraocular lens implantation

Poster 3

Experimental attempt to prevent posterior cataract opacification using hole-IOL

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Purpose: The aqueous humor has inhibitory effect to the growth of lens epithelial cells (LECs). Using this characteristics, aqueous humor perfusion holes were opened to the intraocular lens (IOL) optics, and the inhibitory effects to posterior cataract opacification (PCO) were examined *in vivo*.

Method: IOLs (VA-60BB, HOYA) were prepared and eight holes (3.5 ~ 4.6mm away in the area of ⌀ 0.55mm from the center) were opened (hole-IOL). As a control, no treated IOLs (VA-60BB) were prepared. Japanese white rabbits of 8-week-old, were anesthetized and performed phaco surgery from 2.4mm corneal incision. And the hole-IOL or no treated IOL were implanted. At 2 and 4 weeks postoperatively, PCO were observed using slit lamp microscope and EAS-1000. After anterior observation, paraffin sections were stained using hematoxylin and eosin. The thickness of the lens cell layer grown on the posterior pole were measured and compared during 2 groups.

Result: Development of LECs and severe fibrosis were observed using no treated IOL; however, the changes were small using hole-IOL. The thickness of growth LECs was 149 ± 79μm in no treated IOL, 8.0 ± 1μm in hole-IOL.

Conclusions: The hole-IOL prevent PCO development. Aqueous humor will be a key factor to prevent PCO in future.

Poster 4

Comparison of iris-fixated foldable lens and scleral-fixated foldable lens implantation in eyes with insufficient capsular support

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Purpose: To compare the vision outcome of intraocular lens (IOL) replacement by using iris-fixated (IF) foldable IOL (IF-IOL) and scleral-fixated (SF) (SF-IOL) foldable IOL in patients with insufficient capsular support.

Materials and Methods: A total of 63 eyes of 62 patients with insufficient posterior capsule support underwent placement of IF-IOL or SF-IOL between January 2008 and August 2011 and the patients were prospectively followed. Outcome measures included changes in visual acuity, slit lamp examination, refractive indices and corneal curvatures.

Results: Mean improvement of uncorrected visual acuity (UCVA) was greater in IF-IOL eyes as compared to the SF-IOL group (0.43 D ± 0.19 D vs. 0.35 D ± 0.18 D, $p<0.05$). Moreover, 12 (12/31, 38.7%) IF-IOL group versus 4 (4/32, 12.5%) SF-IOL group had a better improvement on postoperative UCVA to preoperative best corrected visual acuity (BCVA) while 9 (9/31, 28.1%) IF-IOL group versus 18 (18/31, 56.3%) SF-IOL group presented a worse improvement. Furthermore, myopic mean manifest sphere and mean cylinder magnitude were lower in IF-IOL eyes than that in SF-IOL group (-0.47 D ± 0.58 D vs. 0.50 D ± 0.43 D, $p<0.01$; 0.84 D ± 0.53 D vs. 1.23 D ± 0.70 D, $p<0.05$). Corneal astigmatism and surgically induced astigmatism showed no differences between 2 groups. In addition, fewer complications were observed in IF-IOL eyes.

Conclusions: IF-IOL implantation can give a significant improvement in vision with fewer complications than sclera-fixated foldable IOL in patients with insufficient capsular support.

Poster 5

Intraocular lens power calculation using IOLMaster and various formulas in Chinese population with axial length exceeding 30.00 mm

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Purpose: To compare the accuracy of 5 intraocular lens (IOL) power calculation formulas in Chinese population with axial length (AL) exceeding 30.00mm.

Methods: This is a Perspective clinical trial (No: ChiCRT-IPC-15005890). In department of Ophthalmology, Xi'an No.4 Hospital, Xi'an, China, between February 2014 and December 2014, 34 eyes with AL exceeding 30.00mm of 27 Chinese patients were recruited. The biometry of those patients was finished by IOLMaster preoperatively. Eyes with pathology or previous ocular surgery or operative complications or combined signal-noise ratio (SNR) of AL less than 32 or those with missing data were exclude. In each case, the power of the implanted IOL was used to calculate the predicted postoperative refractive error by 5 IOL power calculation formulas: Haigis, SRK II, Hoffer Q, Holladay 1 and SRK/T. The actual postoperative refractive error was measured more than 3 months postoperatively. Linear correlation between the predicted postoperative refractive error and actual postoperative refractive error, absolute error distribute and mean error was analyzed for each formula.

Results: 25 eyes (18 Chinese patients) were studied. 9 eyes (26.5%) were excluded. The correlation coefficient of different intraocular lens calculation formulas were: Haigis formula r=0.858, SRK II formula r=0.501, Hoffer Q formula r=0.775, Holladay 1 formula r=0.898, SRK/T formula r= 0.803.Absolute error of those formulas was different. Haigis formula and Holladay 1 formula had the better concentricity. All those formulas could cause hyperopic refraction, The SRK II was the largest, with a mean of +2.01D. The Haigis, Hoffer Q, Holladay 1, and SRK/T formula showed a slight tendency hyperopia, with a mean of +0.55D, +1.31D, +1.21D, and +0.73D.

Conclusions: In Chinese population with AL exceeding 30mm, the 5 formulas caused a postoperative hyperopic refractive error. Holladay 1 formula had the best correlation coefficient between predict and actual. Haigis formula and Holladay 1 formula had the better concentricity. The SRK II was the least accurate formula for those eyes.

Poster 6

Evaluation of light scattering in 4 types of hydrophobic acrylic intraocular lens

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Purpose: To evaluate of light scattering in 4 types of hydrophobic acrylic intraocular lens (IOL).

Methods: 115 eyes of 85 patients who had undergone phaco-emulsification and aspiration (PEA) and IOL implantation. Four types of hydrophobic acrylic intraocular lens were used in the patients; SP2 (XY-1 model of clinical research, HOYA) in 29 eyes, W-60 (Santen) in 24 eyes, PN6 (Kowa) in 32 eyes and ZCB00V (AMO) in 30 eyes. The IOLs were photographed 1 weeks, 6 months, and 1 years after implantation using EAS1000 (NIDEK) in slit and were measured light scattering in the surface and middle regions of IOL optics. And, IOLs were compared the rate of increase in light scattering from 1 weeks to 1 years after implantation.

Results: The surface scatterings (1 year postoperatively) of SP2, W-60, PN6 and ZCB00V were 1.52±1.44, 8.92±1.58, 2.10±1.05 and 2.85±1.00 CCT respectively. The surface scattering of SP2 was significantly less than one of W-60. The middle scatterings (1 year postoperatively) were 0.50±0.57, 9.08±1.82, 0.25±0.26 and 2.65±1.89 CCT respectively. The middle scattering of PN6 was significantly less than one of W-60. The rate of increase in light scatterings were low in all groups and there were no statistically significance.

Conclusions: Light scatterings in the surface and middle regions of hydrophobic acrylic IOLs were different among IOLs. However, the elevations ratios of light scatterings were fine in recent productions of IOLs.

Poster 7

Cataract surgery in pseudoexfoliation syndrome: impaired mydriasis and postoperative increment in intraocular pressure

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Purpose: To examine the incidence of impaired mydriasis, iris sphincter muscle incision and alteration of intraocular pressure at day 1 post-cataract surgery in patients with pseudoexfoliation syndrome (PEX patient) without glaucoma in Wakayama Medical University Hospital, Japan. The incidence of cataract surgery-related complications is more frequent in PEX patients than in non-PEX subjects. We previously reported more marked refractive error in intraocular lens surgery in PEX patients (Ishikawa N, et al. *J Cataract Refract Surg* 2013).

Methods: (1) Alteration of post-cataract surgery intraocular pressure (IOP) in 27 PEX eyes in 27 consecutive patients and 182 eyes in 106 patients without PEX at day 1 post-operation was retrospectively reviewed. The patients were grouped as "IOP increased", "nor changed" or "IOP decreased" by plus/minus alteration of 2 mm Hg. (2) The intraoperative incidence of impaired mydriasis and zonule rupture was reviewed in a consecutive series of 30 PEX patients (37 eyes) and 81 normal subjects (145 eyes) in another duration.

Results: (1) Mean age of each group of patients was 79.00 ± 4.67 in PEX patients and 73.37 ± 9.44 in non-PEX subjects. Preoperative IOP was 12.22 ± 3.15 mm Hg in PEX patients and 13.33 ± 3.13 mm Hg in non-PEX subjects (N.S.). The numbers of eyes in the group of "IOP increased", "nor changed" or "IOP decreased" were 14, 9 or 4 in PEX eyes and 28, 71 or 63 in eyes of non-PEX subjects ($p < 0.05$). (2) Mean age of each group of patients was 79.41 ± 7.62 in PEX patients and 74.51 ± 8.11 in normal subjects. The number of eyes to receive incision(s) of sphincter muscle was 5 in 37 PEX eyes and 3 in 145 non-PEX subjects ($p < 0.01$). The incidence of zonule rupture was 3 in 37 PEX eyes and 2 in 145 non-PEX subjects ($p < 0.05$).

Conclusions: The IOP significantly increases in eyes with PEX at post-cataract surgery day 1. Cataract surgery in PEX eyes shows higher incidence of impaired mydriasis and requirement of iris sphincter muscle incision, which both potentially make the surgery more complexed.

Poster 8

Estimation of the lens volume of normal eye from the ultrasound biomicroscope images

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Purpose: After measuring the anterior and posterior diameters of the human lens, the diameter of the equatorial portion and the external periphery of the transverse surface of the normal eye, we calculated the volume of the lens as an ellipsoid in comparison with an imperfect ellipsoid.

Methods: The axial length was 24.2mm in a 23-year-old young woman with no physical disease or eye disease. Using an ultrasound biomicroscope (UBM), the lens was photographed from 4 directions: at 12-6, 2-8, 3-9, and 4-10 o'clock. After measuring the anterior and posterior diameter, the equatorial diameter and outer periphery in each direction using high-precision calculation sites, the volume of the lens was determined as an ellipsoid. Then, after modeling to make a 3-dimensional image, the volume of the lens as an imperfect ellipsoid was calculated with a Hira STL viewer.

Results: The volume was 0.133 per unit cm^3 as an ellipsoidal object. After modeling with the 3-dimensional image, the volume of the imperfect ellipsoid was 0.106 per unit cm^3.

Conclusions: The actual lens configuration was that of an imperfect ellipsoid showing a cavity in the posterior direction, making it impossible to measure simply as an ellipsoid. Thus, after constructing it from 3-dimensional image analysis as an imperfect ellipsoidal lens, we considered this method for virtually accurate estimation of volume could be effective for measurement of the volume of a lens of a normal eye.

Poster 9

An optical section-assisted *in vivo* animal model for capsular bend evolution

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Purpose: To establish an optical section-assisted *in vivo* animal model of the capsular bend evolution following cataract surgery.

Methods: A total of 10 rabbits underwent phacoemulsification surgery and intraocular lens (IOL) implantation. On the basis of the relationship between the anterior capsule and IOL, the rabbits were divided into complete overlap (stable) and incomplete overlap (unstable) groups, in which six and four rabbits were included, respectively. The capsular bend optical sections were assessed using ultra-long scan depth optical coherence tomography (UL-OCT), and posterior capsule opacification (PCO) was evaluated using a slit lamp on postoperative days 3, 7, 14, and 28.

Results: Six capsular bend types were identified, namely, anterior (A), middle (M), posterior, detachment (D), funnel (Fun), and furcate adhesion. At day 3, 60% of the sides began to form capsular bends mainly consisting of Fun and A types. At day 7, the Fun types transformed into A or M types. At day 14, the capsular bend comprised A, M, and D types, which were almost maintained until day 28. The earliest lens epithelial cells were found in the unstable and stable groups at days 7 and 28, respectively. The unstable group exhibited higher incidence and faster PCO at day 7 ($p = 0.038$) and 14 ($p = 0.002$).

Conclusions: This animal model does not only mimic capsular bend evolution and PCO processes but also produces OCT optical section images equivalent to and more repeatable than histopathology, thereby providing a promising method for further investigations of PCO.

Poster 10

Histopathological analysis of the ratio of intraocular lens with cellular deposits

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Purpose: We compared the cell adhesion on extracted intraocular lens extracted in various periods in Japanese patients.

Methods: We compared the cell adhesion on extracted intraocular lens extracted in various periods; (early period: from 1987 to 2000 (n = 81), middle period: 2001 to 2007 (n = 105), and late period: from 2008 to 2014 (n = 91) under an approval of Institutional Review Board of Wakayama Medical University. The samples were collected through the IOL Implant Data System Committee of the Japanese Society of Cataract and Refractive Surgery from all over japan that was established at January 1st, 1987. The numbers of specimens of PEMA, PMMA, Acryl, Silicone, or hydrogel-optic parts were 4, 45, 23, 9 or 0 in early period, 4, 11, 72, 11, or 7 in middle period, and then 0, 8, 80, 3 or 0 in late period, respectively. Specimens were stained with hematoxylin-eosin and observed under light microscopy, and analyzed the ratio of IOLs with cellular deposits.

Results: The number of IOLs with adhesion of cellular deposits was 26 (2 PEMA, 18 PMMA, 6 Acryl) in early period, 23 (2 PMMA, 19 Acryl, 1 silicone, 1 hydrogel) in middle period, and 21 (3 PMMA, 18 Acryl) in late period.

Conclusion: Cellular adhesion on the optic parts of extracted IOLs implanted after 2001 was less than on IOLs implanted from 1987 to 2000. Although multiple factors could affect cell adhesion to an implanted IOL, improvement of the optic materials and reduction of surgical invasion due to the advanced surgical technique / phaco machine might be attributable to the outcome.

Poster 11

Management of capsular contraction after complicated cataract surgeries

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Purpose: to evaluate the effect of surgical intervention on capsular contraction after uveitis caused cataract surgeries.

Methods: A total of 16 capsular contractive patients (18 eyes) were underwent surgical management after uveitis caused cataract surgeries in our department from June 2012 to June 2015. Clinical features including the interval between cataract surgeries and capsular released surgeries, best corrected visual acuity (BCVA) and intraocular pressure pre- and post-operation, inflammation reactions post-operation and complications.

Results: Among all the capsular contractive patients received surgical treatment, the average age was 28.4±7.2 years (15~46 years). The average interval between cataract surgeries and capsular released surgeries was 24.8±12.6months (1~120 months). Seven patents (9 eyes) with anterior capsular contraction only were underwent anterior capsular release, average BCVA was 0.11 pre-operation while 0.45 post-operation. Five patients (5 eyes) with intraocular lens (IOL) and capsular subluxation were underwent anterior capsular release and IOL fixation, average BCVA was 0.06 pre-operation while 0.32 post-operation. There were 4 patients (4 eyes) with IOL and capsular complete dislocation, in which 3 patients (3 eyes) were underwent IOL taken out and IOL fixation, average BCVA was 0.01 pre-operation while 0.31 post-operation, while 1 patients (1 eye) were underwent vitrectomy to take out the IOL and IOL fixation since the IOL fallen into posterior pole of vitreous chamber, the BCVA was 0.1 post-operation. Intraocular pressure was transiently increased in 3 eyes (16.7%). Severe inflammation reaction and other complications has not been observed post operation.

Conclusion: Capsular contraction was one of the severe complications post uveitis caused cataract surgeries. Different surgical management chose by varying conditions of contraction was safe and effective. The earlier the contraction was found, the easier the treatment was.

Poster 12

Surgical technique comparison of a limbal versus pars plana approach using the microincision vitrectomy system for removal of congenital cataracts with primary intraocular lens implantation

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Purpose: To compare outcomes of the surgical techniques of a limbal versus a pars plana approach using the 23-gauge microincision vitrectomy system for removal of congenital cataracts with primary intraocular lens (IOL) implantation.

Methods: We retrospectively reviewed all eyes underwent anterior capsulotomy, lensectomy, posterior capsulotomy and anterior vitrectomy through a limbal or a pars plana incision with primary IOL implantation. Refractive correction and amblyopic training began 3 to 5 days postoperatively. Main outcome measures included visual acuity, intraoperative and postoperative complications.

Results: We included 41 eyes of 27 children in limbal group and 41 eyes of 30 children in pars plana group. The mean follow-up period was significantly longer in the pars plana group compared with the limbal group (30.9± 6.3 months vs 56.6 ±10.4 months, $p<0.001$). There was no statistically significant difference in mean logMAR BCVA between the 2 groups ($p> 0.1$). Significantly more eyes had at least 1 intraoperative complication in the limbal group than in the pars plana group (68.8% vs. 18.8%, $p<0.05$), it was mainly distributed at the age range of 2-3 years (7 vs. 1, $p<0.05$). The most common intraoperative complications were: iris aspiration, iris prolapse and iris injury. More eyes in the limbal group had IOL pigmentation, but the difference was not significant (20.8% vs. 4.2%, $p> 0.1$). More eyes in limbal group needed additional intraocular surgery (3 vs. 1).

Conclusions: We recommend the pars plana approach for lower incidence of intraoperative and postoperative complications. The visual results were encouraging and did not differ between the limbal and pars plana groups. The limbal approach should be reserved for children older than 3 years old and caution exercised to minimize disturbance to the iris.

Poster 13

Higher-order aberrations in Japanese eyes with cataracts and eyes with aspherical intraocular lenses

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Purpose: To compare higher-order aberrations (HOA) among cataractous and intraocular lens (IOL) eyes.

Methods: Subjects comprised 1442 eyes with only refractive error and cataract (aged 64.5±9.3 yrs) from Kanazawa Medical University Hospital (KMU) and Monzen Eye Study. Under maximum mydriasis for 4-mm and 6-mm pupils, corneal total HOA (THOA), trefoil, coma, tetrafoil, and spherical were calculated from component maps by wavefront analyzer. Lens opacities were classified as cortical (COR), nuclear (NUC) or posterior subcapsular (PSC) according to WHO classification, retrodots (RD) and waterclefts (WC) according to KMU classification. Presence of COR inside 3-mm of the pupil center was classified as CEN+ and outside as CEN-. Likewise, presence of WC was classified as central and peripheral.

Results: Corneal THOA in eyes with single opacity and IOL eyes was identical in transparent eyes of the same age. For 4-mm entrance pupils, 1) COR2(CEN+), COR3(CEN+), NUC1, NUC2, and WC(central) of ocular THOA, 2) COR2(CEN+) and WC(central) of ocular trefoil, 3) NUC1 and NUC2 of ocular coma, 4) COR2(CEN+), COR3(CEN+), NUC1, WC(central), and WC(peripheral) of ocular tetrafoil, 5) WC(central) of ocular spherical, in single opacity eyes and IOL eyes were significantly higher, and NUC1, NUC2, IOL (60s), and IOL (70s) of ocular spherical were significantly lower than in transparent eyes of the same age ($p<0.05$). For 6-mm entrance, 1) COR2(CEN+), COR3(CEN+), NUC1, and NUC2 of ocular THOA, 2) WC(central) of ocular tetrafoil, in single opacity eyes and IOL eyes were significantly higher, and NUC1, NUC2, RD3, IOL (60s), and IOL (70s) of ocular spherical significantly lower than in transparent eyes of the same age ($p<0.05$).

Conclusions: THOA markedly increased in eyes with NUC. THOA increased as CEN+ and as WC(central) increased in severity, respectively. In eyes with RD, mild cortical opacity, posterior subcapsular opacity, and WC(peripheral), THOA increase was slight. The possibility is high that other factors reduce visual function in these eyes.

Poster 14

Influence on visual function by high-order aberration, forward and backward light scattering in eyes with nuclear opacity

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Purpose: We examined correlations among higher-order aberrations (HOA), forward and backward light scattering (FLS and BLS) and visual function in eyes with nuclear opacity (NUC) and transparent lens (TPL).

Methods: From Monzen Eye Study (2013-2014) and Kanazawa Medical University Hospital (July 2013-October 2015), 42 NUC (29 subjects 66.4±11.0 yrs old) and 42 TPL (42 subjects 57.2±8.7 yrs old) eyes were examined. Lens opacity was graded under maximum mydriasis by one examiner using WHO classification system. Total HOA (THOA) was measured using KR-9000PW (TOPCON). FLS (log(s)) was calculated from C-Quant (OCULUS) measurements. BLS was calculated from peak height of the anterior nucleus using EAS-1000 (NIDEK). Contrast visual acuity (CVA) under 25% daytime (DAY) and evening (EVE) was measured using CAT-2000 (NEITZ).

Results: There was no significant correlation between THOA/FLS and age in either group. BLS was significantly higher with age in TPL eyes ($P<0.001$). The average THOA, FLS, and BLS were 0.128±0.063μm and 0.323±0.116μm, 1.13±0.2 and 1.51±0.2, 80.2±18.5cct and 151.4±44.4cct in TPL and NUC eyes, respectively, being significantly higher in NUC eyes ($P<0.001$). In NUC eyes, best corrected VA (BCVA) was significantly decreased with increase of THOA ($P<0.05$), FLS ($P<0.001$), and BLS ($P<0.05$), and the multiple correlation coefficient of 0.591 by multiple linear regression analysis was significant. BLS had a much greater impact on BCVA. The multiple linear regression analysis between CVA and THOA/FLS/BLS showed significant correlations, and DAY and EVE were markedly affected by FLS and BLS, respectively.

Conclusions: All increases in HOA, FLS, and BLS influenced visual function in eyes with nuclear opacity, and it was especially suggested that FLS and BLS were correlated with visual function decrease under 25% contrast of daytime and evening, respectively.

Poster 15

Lenticular findings in emergency workers at Tokyo Electric Power Fukushima Nuclear Power Plant at 4 years post-exposure

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Purpose: We examined change in the crystalline lenses of workers at the Fukushima Nuclear Power Plant (NPP) of Tokyo Electric Power Company (TEPCO) which was damaged following the Great East Japan Earthquake, at about 4 years post-exposure.

Methods: Of 900 workers exposed to 50mSv and over, 331 (662 eyes) employed at TEPCO head office, Fukushima NPP and Kashiwazaki-Kariwa NPP had eye examinations in 2013, and 510 (1020 eyes) in 2014 and their lenses were documented using EAS-1000 (NIDEK) and cataract camera (LOVEOX). From the retro-illumination and slit images, one doctor graded the three main types of cataracts according to WHO classification system, retrodots (RD) and water clefts (WC) according to Kanazawa Medical University classification system, and vacuoles (VC) according to their location.

Results: The rate of transparent lenses in 2014 (76.7%) was decreased from that in 2013 (91.8%). The prevalences of lens opacities in 2014 (2013) were 2.1% (1.5%), 0.6% (0.5%), 0% (0%), 0% (0%), 0.4% (0%), 2.0% (0.5%), 12.7% (2.4%), 5.6% (2.0%) for cortical cataract (COR), COR in the pupillary area, nuclear cataract, posterior sub-capsular cataract (PSC), RD, WC, VC, and VC in the pupillary area, respectively.

Conclusions: There was no case of cataract influencing visual function at 4 years post-exposure, but prevalence of VC was increased markedly, which is highly likely an initial stage in PSC indicating the possibility of developing increased PSC and decreased visual function.

Poster 16

The repeatability assessment of three-dimensional capsule-intraocular lens complex measurements by means of high-speed swept-source optical coherence tomography

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Purpose : To rebuild the three-dimensional (3-D) model of the anterior segment by high-speed swept-source optical coherence tomography (SSOCT) and evaluate the repeatability of measurement for the parameters of capsule-intraocular lens (C-IOL) complex.

Methods : Twenty-two pseudophakic eyes from 22 patients were enrolled. Three continuous SSOCT measurements were performed in all eyes and the photos obtained were used for 3-D reconstruction. The output data were used to evaluate the measurement repeatability. The parameters included postoperative aqueous depth (PAD), the area and diameter of the anterior capsule opening (Area and D), IOL tilt (IOL-T), horizontal, vertical, and space decentration of the IOL, anterior capsule opening, and IOL-anterior capsule opening.

Results : PAD, IOL-T, Area, D, and all decentration measurements showed high repeatability. Repeated measure analysis showed there was no statistically significant difference among the three continuous measurements (all $P>0.05$). Pearson correlation analysis showed high correlation between each pair of them (all $r>0.90$, $P<0.001$). ICCs were all more than 0.9 for all parameters. The 95% LoAs of all parameters were narrow for comparison of three measurements, which showed high repeatability for three measurements.

Conclusions : SSOCT is available to be a new method for the 3-D measurement of C-IOL complex after cataract surgery. This method presented high repeatability in measuring the parameters of the C-IOL complex.

Poster 17

Visualizing fiber cells in a whole living lens in real-time

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Purpose: To develop a microscopy method to enable changes in lens fiber cells volume in intact non-fixed lenses to be visualised in real-time.

Methods: A variety of microscopy techniques were to trialled to visualise fiber cell morphology in rat and bovine lenses organ cultured in the absence and presence of pharmacological reagents (Cl^- channel inhibitor NPPB, NKCC1 inhibitor bumetanide and Na^+ pump inhibitor ouabain) that regulate ion channel and transporter function. The best microscopy method involved partial closing the aperture of the light source to produce a narrow cone of light that because of orientation of the fiber cells results in the narrow angle of light effectively illuminating fiber cells at an angle. Images obtained using this off-angle light (OAL) approach in whole lenses were compared to images obtained by confocal microscopy of organ cultured lens sections labelled with the Wheat Germ Agglutinin.

Results: Conventional microscopy techniques failed to resolve lens fibre cell structure due to the low contrast of lens tissue. In contrast, OAL microscopy utilized the light scattering and interference caused by the relative angle of illumination of the fiber cells to visualize the ordered structure of fiber cells columns. This approach was most effective in the outer cortex of control lenses, but cells in the inner cortex and core could be also resolved in lenses after exposure to pharmacological inhibitors that changed the water content and therefore the contrast of the images the lens core. In the lens cortex, OAL microscopy allowed spatially distinct damage phenotypes induced by NPPB, bumetanide and ouabain to be observed that were consistent with damage phenotypes seen in fixed lenses.

Conclusions: OAL microscopy enables the non-invasive visualization of dynamic changes to fiber cell morphology in whole lens with high resolution and sensitivity.

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Poster 18

The changes of hydrogen peroxide in aqueous humor and glutathione peroxidase in the anterior capsule after intake of a lutein-containing antioxidant supplement in a gender-differentiated analysis

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Purpose: We had reported the increases in the levels of superoxide scavenging activity in aqueous humor after intake of a lutein-containing antioxidant supplement in both genders. Interestingly, the levels of hydrogen peroxide (H_2O_2) also increased in females. Determining how the increased H_2O_2 affected the lens epithelial cell (LECs), we investigated the correlation between H_2O_2 in aqueous humor and glutathione peroxidase (Gpx), which is known as one of H_2O_2 scavengers, in the anterior capsule of lens with the intake of the same supplement.

Methods: Twenty one males and 41 females who were classified into same grades of cataracts in both eyes were included. The aqueous humor and anterior capsule were collected as pre-intake samples during cataract surgery. Ocuvite+ Lutein® was administered orally beginning the day after surgery. Six weeks later, the post-intake samples were collected during cataract surgery of the opposite eye. The levels of H_2O_2 in aqueous humor were measured using colormethod of oxidized titan while the mRNA expressions of Gpx in the anterior capsule were measured using RT-PCR. The patients were divided into two groups according the changes in H_2O_2: the H_2O_2-increase group and the H_2O_2-decrease group. The differences between these two groups were investigated.

Results: Compared to that for the H_2O_2-decrease group, the change in Gpx expression for the H_2O_2-increase group was significantly higher in females ($P<0.05$). However, there were no statistical differences in males.

Conclusion: According to these results, when H_2O_2 is introduced into the LECs, there is an increased production of Gpx which causes increased scavenging of H_2O_2 in females. However, this was not found in males.

Poster 19

The capsular thickness and cells attached to the anterior lens capsules in dogs with mature cataract

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Purpose: We investigated the thickness of the anterior lens capsule (ALC) and the morphological structures and mineral element composition of the cells attached to ALC in dogs with mature cataract.

Methods: Seventy anterior lens capsules from 70 dogs with primary mature cataract were obtained by phacoemulsification. With the approval of our university's animal research committee, the ALCs of 5 clinically healthy beagles were removed immediately after the dogs were euthanized for purposes other than our study, and used as normal, control capsules. The thickness of the capsule was measured by scanning electron microscopy (SEM). The capsules were stained with toluidine blue and observed by phase contrast microscopy. The cells attached to the capsules were observed by SEM, and analyzed using energy-dispersive X-ray spectroscopy (EDS). Spearman's rank correlation was used for statistical analysis, and a P-value less than 5% was considered to indicate significance.

Results: There was a positive correlation between age and ALC thickness (Spearman's $\rho = 0.5338$, $P < 0.001$), and the slope of the regression line was 3.895 μm/year. The cell types observed in the ALC of dogs with mature cataract were classified as cuboidal, spindle, fiber-like, pyknotic, and necrotic, while those seen in the control capsules were cuboidal and pyknotic. Upon SEM, the epithelial cells in the ALCs of eyes with mature cataracts were found to be more loosely packed than those in control capsules. EDS revealed that capsules from eyes with mature cataract showed the presence of calcium-rich particles attached to the cells.

Conclusions: The morphological changes in the epithelial cells of the ALC in eyes with mature cataract may be the cause of the presence of calcium-rich particles. In eyes with cataract, the thickness of the ALC increases with age.

Poster 20

Comparison of free radical scavenging activities between original and generic anti-cataract eye drops

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Purpose: Oxidative stress has been known as a risk factor for development and progression of cataract. The purpose of this study was to compare free radical scavenging activities between original and generic anti-cataract eye drops, which contained pirenoxine as an active pharmaceutical ingredient (API).

Methods: We used anti-cataract eye drops containing 0.005% pirenoxine. The original and generic drugs were Catalin® and Kary Uni®, respectively. Free radical scavenging activities of superoxide and hydroxyl radicals were measured by electron spin resonance (ESR), and compared between the two eye drops.

Results: Both the original and generic eye drops showed the same activities in superoxide scavenging. In addition, both drugs showed scavenging activities for hydroxyl radicals, with a significantly higher level for the generic than for the original eye drop ($p<0.0001$).

Conclusions: The original and generic anti-cataract eye drops with pirenoxine as an API were both shown to have scavenging activities for superoxide and hydroxyl radicals, which are representative reactive oxygen species produced during oxidative stress in the lens. The difference in the hydroxyl radical scavenging activities between these two eye drops suggested that vehicles of the eye drops may have contributed to their activities.

Poster 21

Anti-cataractogenic effect of L-carnosine: metabolomics, proteomic and cytotoxicity investigation

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Purpose: L-carnosine is a naturally occurring dipeptide in mammalian skeletal muscles and other tissues; recently it has gained much popularity as an oral supplement and anticataractogenic agent due to its purported antioxidant activity. Conclusive research to support such claims is lacking. This work offered a critical appraisal of the major attributes of L-carnosine pertaining to possible anticataractogenic properties.

Methods: Direct in vitro antioxidant free radical scavenging properties were assayed using TEAC, CUPRAC and DPPH assays. In direct ex-vivo and in vivo antioxidant assays were studied using measuring glutathione bleaching capacity and sulfhydryl (SH-) capacity and hydrogen-peroxide lens epithelial assays. Porcine lenses incubated in high galactose media were also used to study the effects of L-carnosine. MTT cytotoxicity assay was also performed using lens epithelial cells.

Results: The results showed that L-carnosine is a highly potent antiglycating agent with very weak antioxidant capacity. MTT assay showed a non-significant decrease of lens viability compared with control. The incubated lenses in high galactose media and treated with 20 mM L-carnosine showed a dramatic inhibition of advanced glycation end product formation as evidenced by NBT assay and boronate affinity chromatography.

Conclusion: L-carnosine demonstrated a potent antiglycation effect with high lens epithelial cell tolerability. This might offer a new horizon for delaying the onset of diabetic cataract.

Poster 22

The effect of astaxanthin on VEGF levels and peroxidation reactions in the aqueous humor

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Purpose: In this study, we measured various substances related to reactive oxygen species (ROS) and VEGF levels in the human aqueous humor obtained from patients who took astaxanthin (AX), and also discussed the relationship between AX intake and various factors.

Methods: Subjects of this study were 35 patients who underwent bilateral cataract surgery on one side before and the other side after the intake of AX (6 mg/day for 2 weeks). Their aqueous humor was taken during the surgery and subjected to measurements of VEGF level, $O_2^{\bullet-}$ scavenging activity, H_2O_2 level, and total hydroperoxides (TH) level. We evaluated the relationship between VEGF and the oxidation-related parameters as well as the patient's diabetic status, age, and sex.

Results: VEGF levels did not change significantly but $O_2^{\bullet-}$ scavenging activity and TH level significantly ($p<0.05$) increased and decreased, respectively. Both pre- and post-AX intake, VEGF and TH levels were positively correlated (Pearson: r=0.42, $p<0.05$; r=0.55, $p<0.01$, respectively). Analysis of VEGF levels and $O_2^{\bullet-}$ scavenging activities gave a negative correlation but only pre-AX intake (r=-0.37, $p<0.05$). Differences in levels pre- and post-AX only showed association between VEGF and TH (r=0.49, $p<0.01$) analyzed by multiple linear regression. Using multivariate analysis, pre-AX VEGF level was associated with two factors of TH and $O_2^{\bullet-}$ scavenging activity (r=0.49, $p<0.05$), and post-AX VEGF level with two factors of TH and sex (r=0.60, $p<0.01$).

Conclusions: AX intake may have affected VEGF level through its antioxidant effects by increasing $O_2^{\bullet-}$ scavenging activity and suppressing peroxide production.

Poster 23

The betaine-homocysteine S-methyltransferase 1 (BHMT1) involved in human age-related nuclear cataracts

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Purpose: The goal of this study was to quantitatively identify the differential level of BHMT1 in nuclear cataracts of different ages and normal lens nuclei in humans.

Methods: 48 human lens nucleus samples with hardness grades III ~ IV were obtained during cataract surgery by extracapsular cataract extraction (ECCE). 7 normal transparent human lens nuclei were obtained from donors during corneal transplantation surgery. Lens nuclei were divided into seven groups according to age and optic axis: Group A (mean axial length 23.0±0.5 mm; average age 80.8 ±1.2 years), Group B (mean axial length 22.7±0.7 mm; average age 57.0±4.0 years), Group C (mean axial length 23.0±0.6 mm; average age 80.3±4.5 years), Group D (mean axial length 22.9±0.6 mm; average age 56.9±4.2 years), Group E (mean axial length 22.9±0.5 mm; average age 78.1±2.5 years) and Group F (mean axial length 22.7±0.9 mm; average age 57.6±3.3 years).Water-soluble, water-insoluble and water-insoluble-urea-soluble protein fractions were extracted from samples. The proteomic profiles of each fraction were further analyzed using 8-plex isobaric tagging for relative and absolute protein quantification (iTRAQ) labeling combined with two-dimensional liquid chromatography and tandem mass spectrometry (2D-LC-MS/MS). The data were analyzed with the ProteinPilot software for peptide matching, protein identification and quantification. Differentially expressed proteins were validated by Western blotting.

Results: In age-related nuclear cataracts (ARNC), the expression levels of betaine-homocysteine S-methyltransferase 1(BHMT1) was down-regulated, suggesting new roles for BHMT1. The mass spectrometric analysis results were consistent with the Western blot validation. Pajares (Cell. Mol. Life Sci. 2006) hypothesized that the presence of BHMT1 in the lens could be related to its role in betaine removal when osmotic stress disappears. BHMT1 has been identified during the search for protein-protein interactions in several systems.

Conclusion: This is the first report to indentified BHMT1 that may be involved in ARNC pathogenesis.

Poster 24

Inverse finite element analysis of mouse lens compression for determining elastic moduli

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Purpose: Presbyopia is the gradual loss of accommodation ability with age. This is generally thought to be due to age-related lens stiffening. Compression testing has been used to evaluate lens stiffness; however, stiffness is an extrinsic metric as it is affected by size, shape, and mechanical heterogeneity. Thus, stiffness is not a reliable metric for comparing lenses of different genotypes or ages. To overcome this difficulty, an inverse finite element analysis of a compression test was used to determine the elastic modulus (an intrinsic measurement of stiffness) of the lens.

Methods: A 17-week-old mouse lens was evaluated using compression testing. The dimensional values of the lens were assigned from photographs of the lens collected during the experiment. Inverse finite element analysis was used to estimate and determine the elastic moduli of the nucleus, cortex, and capsule by comparing the experimental force-displacement data with the model-predicted force-displacement relationship up to 10% axial compression.

Results: Preliminary results show a similar positive trend in both the experimental and computer-simulated data with estimated values of 57 kPa, 39 kPa, and 48 kPa respectively. Additional mechanical tests were used to independently confirm the elastic moduli of the lens capsule and nucleus.

Conclusions: Identifying the corresponding mechanical properties of lenses from mice having various lens-specific genotypes will provide better insight on how specific proteins contribute to developing presbyopia. UTSA supports this study, partially funded by GM060655.

Poster 25

Human age related cataract and decorin: correlations among opacity severity, age and decorin expression

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Purpose: Previously, we reported that DCN (decorin proteoglycan) was highly expressed among lens epithelial cells (LECs) in a posterior capsule opacification (PCO) rat model, though its role was unclear. Here, we investigated the expression of DCN in cataractous aqueous humor and LECs of humans.

Method: The study was approved by Institutional Review Board, Kanazawa Medical University. Subjects comprised 82 cataractous eyes treated at Kanazawa Medical University Hospital, Ishikawa, Japan, from April through August, 2015. Age at time of surgery, along with subtype and severity of opacity classified using WHO cataract classification system were documented. The concentrations of DCN protein in aqueous humor were measured using Human DCN ELISA method. DCN mRNA expression in LECs were measured using real-time RT PCR.

Result: Although DCN was detected in human aqueous humor, its concentration varied among subjects and showed no relationship with age, cataract subtype or grade. Expression of DCN mRNA in LECs also differed markedly among subjects, however, its expression was significantly increased in posterior capsular (PSC) cataract grade 2-3.

Conclusion: As far as the authors are aware this is the first study to report that DCN was secreted in human aqueous humor and the increased levels of DCN in human LECs of patients with advanced posterior capsular cataract. DCN may be related to the progression of PSC cataract.

Poster 26

Suppression of injury-induced epithelial-mesenchymal transition in tropomyosin 2 mutant mouse lenses created by CRISPR-CAS9

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Purpose: Injury to lens epithelial cells (LECs) leads to epithelial-mesenchymal transition (EMT) with resultant fibrosis. In this study, we investigated the role of tropomyosin (Tpm) 2 in EMT during wound healing in mouse lens by generating Tpm2 hetero-knock-out (*Tpm2*$^{+/-}$) mice, using the clustered regularly interspaced short palindromic repeat/Cas9 (CRISPR/Cas9) system.

Method: Experiments accorded with National Institutes of Health Guide for the Care and Use of Laboratory Animals. We generated CRISPR/CAS9 -mediated Tpm2 mutations in germline C57BL/6NCr mice. *Tpm2*$^{+/-}$ mice were used for study because theTpm2 gene null-knockout mouse exhibits viviparous lethality. The crystalline lens was injured by needle puncture in *Tpm2*$^{+/-}$ and wild-type (*WT*) mice of the C57BL/6 background. The eyes were isolated and processed for paraffin section at day 5, or 10 post-injury. Immunohistochemistry was employed to detect α-smooth muscle actin (αSMA), a marker of EMT and Tpm1/2.

Results: NormallyTpm2 and αSMA were not expressed in *Tpm2*$^{+/-}$ or *WT* mice. LECs assumed an elongated, fibroblast-like morphology, implicating the process of EMT at days 5 and 10 in *WT* mice in contrast to in *Tpm2*$^{+/-}$ mice. The multilayered fibroblast-like lens cells at the capsular break of *WT* mice at day 5 and day 10 expressed αSMA and Tpm1/2. In *Tpm2*$^{+/-}$ mice, expression of αSMA and Tpm2 were observed in fibroblastic LECs, however, the area of immune-positive cells was smaller than in the LECs of *WT* mice.

Conclusion: *Tpm2*$^{+/-}$ mice can be created using the CRISPR/Cas9 system. Tpm2 is involved in the progression of EMT in wound healing of mouse LECs. Inhibition of Tpm2 may suppress PCO.

Poster 27

The inhibitory effect of Zebularine on the posterior capsule opacification after phacoemulsification in a rabbit model induced by transforming growth factor-beta 2

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Purpose: To determine the effect of Zebularine, a DNA methylation inhibitor, on the posterior capsule opacification after cataract surgery in a rabbit model induced by transforming growth factor-beta 2 (TGF-beta 2).

Methods: Phacoemulsification and intraocular lens implantation were performed in 12 rabbits. Different medications were administered into the anterior chamber at the end of cataract surgery as follows: 1/3 of which were injected with balanced salt solution, 1/3 with TGF-beta 2 (4 ng/mL, 0.1 mL), and 1/3 with TGF-beta 2 (4 ng/mL, 0.1 mL) and Zebularine (100 ng/mL, 0.1 mL). Slit-lamp photography was performed 1, 2, and 3 months postsurgery to record the degree of posterior capsule opacification determined by clinical evaluation. Moreover, to compare the fibrotic changes of posterior capsule after the cataract surgery among three groups, we obtained the capsuls of each rabbit and performed the following immunohistochemical analysis on the histological sections, including TGF-beta 2, TGF-beta RII, and a series of mesenchymal markers (alpha-smooth muscle actin, type IV collagen, vimentin, and matrix metalloprotein 2).

Results: Compared to the other two groups, the TGF-beta 2 group presented with more severe posterior capsule opacification. However, in the group treated with TGF-beta 2 and Zebularine, the fibrotic change of posterior capsule was greatly alleviated. Meanwhile, immunohistochemical analysis showed a lower expression of TGF-beta 2, TGF-beta RII, and mesenchymal markers in the Zebularine-treated group, compared to the TGF-beta 2 group.

Conclusions: High TGF-beta 2 concerntration in the anterior chamber plays a role in the development of posterior capsule opacification in rabbit model. Our findings suggested that the fibrosis process of posterior capsule be inhibited by Zebularine, possibly related to the inhibition of mesenchymal fibrosis. DNA methylation inhibitor may provide a brandnew direction for the treatment of posterior capsule opacification after phacoemulsification.

Poster 28

Mutation of phosphorylation sites in αA-crystallin does not impair chaperone function or anti-apoptotic function

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Purpose: Post-translational modifications including phosphorylation, acetylation, deamination, and truncation affect structural and functional properties of α-crystallin. The current studies were performed to study phosphorylation effects on αA-crystallin serine residues S45, S59 and S122, and to determine if human αA- and αB-crystallins have different functional consequences of phosphorylation at similar amino acids.

Methods: Substitution of serines with aspartate in recombinant αA-crystallin yielded phosphorylation mimics proteins αA-S45D, αA-S59D, αA-S122D, and a triple D3 mutant (TM). αA-Crystallins were over-expressed in E. coli and purified by sequential column chromatography. Crystallins were analyzed by Superose 6 gel filtration (GF) to characterize oligomeric complexes (OC) formation. Chaperone-like activity (CLA) was measured by determining the ability of αA-crystallin mutants to suppress chemically-induced and heat-induced client protein aggregation. Cell lines expressing αA-crystallins were constructed and used to test anti-apoptotic activity of mutant forms of the protein.

Results: As evidenced by GF elution profiles, wild-type and mutant αA-crystallin proteins formed OC ranging in apparent molecular weight from 0.55-1.3 MDa. All αA-crystallins demonstrated CLA by preventing client proteins from heat and chemically induced protein aggregation *in vitro*. Analysis of anti-apoptotic activity in cells, all proteins retained functional anti-apoptotic activity.

Conclusions: Substitution of serines 45, 59, and 122 with aspartate in αA-crystallin is not detrimental to OC formation, CLA, or anti-apoptotic function. These findings suggest that phosphorylation of serine residues in αA-crystallin play an additional role in protein function.

Poster 29

αA-crystallin point mutations affect different types of F-actin networks

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Purpose: Several αA-crystallin mutations produce various lens phenotypes in mice, such as cataract, stunted growth, and small lenses. This project aims to determine the altered interaction between these αA-crystallin mutants, wild-type (WT) αB-crystallin, and cytoskeletal proteins in cultured primary lens epithelial cells (LECs) to elucidate part of the molecular basis for these lens phenotypes.

Methods: Primary LECs were harvested from: WT, αA(Y118D), αA(R54C), αA(R54H), αA-/-, and αB-/- mice, all in the C57BL/6J (B6) background strain. Cultured cells at P1 were then labeled for immunofluorescence and imaged by confocal microscopy.

Results: Primary LECs were confirmed to express endogenous αA-crystallin and αB-crystallin. Cells were stained for αA-crystallin, actin, microtubules (MTs), and αB-crystallin. In WT cells, αA-crystallin was enriched near the leading edge with lamellar F-actin. In αA(Y118D) cells, αA-crystallin formed substantial aggregates with F-actin. αA(R54C) cells display enlarged cell size with an abundance of actin stress fibers. αA-crystallin appeared to surround actin stress fibers. Finally, the αA(R54H) mutant protein appeared to form tube-like structures surrounding actin fibers. Almost none of the WT or mutant αA-crystallin proteins co-localized with MTs in these cultured cells, and only partly with αB-crystallin.

Conclusions: αA-crystallin is typically associated with lamellar actin in B6 WT cells, but not actin stress fibers. Endogenous αA-crystallin point mutants distinctly impair the F-actin network to affect the cytoskeleton and cell size in cultured LECs. αA(Y118D), with enhanced chaperone-like activity, forms aggregates with F-actin, while the αA(R54C) and αA(R54H) mutants, with reduced chaperone-like activity, appear to promote actin stress fibers by potentially binding to actin fiber coating proteins. αA-crystallin may play distinct roles in the regulation of actin networks in lens cells to facilitate lens formation, or to trigger cataratogenesis.

Poster 30

Screening and verification of genes and proteins involved in oxidative damage on human lens epithelial cells cultured in physiological hypoxia

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Purpose: HLECs are usually cultured in 21% O_2, which is totally different from physiological condition. This study aimed to compare the anti-oxidative ability of HLECs cultured in different oxygen levels and find out the key genes and proteins associated with oxidative stress in these two different environments.

Methods: HLEC-B3 was incubated for in 21% O_2 and 1% O_2 condition, respectively. SOD, CAT activity and GSH content were assayed. Then the cells were treated with different concentrations of hydrogen peroxide for 24h. The total RNA of the treated cells was collected for microarray analysis and mRNA chip analysis to screen the differentially expressed genes. GO analysis was used for mRNA functional classification. The proteomic profiles of each group were further analyzed using 8-plex iTRAQ labeling combined with 2D-LC-MS/MS.

Results: The amount of GSH and the activities of SOD and CAT were lower in the 1% O_2 group compared with the 21% O_2 group (P<0.05). Cell viability of the 1% O_2 group was lower than the 21% O_2 group (P<0.05). Two microRNAs were found out as the crucial genes which may lead to the difference in anti-oxidative ability between the two groups. mRNAs were differentially expressed in different oxygen environments. GO analysis reported that the most significant gene categories are response to hypoxia, regulation of cell death and regulation of apoptosis. The target mRNA of our chosen microRNA participated in the process of cell proliferation and EMT. Some proteins identified by iTRAQ were associated with the target microRNAs.

Conclusions: The ability of anti-oxidation in HLECs is weaker in physiological condition. The different responses to hypoxia, regulation of cell death and regulation of apoptosis appeared on the expression level of mRNA when oxidative damage happened on HLECs cultured in two different environments, indicating the underlying discrepancy of anti-oxidative mechanism.

Poster 31

Next generation sequencing of somatic variants in human lens epithelial cell DNA: a feasibility study

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Purpose: The notion that somatic mutations accrue in lens epithelial cells and contribute to cataract formation is consistent with the findings of several epidemiological studies. It has been shown, for example, that cataract is more frequent in lenses exposed to UV-B or ionizing radiation. Further, patients with defective DNA repair pathways have an increased cataract risk. It has been difficult to demonstrate the presence of somatic mutations directly, however, because mutant sequences are expected to be present at low frequency compared to wildtype. Here, we explored the use of Next Generation Sequencing (NGS) for detecting somatic mutations in human lens epithelia.

Methods: DNA was extracted from entire human lens epithelia or regions thereof. High throughput sequencing was performed on an Illumina HiSeq platform using the WUCaMP v2 gene panel (a validated set of 150 genes implicated in cancer). To screen for somatic variants we used VarScan2 software in somatic mode and the Integrative Genomics Viewer (IGV) visualization tool for validation.

Results: We found from 1 to 4 somatic variants in each of 4 pairs of epithelia. There was no difference in the number of variants detected in the (sun-exposed) central epithelium compared to the (shaded) peripheral epithelium (n=7 epithelia). Interestingly, analysis of the lower nasal quadrant, identified 5 somatic variants (of which 3 were C-to-T "UV-B signature" mutations) compared to a single variant in the remaining portion of the epithelium.

Conclusions: This is first report of somatic mutations in human lens epithelial cell DNA. Our results suggest that NGS technology can be employed successfully to identify sequence changes that affect only a small proportion of cells in the epithelium. This approach should prove useful in assessing the contribution of somatic mutations to cataract etiology.

Poster 32

Structural and functional modifications of arginine mutation in αA-crystallin

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Purpose: Arginine mutation in αA-crystallin R12C, R21L, R49C and R54C are the most frequent in congenital cataract patients worldwide. To determine their effects on structural and functional modifications will aid us to develop remedy to prevent or treatment of congenital cataractogenesis.

Methods: Recombinant αA-crystallin R12C, R21L, R49C and R54C were expressed in *E.coli* and purified *via* size exclusion chromatography, their oligomeric size by HPLC, secondary structure by CD surface hydrobhobicity, tryptophan fluorescence, Bis-ANS binding, chaperone activity by sepectrophotometry, The mammalian two-hybrid assays and quartz crystal microbalance (QCM) were performed to determine their protein-protein interactions.

Results: The results shows that when compared to the wildtype αA-crystallin, the arginine mutants were formed high molecular weight cross-linked products and aggregates. A nil/slight secondary structural confirmation in β-sheet was noticed, the tryptophan fluorescence intensity surface hydrophobicity, chaperone activity and protein-protein interactions were decreased.

Conclusions: Arginine mutation in αA-crystallin plays a significant role in the development of HMW aggregates which are shifting their structural and functional modifications leads to cause early cataract formation. Further, the site of mutation alters the level of impacts. Aggregation prevention/removing drugs are the need of hour for prevention of cataract formation.

Poster 33

Identification of UVB responsive genes in *in vivo* mouse lens by cDNA microarray

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Purpose: Epidemiological and experimental studies have revealed that exposure to ultraviolet B (UVB) light can induce cataractogenesis. The present study investigated UVB induced change in gene expression in *in vivo* mouse lens.

Methods: The right eyes of seven 8-week old C57BL/6J mice were exposed to 30mJ/cm^2 UVB while the left were shielded by aluminum foil as unexposed controls. Total RNA was isolated from the lens epithelium-enriched fraction of treatment and control lenses 24 h later, and compared for change in global gene expression using Affymetrix Mouse Gene array. Immunohistochemical analysis of lenses was performed to identify cells expressing candidate genes.

Results: Sixty eight genes (probe sets) were commonly up-regulated more than 1.5-fold and two commonly down-regulated more than 0.6-fold by UVB exposure in two independent experiments (3 and 4 mice), respectively. Of the 68 up-regulated genes, two (Cdkn1a: cyclin-dependent kinase inhibitor 1A (p21) and Otx2: homeobox protein Otx2) were selected because of their higher up-regulation and novelty, and the cells expressing them were identified by immunohistochemical analysis. Immunofluorescence histochemistry demonstrated that both p21 and Otx2 genes were expressed mainly in lens epithelial cells. Cdkn1a encodes a potent cyclin-dependent kinase inhibitor p21 whose up-regulation may cause cell cycle arrest of lens epithelial cells. Otx2 encoded a transcription factor which is involved in the development of the brain and sense organs, and its up-regulation may cause a change in lens cell characteristics.

Conclusion: The data revealed novel and previously identified differences in gene expression among UVB-exposed and unexposed lens *in vivo*. Mouse lens epithelial cells *in vivo* responded to a single dose of UVB radiation by enhancing multiple genes. The results may contribute to compilation of a list of genes involved in UVB-induced lens cataractogenesis.

Poster 34

Effect of UV-B irradiation on the secondary structural properties of α-crystallin pior to its chaperone-like activity

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Purpose: To evaluate the injury of UV-B irradiation on the secondary structural properties of α-crystallin and its chaperone activity.

Methods: α-crystallin was isolated from bovine lenses using fast protein liquid chromatography (FPLC). The purified α-crystallin was subjected to UV-B irradiation (308 nm; 670μW/cm^2; 2, 4, 8, 12, 24, 36, 48, 60 hours, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 days). We measured the Fourier transform infrared (FTIR) spectroscopy in amide I band (1600 − 1700 cm^{-1}) and the chaperone activity of both irradiated and non-irradiated α-crystallins.

Results: With the extended irradiation, the FTIR spectrum showed the IR peak blue-shifts from 1631 cm^{-1} to 1645 cm^{-1}, which indicated the conversion process of protein secondary structure from β-sheets to random coils. When the irradiation time was below 2 days, the IR spectra had a larger change in intensity: a decrease around 1631 cm^{-1} and an increase close to 1645cm^{-1}, compared to the spectra above 2 days. The photodegradation of tyrosine residues, caused by the extended irradiation, also had a transition point at the 2nd day, which was very similar to the variation of β-sheets. The chaperone activity of α-crystallin with the irradiation time fewer than 2days remained intact. However, this activity was reduced to 70% after irradiation time at the 3rd day and decreased with increasing doses of UV-B irradiation. Moreover, this activity was reduced to 30% after irradiation time over 6 days and remained constant.

Conclusions: Considering the perfect synchronization of protein secondary structure, Tyrosine residues and chaperone activity, it could be indicated that the intramolecular anti-parallel β-sheet structures of α-crystallin were susceptible to the damaging effect of UV-B irradiation (308 nm), leading to the subsequent decrease of its chaperone activity, and the chaperone active site on α-crystallin was close to the hydrophobic end of the β-sheet (109~122).

Poster 35

Gja8 (Cx50) is important for ball and socket formation in differentiating lens fibers

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Purpose: Gja8 (Cx50) knockout (KO) lenses are small, have impaired denucleation, and develop mild cataracts. These phenotypes may be attributed to impaired lens epithelial cell proliferation and fiber cell differentiation. We are further investigating the molecular and cellular mechanisms of the lens defects in Gja8 KO lenses.

Methods: We have examined the morphology of peripheral elongating and maturing fibers in wildtype (WT) and Gja8 KO lenses using immunofluorescent labeling and confocal microscopy of vibratome sections.

Results: WT peripheral fibers have distinct ball and socket (BS) structures primarily on their broad sides. KO lenses lacked BS, and instead show numerous smaller knob-like structures. Large plaques of Cx46 gap junctions (encoded by Gja3), are seen on WT BS. In KO fibers Cx46 plaques are smaller and do not exclude WGA staining. Other BS associated molecules including ZO-1, PMP22 and beta-dystroglycan (DAG) are also mislocalized in the KO fibers. Moreover, BS still form in Gja3 KO lenses.

Conclusions: Our data suggest that Cx50 is critical to the formation of mature BS structures and to the targeting of BS-associated proteins. Therefore Cx50 may act as adhesion sites for stabilizing BS and as protein scaffolding sites, likely through its C-terminal PDZ-domain binding motif. The loss of high surface area BS structures enriched in connexins for metabolite transport may impede rapid growth of peripheral fibers and contribute to the small lens phenotype.

Poster 36

Tropomodulin 1 regulation of actin is required for the formation of large paddle protrusions between mature lens fiber cells

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Purpose: The complex interface between mature lens fiber cell membranes is characterized by specialized ball-and-socket processes and larger paddle protrusions, which are hypothesized to be important for lens mechanical integrity. We previously demonstrated that tropomodulin 1 (Tmod1), an F-actin capping protein, plays an important role in fiber cell packing and lens stiffness. We hypothesize Tmod1 is needed for formation of complex fiber cell interdigitations that promote cell-cell interactions.

Methods: Using wild-type (WT) and Tmod1(-/-) mice, we characterized mature lens fiber cell structure using electron microscopy (EM) and immunostaining of single fiber cells for F-actin and F-actin-binding proteins.

Results: EM of WT lenses reveals rows of fiber cells with coordinated paddle protrusions that are decorated by smaller protrusions of equal size and spacing, likely representing the finger-like portions of balls-and-sockets. In contrast, Tmod1(-/-) lens fibers have disorganized paddles with irregular protrusions. Immunostaining of WT inner fiber cells demonstrates that paddles and protrusions are rich in F-actin. Tmod1(-/-) fiber cells have F-actin-positive protrusions, but very few paddles. In WT lens fibers, Tmod1 is localized in large puncta at the base of paddles. Alpha-actinin, an F-actin cross-linker is located on WT and Tmod1(-/-) fiber membranes, but large α-actinin puncta are only observed at the base of WT paddles. However, Arp3, an initiator of F-actin assembly, is at the base of small protrusions in both WT and Tmod1(-/-) lens fibers.

Conclusions: These results suggest Tmod1 is required for normal formation of lens fiber cell paddles and may stabilize α-actinin cross-linked F-actin at the base of large paddles to maintain their structure. Formation of small protrusions to create balls-and-sockets between lens fiber cells may be facilitated by Arp3. This is the first work to reveal proteins required for the formation of paddles between lens fibers and suggests that paddles are needed for lens integrity.

Poster 37

Effects of activation of Src-family tyrosine kinases on human LEC apoptosis and EMT under high glucose environment

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Purpose: Subcapsular cataract is one type of diabetic cataracts. Understanding the changes of LECs under high glucose is crucial for prevention of cataract. We will investigate c-Src kinase on apoptosis and epithelial-to-mesenchymal transition (EMT) of LECs cultured in high glucose.

Methods: Human LECs (HLE-B3) were cultured for 24 hours with DMEM containing 5.5 mmol/L glucose (Control), DMEM containing 35.5 mmol/L glucose (High glucose) and DMEM containing 35.5 mmol/L glucose with 10 μmoL/L PP1, a specific inhibitor of c-Src (PP1 group). In 3, 6, 12 and 24 hours after culture, apoptosis of the cells was detected by flow cytometry assay. Activation of c-Src (p-Src^{418}), alterations of protein Bcl-xL, survivin, caspase-3, E-cadherin and α-SMA were assayed by Western blot analysis.

Results: Elevated expressions of p-Src^{418} were found in High glucose group and peaked at 6 hours after culture, which was significantly higher than that in Control and PP1 groups, respectively (P<0.01). The apoptotic rates in PP1 group were higher than that in High glucose and Control groups (P<0.05), but no remarkable differences were seen between High glucose and Control groups in 6, 12 and 24 hours (P>0.05). Comparing with High glucose and Control groups, the expressions of Bcl-xL and survivin were significantly increased in PP1 group, but caspase-3 were declined in 6 and 12 hours (P<0.05). LECs showed slender in shape in 24 hours in High glucose group, but their appearance was close to normal n PP1 group. The expression of E-cadherin reduced and α-SMA increased significantly in 6 hours in High glucose group comparison with PP1 and Control groups (P<0.05).

Conclusions: High glucose activates c-Src kinase, therefore inhibits apoptosis and induces EMT of LECs. However, PP1 induces apoptosis and impedes the process of EMT of them. PP1 may maintain epithelial characteristics even under the stress of high glucose.

Poster 38

Induction of inflammatory mediators by elevated aldose reductase gene expression in the lens

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Purpose: The purpose of our study was to examine the relationship between elevated expression of aldose reductase (AR) and changes to the lens transcriptome.

Methods: Using RNA-Seq, we examined the transcriptome of transgenic mouse lenses engineered for over-expression of human AR in comparison with age/sex matched nontransgenic controls. Transcript abundance was determined using CUFFLINKS and validated by qPCR of selected targets. Changes in gene expression were also examined in primary LEC cultures from relevant strains.

Results: RNA-Seq data indicated that genes associated with inflammatory signaling were among the most prominent differentially-expressed transcripts in AR-Tg lenses. These included (fold-changes) monocyte chemotactic protein MCP-1 (9.5), monocyte-specific chemokine MCP-3 (44), fractalkine *Cx3Cl1* (4.5) and vascular endothelial growth factor (2.4). As measured by ELISA, we observed a two to five-fold increase in expression of pro-inflammatory cytokines (TNFα, MCP-1, Cx3Cl1, VEGF) in eyes of AR-Tg mice compared to controls. AR over-expression in lens led to a 6-fold increase in transcripts for Egr-1, a transcription factor known to stimulate expression of proinflammatory genes. Relative to wild type controls, increased Egr-1, both native and acetylated forms, were also observed in primary cultures of LEC from AR-Tg mice. AR appears to drive upregulation of Egr-1 expression, as significantly lower levels were observed in LEC cultures from ARKO mice or in cultures treated with AR inhibitors.

Conclusions: Activation of AR gene expression leads to transcriptional activation of a variety of proinflammatory genes that have previously been shown to be increased in the diabetic eye. Egr-1, a transcription factor that becomes activated and hyperacetylated in AR-Tg lenses, may play a key role in driving expression of proinflammatory genes.

Poster 39

Identify novel genetic modifiers on mouse chromosome 2 for cataractogenesis

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Purpose: Our previous data reveal that multiple genetic traits regulate the severity of cataracts in Gja3 knockout (-/-) mice between the B6 and 129 strain backgrounds. This study aims to identify novel genetic modifiers on mouse chromosome 2 that control the severity of nuclear cataracts in Gja3-/- mice.

Methods: Chromosome substitution by homologous counterparts between 129 and B6 inbred strains for localizing the chromosomal location of novel genetic modifiers; slit-lamp examination *in vivo* and lens light scattering measure *in vitro* for evaluating cataract severity; morphological, immunological and biochemical analyses for studying the underlying molecular basis.

Results: A co-dominant cataract suppressor, periaxin, has been previously identified on chromosome 7 (Chr7) of the B6 strain. Gja3-/- mice with homozygous 129 Chr7 (Chr7-129/129) had severe nuclear cataracts. However, one mouse line with Chr7-129/129 genotype displayed mild cataracts; in addition, cataract severity seemed to be associated with coat colors-agouti-coated offspring displayed severe cataracts while black-coated offspring displayed mild cataracts. We confirmed that D2Mit48, a marker close to the agouti gene loci on mouse Chr2, was a good indicator for coat color and cataract severity. Genotyping of more backcrossed offspring indicated two regions that may host genetic modifier(s); one is between the markers D2Mit113 and D2Mit168, and the other between the markers D2Mit22 and D2Mit101. Immunostaining showed altered distribution of 129 periaxin in fiber cells of lenses with severe cataracts, compared to the lenses with mild cataracts.

Conclusions: Two intervals on mouse chromosome 2 have been identified from the B6 strain background to alleviate the degree of nuclear opacity in Gja3 knockout mice. We are in the process of fine-mapping to narrow down the region and determining whether these genetic modifiers utilize similar and/or different molecular and cellular events related to the function of periaxin or other membrane/cytoskeleton organization to inhibit cataract formation.

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