Program & Abstracts

International Conference on the Lens

December 10-15, 2023
Kailua Kona, Hawaii


Welcome!
E KOMO MAI

ICL 2023 – MEETING AT A GLANCE

Sunday, December 10th	
3:00 pm	***Registration @ Kaleiopapa Convention Center***
5:00 pm	*Welcome Reception at Pa'akui Point*
	Opening Session @ Kaleiopapa Convention Center
7:00 pm	Recognition of Cataract Research Award and Travel Award Recipients
7:30 pm	Keynote Lecture
Monday, December 11th	
7:30 am	***Registration @ Kaleiopapa Convention Center***
8:20 am	Welcoming Remarks
8:30 am	Session 1: Lens Development
10:00 am	*Break – Tea/Coffee*
10:30 am	Session 2: Lens Cell Biology and Matrix Biology
12:00 pm	*Lunch*
1:15 pm	Session 3: Omics Approaches in Lens Research
2:45 pm	*Break – Tea/Coffee*
3:00 pm	Session 4: Function and Homeostasis of Crystallins
4:30 pm	Poster Session
6:00 pm	*Luau dinner and show*
Tuesday, December 12th	
8:30 am	Session 5: Lens Fiber Differentiation and Autophagy
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10:30 am	Session 6: Lens Physiology and Channel Proteins
12:00 pm	*Lunch*
1:15 pm	Session 7: Physiological Optics and Biomechanics of the Lens
2:45 pm	*Break – Tea/Coffee*
3:00 pm	Session 8: Biochemistry and Biophysics of Crystallins
Wednesday, December 13th	
8:30 am	Session 9: Lens Regeneration and Cross-talk
10:00 am	*Break – Tea/Coffee*
10:30 am	Session 10: Lens Fibrosis and EMT
12:00 pm	*Afternoon Free / Golf Tournament*

Thursday, December 14th	
8:30 am	Session 11: Lens Genetics and Cataract
10:00 am	*Break – Tea/Coffee*
10:30 am	Session 12: Biology of the Aging Lens and Redox Biology
12:00 pm	*Lunch*
1:15 pm	Session 13: Pathology and Regulatory Pathways
2:45 pm	*Break – Tea/Coffee*
3:00 pm	Session 14: Lens Clinical Studies and Treatment
4:30 pm	*Break*
5:30 pm	**The Kinoshita Lecture**
6:30 pm	*Cocktails Bay View Photo Garden*
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Kinoshita Lectureship

Fielding J. Hejtmancik, MD. PhD
National Eye Institute, NIH
Bethesda, MD

Cataract Research Award

Gus Grey, PhD
University of Auckland
Auckland, New Zealand

Henry Fukui Award

Katia del Rio-Tsonis, Ph.D.
Miami University
Oxford, Ohio

Alvira and Venkat Reddy Award

Norihiro Watanabe, M.D.
Nippon Medical School
Kanagawa, Japan

Frederick Bettelheim Award

Liliana Quintanar, Ph.D.
Center for Research and
Advanced Studies (Cinvestav)
Mexico City, Mexico

Gerald Robison Award

Sarah Y. Coomson, M.Sc.
University of Delaware
Newark, Delaware

Young Investigator Awards

Sepideh Cheheltani, M.S.
University of Delaware
Newark, Delaware

Electra Coffman, B.A.
The Ohio State University
Columbus, Ohio

Christian Zevallos Delgado, M.S.
University of Houston
Houston, Texas

Jia-Ling Fu, B.Sc.
Sun Yat-sen University
Guangzhou City, China

Sayan Ghosh, Ph.D.
University of Pittsburgh
Pittsburgh, Pennsylvania

Suhotro Gorai, M.S.
University of Delaware
Newark, Delaware

Shiwali Goyal, Ph.D.
National Eye Institute, NIH
Bethesda, Maryland

Peter N. Huynh, Ph.D.
Indiana University at Bloomington
Bloomington, Indiana

Johanna L. Jones, Ph.D.
University of Tasmania
Hobart, Australia

Bo Ma, Ph.D.
University of Texas Health Science Center
San Antonio, Texas

Natsuko Maeda, M.D.
Nippon Medical School
Kanagawa, Japan

Daisuke Sasaki, B.S.
Kyoto University
Kyoto, Japan

Jiayue Sun, M.S.
Kyoto University
Kyoto, Japan

Jared A. Tangeman, Ph.D.
Johns Hopkins University
Baltimore, Maryland

Qian Wang, Ph.D.
Columbia University
New York, New York

INTERNATIONAL CONFERENCE ON THE LENS

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

Organizing Committee

Scientific

Salil Lachke, Ph.D.
University of Delaware

Administrative

Peter F. Kador, Ph.D.
Meredith, New Hampshire

Thomas White, Ph.D.
SUNY Stony Brook

J. Samuel Zigler, Ph.D.
Middletown, Maryland

Acknowledgements

We gratefully acknowledge support for the meeting received from:

NFER
THE National Foundation for Eye Research

PROGRAM

All Sessions held in Kaleiopapa Convention Center

Sunday, December 10th

3:00 pm ***Registration @ Kaleiopapa Convention Center***

5:00 pm ***Welcome Reception at Pa'akui Point***

Opening Session @ Kaleiopapa Convention Center

7:00 pm **Recognition of Cataract Research Award and Travel Award Recipients**

7:30 pm **Keynote Lecture – Introduction by Peter Kador**

Pawan Sinha: How Restoration of Vision through Cataract Surgery in Children Reveals How Vision Works in the Brain

Monday, December 11th

7:30 am **Registration @ Kaleiopapa Convention Center**

8:20 am **Welcoming Remarks**

8:30 am **Session 1: Lens Development**

Organizer: Xin Zhang, Co-Moderator: Xiangjia Zhu

1. Xin Zhang: Regulation of cell adhesion at the crossroad of lens development
2. Mason Posner: Identifying novel regulators of lens development using zebrafish
3. Yanzhong Hu: Regulation of Heat Shock Factor 4 in lens development
4. Sarah Coomson: The RNA-binding protein Elavl1 is necessary for proper lens development and its deficiency causes cataract
5. Xiangjia Zhu: What makes a bigger lens in high myopia?

10:00 am *Break – Tea/Coffee*

10:30 am **Session 2: Lens Cell Biology and Matrix Biology**

Organizer: Cathy Cheng, Co-Moderator: Julie Lim

1. Cathy Cheng: Changes in the F-actin network localization between cortical and nuclear lens fibers
2. Rosica Petrova: Localization of AQP5 water channels to the broadside gap junction plaques that mediate the outflow of water from the nucleus of rodent lenses
3. Steve Bassnett: The effect of fiber cell compaction on the power and optical quality of the mouse lens
4. Qian Wang: Grb2 functions in synergy with Frs2 and Shp2 in FGF-induced lens development
5. TJ Plageman: Elucidating the function of the lens fiber cell tricellular junction adherens junctional protein δ-catenin

12:00 pm *Lunch*

Monday, December 11th (Continued)

1:15 pm **Session 3: Omics Approaches in Lens Research**

Organizer: Marc Kantorow, Co-Moderator: Ales Cvekl

1. Salil Lachke: Spatiotemporal transcriptome meta-analysis to uncover regulatory relationships in isolated lens epithelium and fibers from embryonic, juvenile, adult and aged mice
2. Ales Cvekl: Hi-C analysis of long-range chromatin contacts and looping between mouse ES cells, lens epithelium and lens fibers
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4. Marc Kantorow: Regulation of lens fiber cell differentiation by hypoxia-regulated epigenetic programming
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2:45 pm *Break – Tea/Coffee*

3:00 pm **Session 4: Function and Homeostasis of Crystallins**

Organizer: Krishna Sharma, Co-Moderator: John Clark

1. John Clark: The Significance of Crystallins in the Optical Function of the Lens
2. Sayan Ghosh: Exploring the multifaceted roles of βA3/A1-crystallin: from lens structural protein to non-lens functions in the retina
3. Hassane Mchaourab: Transcriptional coupling between Nrf2 and αB-crystallin in the lens and heart of zebrafish under proteostatic stress
4. Laxman Mainali: Association of αA-, αB-, and α-Crystallin with the Model of Human Lens-Lipid Membranes is Modulated by Cholesterol Content in the Membranes
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4:30 pm - 5:30 pm **Poster Session**

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2. Electra Coffman: Arvcf Stabilizes N-Cadherin and Cytoskeletal Proteins in the Apical Junctional Complex of the Lens Fiber Cell
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4. Kohei Miyata: Investigation of pupillary response in Synergy® implanted eyes
5. Reona Mouri: Investigation of pupillary response in Lentis Comfort® implanted eyes
6. Natsuko Maeda: ACOMOREF2® in intraocular lenses eyes
7. Tomoko Shiroyama: Backgrounds and post cataract surgery outcomes in patients over ninety years old
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6:00 pm ***Luau Dinner and Show***

Tuesday, December 12th

8:30 am **Session 5: Lens Fiber Differentiation and Autophagy**

Organizer: Amer Riazuddin, Co-Moderator: Quili Fu

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10:00 am *Break – Tea/Coffee*

10:30 am **Session 6: Lens Physiology and Channel Proteins**

Organizer: Tom White, Co-Moderator: Paul Donaldson

1. Tom White: Probing the role of connexin 50 channels in early postnatal lens physiology using single-cell RNA sequencing
2. Paul Donaldson: Physiological modulation of the crystalline lens water transport changes the stiffness of the ex vivo non-decapsulated bovine lenses measured using a lens spin test system
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4. Kevin Schey: Controlling Aquaporin Function in the Lens
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12:00 pm *Lunch*

1:15 pm **Session 7: Physiological Optics and Biomechanics of the Lens**

Organizer: Matthew Reilly, Co-Moderator: Bianca Maceo Heilman

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2. Bianca Maceo Heilman: Average refractive index of human and non-human primate lenses: age and accommodation dependence
3. Susana Marcos: Crystalline lens optical, geometrical and mechanical properties from OCT quantitative imaging in vivo
4. Pooja Rathaur: Composition and Structural Organization of the Human Ciliary Zonule
5. Christian Zevallos-Delgado: Crystalline lens biomechanics

2:45 pm *Break – Tea/Coffee*

Tuesday, December 12th (Continued)

3:00 pm **Session 8: Biochemistry and Biophysics of Crystallins**

Organizer: Eugene Serebryany, Co-Moderator: Ariel Alperstein

1. Eugene Serebryany: The benefits and drawbacks of high Cys content in gamma-crystallins
2. Jiayue Sun: Analysis of the Susceptible Tryptophan Residues on the Greek Key Motifs of βB2-Crystallin
3. Liliana Quintanar: Metal Induced Aggregation of Human Lens Crystallins: The Bioinorganic Chemistry of Cataract Disease
4. Ariel Alperstein: Towards two-dimensional infrared microscopy of crystallin protein protective chaperone activity

Wednesday, December 13th

8:30 am **Session 9: Lens Regeneration and Cross-talk**

Organizer: Katia Del-Rio Tsonis

1. Jared Tangeman: Integrated single-cell multiomics delineates the gene regulatory networks guiding early lens development
2. Kelly Tseng: Regenerating During Development: Understanding Vertebrate Eye Regrowth Using the Frog Embryo
3. Konstantinos Sousounis: DNA Damage Response during Salamander Lens Regeneration
4. Katia Del-Rio Tsonis: A Lens Regeneration Paradigm
5. Sepideh Cheheltani: Effect of Cap2 on the regulation of actin cytoskeleton and fiber cell organization in the lens

10:00 am *Break – Tea/Coffee*

10:30 am **Session 10: Lens Fibrosis and EMT**

Organizer: Janice Walker, Co-Moderator: Eri Kubo

1. Janice Walker: A role for UTX/KDM6B lysine demethylase enzymes in driving the progression of the fibrotic response to lens injury
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12:00 pm ***Afternoon Free / Golf Tournament***

Thursday, December 14th

8:30 am **Session 11: Lens Genetics and Cataract**

Organizer: Elena Semina, Co-Moderator: Fielding Hejtmancik

1. Fielding Hejtmancik: The c.119-123dup5bp mutation in human γC-crystallin, destabilizes the protein and activates the unfolded protein response to cause highly variable cataracts
2. Hélène Choquet: Transcriptome-wide association study based on 54 tissues identifies novel candidate susceptibility genes for cataract
3. Elena Semina: Dido1 is involved in vertebrate lens development, with a possible role in human disease
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5. Johanna Jones: Analysis of isolated congenital cataract genes in Australian families

10:00 am *Break – Tea/Coffee*

10:30 am **Session 12: Biology of the Aging Lens and Redox Biology**

Organizer: Xingjun Fan, Co-Moderator: Rachel Martin

1. Marjorie Lou: The Ying-Yang effect, or the damage and benefit of reactive oxygen species (ROS)to the lens – A review
2. Rachel Martin: From calcium storage to vision, by way of biomineralization: Tunicate βγ-crystallin
3. Julie Lim: Interactions between the lens and ocular humors: does the lens contribute to the maintenance of high levels of ascorbic acid in the vitreous?
4. Lee Goldstein: Tracking molecular aging and hyperglycemia in murine and human lenses by in vivo quasi-elastic light scattering spectroscopy
5. Xingjun Fan: Deficiency in glutathione peroxidase 4 (GPX4) results in abnormal lens development and newborn cataract

12:00 pm *Lunch*

1:15 pm **Session 13: Lens Pathology and Regulatory Pathways**

Organizer: Vasanth Rao, Co-Moderator: Melinda Duncan

1. Alan Shiels: A TRPM3 mutation disturbs cation homeostasis and gene expression in the lens
2. David Li: A Chaperone System Guards Normal Lens Development and Prevents Cataractogenesis
3. Peter Huynh: Eph and ephrin gene expression changes in the ocular lens of aging and knockout mice
4. Melinda Duncan: The molecular basis of aniridic cataract
5. Vasanth Rao: Regulatory Mechanisms of Myosin II Activity and Their Influence on Lens Function

2:45 pm *Break – Tea/Coffee*

Thursday, December 14th (Continued

3:00 pm **Session 14: Lens Clinical Studies and Treatment**

Organizer: Hiroshi Sasaki, Co-Moderator: Hiroyuki Matsushima

1. Natsuko Hatsusaka: The risk of developing pterygium and various cataract types due to ocular UV exposure during childhood
2. Norihiro Watanabe: The microstructure for zonular fibers under ocular inflammation
3. Shinichiro Kobayakawa
4. Hiroyuki Matsushima: Current Status of Intraocular Lens Long-Term Stability
5. Mayumi Nagata: Differences in Intraocular Inflammation in the Anterior chamber Depending on the Position of Intraocular Lens

4:30 pm *Break*

5:30 pm **The Kinoshita Lecture *6:30 pm***

Cocktails Bay View Photo Garden

7:00 pm Conference Banquet Bay View Photo Garden

Friday, December 15th

8:30 am **Session 15: Pharmacological Approaches to Lens Disease**

Organizer: Vincent Monnier, Co-Moderator: Barbara Pierscionek

1. Barbara Pierscionek: Functional approaches to investigating novel anti-cataract therapies
2. Gus Grey: Ion, pharmaceutical and nutrient transport in the lens resolved with imaging mass spectrometry
3. Vincent Monnier: Is the prevention of γ-crystallin aggregation with small molecules a valid strategy for cataract prevention?
4. Juliet Moncaster: Microtubule-Associated Protein Tau (MAPT) in mouse non-transgenic and transgenic Alzheimer's Disease lenses
5. Peter Kador: Pharmaceutical Prevention of Sugar Cataracts

10:00 am **Closing Remarks and Depart**


Sunday, December 10th
ALOHA

Keynote Lecture

How Restoration of Vision through Cataract Surgery in Children Reveals How Vision Works in the Brain

Pawan Sinha[1]

[1] *Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA*

Expanding human knowledge can be tied intrinsically to betterment of the human condition. As scientists, we hope to be able to merge our personal desire to be good Samaritans with our professional desire to be good researchers. This aspiration to braid science and service can only come to fruition, however, if we actively identify opportunities that mesh the two—the kind of science that necessitates service. I shall describe an effort launched by my lab at MIT as an example of what such initiatives might look like. The genesis of this project lies in the confluence of a crucial humanitarian mission and a fundamental scientific quest.

India is home to the world's largest population of blind children. The visual handicap, coupled with extreme poverty, greatly compromises the children's quality of life, prospects for education, employment and even basic survival. The humanitarian mission of Project Prakash is to bring light into the lives of curably blind children (most suffering from congenital cataracts) by providing them sight-restoring surgeries.

Embedded in this mission is an unprecedented opportunity to study one of the deepest scientific questions: How does the brain learn to extract meaning from sensory chaos? By following the development of visual skills in these unique children who are just setting out on the enterprise of learning how to see, we have gained insights into fundamental questions regarding visual learning and brain plasticity. Besides their intrinsic scientific value, these findings have also shaped our thinking in domains such as autism and artificial intelligence.

True to its name (Prakash in Sanskrit means light), the project has helped illuminate lives while also illuminating science.

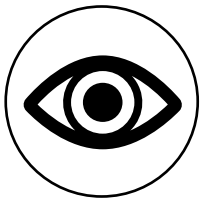
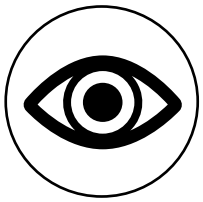

Monday, December 11th

Monday, December 11th

Session 1: Lens Development

Organizer: *Xin Zhang*
Co-Moderator: *Xiangjia Zhu*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker		Title
8:30 a.m.		**Xin Zhang**	Regulation of cell adhesion at the crossroad of lens development
8:48 a.m.		**Mason Posner**	Identifying novel regulators of lens development using zebrafish
9:06 a.m.		**Yanzhong Hu**	Regulation of Heat Shock Factor 4 in lens development
9:24 a.m.		**Sarah Coomson**	The RNA-binding protein Elavl1 is necessary for proper lens development and its deficiency causes cataract
9:42 a.m.		**Xiangjia Zhu**	What makes a bigger lens in high myopia?

Regulation of cell adhesion at the crossroad of lens development

Xin Zhang[1,2]

[1]*Department of Ophthalmology, Pathology & Cell Biology, Columbia University, New York, NY, USA*
[2]*Department of Pathology & Cell Biology, Columbia University, New York, NY, USA*

Purpose: Cell adhesion plays a crucial role in cell shape, motility and differentiation. While numerous factors associated with cell adhesion have been shown to be significant in tissue morphogenesis, the role of negative regulators has not been well studied. In this study, we investigate the importance of restricting cell adhesion in lens development.

Methods: We employed Cre/LoxP system to generate genetic deletions specifically in the lens. The mutants are analyzed by histology and immunohistochemistry.

Results: Abl and Src are two crucial kinases involved in regulating the focal adhesion complex, which plays a key role in cellular binding to the extracellular matrix. Our study reveals that the deletion of Abl leads to hyperactive focal adhesion signaling, resulting in the formation of a lens stalk due to defective lens vesicle closure. The downstream mediators of Abl signaling are the adaptor proteins Crk and the Rho GTPase Rac1, as the Abl lens stalk phenotype can be rescued by the deletion of either Crk or Rac1. On the other hand, the loss of Csk results in the hyperactivation of Src. Although Csk mutant itself exhibits only mild lens stalk defects, the combined deletion of Abl and Csk leads to lens hernia and the explosive loss of lens materials. These findings demonstrate that Abl and Csk synergistically regulate the cellular mechanics necessary for lens vesicle closure.

Conclusions: These results indicate that Abl and Csk, acting as negative regulators of focal adhesion, are significant contributors to the dynamic regulation of cell adhesion at the closure of the lens vesicle.

Identifying novel regulators of lens development using zebrafish

Mason Posner[1], Dylan R. Farnsworth[2]

[1]*Department of Biology and Toxicology, Ashland University, Ashland, OH, USA*
[2]*The RNA Institute, University at Albany, State University of New York, Albany, NY*

Purpose: While recent progress has been made towards elucidating the molecular pathways that guide vertebrate lens development, questions remain about the mechanisms that control the differentiation of organelle-free fiber cells. We have used single cell-RNA sequencing of zebrafish embryos and larvae to identify novel genes hypothesized to play a role in lens development, with subsequent CRISPR gene editing to test their importance.

Methods: We used single cell RNA-sequencing data from 1 to 5-day old zebrafish and bulk RNA-Seq data from mouse lens tissue to identify genes with lens preferred expression. A mixture of four guide RNAs designed to target Cas9 cleavage to individual genes of interest was injected into zebrafish zygotes and resulting larvae were examined by DIC microscopy to identify physical defects in the lens.

Results: A list of the top 200 genes expressed in zebrafish epithelial and fiber cells included 16 genes with no currently known lens function. We CRISPR targeted nine of these genes, finding strong lens phenotypes in one (c1qbp), weak phenotypes in five (hmx1, id3, sox13, endou2, bmp5), and no defect in three (pabpc4, ssb, slc7a11). We screened five genes with enhanced expression in mouse lens cells, finding strong lens phenotypes in three (cebpg, csdc2a and csde1), a weak phenotype in one (carhsp1) and no defect in one (hmga2).

Conclusions: Two genes that led to the strongest lens structural damage after CRISPR editing were for a mitochondria related protein linked to autophagy (c1qbp) and a transcription factor regulating cellular stress response (cebpg). We have begun to analyze the histological and molecular changes occurring in the lenses of cebpg crispant larvae. Overall, our data demonstrate an efficient workflow for using zebrafish to identify novel genes with roles in vertebrate lens development. Future work will dissect the molecular interactions of their gene products.

Regulation of Heat Shock Factor 4 in lens development

Yanzhong Hu[1], Jing Li[1], Xiukun Cui[1], Jing Zhang[1], Hui Li[1]

[1]Division of Eye Degeneration Disease, The National Jointed Laboratory of Antibody Drug Engineering, Henan University School of Medicine, Kaifeng, Henan China

Purpose: Development of lens is regulated by temporospatially turning on or off a series of transcriptional factors, such as Pax6, Six3, sox2/sox3, FoxE3, L-Maf/c-MAF, Pitx3, ProX1, SOX2 and HSF4. Genetic Variations in the DNA binding domain of HSF4 results in the hereditary autosomal dominant cataracts in human. Mice lacking HSF4 gene suffer congenital cataracts at postnatal age. The dominant pathological characteristics of HSF4-null cataracts are hyperproliferation of lens epithelial cells and abnormal terminal differentiation of lens fiber cells including the delayed degradation of nucleus, mitochondria and endoplasm reticulum. However, the molecular mechanism underlying HSF4 regulation on those cellular processes remains unclear.

Methods: C57BL/6 mice with Hsf4del-42 mutation were generated with CRISPR-Cas9, in which a 126 bp in exome 3, encoding 42 amino acids from ? to ? of HSF4 protein were deleted in frame from HSF4 genome. Lens tissues from Hsf4del-42 mice was characterized by immunoblotting, immunofluorescence, immunoprecipitation and RT-pCR.

Results: HSF4del-42 mice exhibited cataracts at P17, and the opacification lens become small in size with vacuous and fibrosis in lens fiber with age increase. The HSF4del-42 lens showed the delayed degradation of nuclei and mitochondria in lens fibers. The results of immunoblot or immunofluorescence demonstrated that the autophagic pathways were blocked reflecting by accumulation of LC3I/II and P62. RNA-seq results demonstrate that 798 genes were downregulated including gamma-crystallins, beta-crystallins, heat shock protein 25 and autophagic protein ATP9a. HSF4 could bind to and activate ATP9a promoter. Deletion of Hsf4 or knockdown ATP9a by siRNA cause the autophagosome accumulation in lens epithelial cells. In addition, we found that HSF4 participated in downregulating ROS production in lens. The ROS level was upregulated in the postnatal $HSF4^{del-42}$ lens and in mELC/Hsf4-/- cell lines as compared to that in the wildtype counterparts. HSF4 deletion upregulated the expression of NOX2, NOX2, and downregulated HO-1 expression. In addition, we found that Keap1protein was accumulated, while NRF2 was downregulated in $HSF4^{del-42}$ lens in vivo and in mLEC/Hsf4-/- cell line in vitro. Overexpression of HSP25 could downregulate keap1 protein in the mLEC/Hsf4-/- cells.

Conclusions: 1) HSF4 regulates the autophagic efflux via controlling the expression of ATP9a, which may be associated with organelles' degradation during lens fiber cell terminal differentiation. 2) HSF4 participates in downregulating ROS level during lens development via directly attenuating the expression of NOX2 and NOX4 or indirectly modulating keap1-Nrf2 pathway.

The RNA-binding protein Elavl1 is necessary for proper lens development and its deficiency causes cataract

Sarah Coomson[1], Sandeep Aryal[1], Salil A. Lachke[1,2]

[1]Department of Biological Sciences, University of Delaware, Newark, DE, USA
[2]Center for Bioinformatics and Computational Biology, University of Delaware, Newark, DE, USA

Purpose: In the last decade, several conserved RNA-binding proteins (RBPs) (e.g., Caprin2, Celf1, Rbm24, Tdrd7) have been identified as key regulators in lens development. RBPs participate in distinct post-transcriptional control mechanisms (e.g., mRNA splicing, transport, stability, translation) to determine the cellular proteome. Toward a deeper understanding of RBPs in lens development, we applied iSyTE and identified robust expression of Elavl1 in the lens. Elavl1 directly binds AU-rich elements (AREs) in target mRNAs to primarily regulate their stability. Notably, >60% of iSyTE-identified high-priority lens genes (n=528) have AREs, further supporting the examination of Elavl1 in lens development.

Methods: Elavl1 expression in mouse lens was validated by RT-PCR, Western blotting, immunofluorescence and public RNA-seq datasets. A mouse lens-specific conditional knockout (*Elavl1*^{cKO}) model was generated using the *Pax6GFPCre* line. *Elavl1*^{cKO} lenses were subjected to histological analysis to examine morphological changes and to high-throughput RNA-sequencing (RNA-seq) to examine the lens transcriptome alterations.

Results: Elavl1 is robustly expressed in the lens from early development stages and is detected in both epithelial and fiber cells. Starting from E16.5, lens defects were detected in *Elavl1cKO* mice, which later progressed to early-onset cataract. Histology confirmed the small lens size and abnormalities in fiber cell region in *Elavl1*^{cKO} mice. Temporal analysis by RNA-seq identified novel genes as well as those with known functions in the lens to be misexpressed. Interestingly, 67% of the misexpressed genes (some with key function in the lens) have AREs (e.g., *Caprin2, Cdh1, Pon2, Sptbn1 and Vit*), and thus may represent Elavl1 direct binding targets. Together, these data provide molecular insights into *Elavl1*^{cKO} lens pathology.

Conclusions: This report characterizes the function of the conserved RBP Elavl1 in mouse lens development. Further, these data demonstrate that Elavl1 is necessary from early stages for proper levels of specific mRNAs in lens development, alteration of which causes fiber cell defects and cataract.

Funding: R01EY021505

What makes a bigger lens in high myopia?

Xiangjia Zhu[1], Yu Du[1], Dan Li[1], Jie Xu[2], Qingfeng Wu[3], Wenwen He[1], Keke Zhang[1], Jie Zhu[4], Linying Guo[5], Ming Qi[6], Ailin Liu[7], Jiao Qi[1], Guangyu Wang[2], Jiaqi Meng[1], Zhenglin Yang[8], Kang Zhang[2], Yi Lu[1]

[1]Eye Institute, Eye & ENT Hospital, Fudan University, Shanghai, China
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[4]Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, China
[5]Department of Radiology, Eye & ENT Hospital, Fudan University, Shanghai, China
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Purpose: High myopia is a leading cause of blindness worldwide, Aberrant growth of lens in high myopia may be one of the important pathological changes that induce a series of perioperative problems of lens replacement surgery. Larger lens requires increased production and accumulation of structural proteins, as well as dysregulation of lens fiber alignment. Here, we intended to investigate the underlying mechanism of pathological lens growth in high myopia.

Methods: Morphological data of lens from highly myopic and emmetropic individuals were collected using MRI and scanning electron microscope, and lens epithelial tissue was characterized by gene expression profiling, RT-qPCR, Western blotting, immunostaining, ChIP-qPCR, and parallel reaction monitoring. Defocus-induced and interphotoreceptor retinoid-binding protein knockout highly myopic mouse models were used to validate the findings.

Results: An increased lens size in highly myopic eyes associated with up-regulation of β/γ-crystallin expressions is observed in both human participants and two independent highly myopic mouse models. Further mechanistic studies revealed an essential role for transcription factor MAF, in regulating β/γ-crystallin gene expressions by its direct binding to their promoters and by activation of downstream TGF-β1-Smad signaling. Additionally, an accelerated nuclear cataract in highly myopic eyes is associated with highly polarized lens fiber alignment, mediated by TGF-β1-induced methyltransferase METTL3 upregulation, increased m6A modifications of DVL2 read by IGF2BP3, and stabilization of DVL2 mRNA that boosts planar cell polarity (PCP) signaling pathway.

Conclusions: These data demonstrate morphological changes of lens as a characteristic feature of high myopia, and point to the dysregulation of a MAF-TGF-β1-crystallin axis as well as TGF-β1-METTL3-PCP signaling axis as a novel molecular mechanism underlying the pathological lens growth, which could provide a potential guide for future therapeutic interventions.

Monday, December 11th

Session 2: Lens Cell Biology and Matrix Biology

Organizer: *Cathy Cheng*
Co-Moderator: *Julie Lim*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker		Title
10:30 a.m.		**Cathy Cheng**	Changes in the F-actin network localization between cortical and nuclear lens fibers
10:48 a.m.		**Rosica Petrova**	Localization of AQP5 water channels to the broadside gap junction plaques that mediate the outflow of water from the nucleus of rodent lenses
11:06 a.m.		**Steve Bassnett**	The effect of fiber cell compaction on the power and optical quality of the mouse lens
11:24 a.m.		**Qian Wang**	Effect of Cap2 on the regulation of actin cytoskeleton and fiber cell organization in the lens
11:42 a.m.		**TJ Plageman**	Elucidating the function of the lens fiber cell tricellular junction adherens junctional protein δ-catenin

Changes in the F-actin network localization between cortical and nuclear lens fibers

Catherine Cheng[1] and Michael P. Vu[1]

[1]*School of Optometry and Vision Science Program, Indiana University, Bloomington, IN, USA*

Purpose: Specialized interlocking structures are required for normal biomechanical properties of the lens and have been extensively described using electron microscopy techniques. The 3D architecture of the lens and its interlocking fiber cells make it difficult to visualize and image. We developed a novel fiber cell immunostaining method that preserves fiber cell morphology and allows for protein localization with impressive resolution of these intricate cells.

Methods: Lens fiber cells, from 8-12-week-old-wild-type C57BL6/J background mice, were imaged after staining with wheat germ agglutinin (WGA, cell membrane) and phalloidin (F-actin) using super-resolution confocal microscopy. We characterized cortical differentiating and mature fiber cells and nuclear fiber cells from the center of the lens.

Results: We visualized the membranes of the lens fiber cells and were able to clearly discern the complex interdigitations between neighboring fiber cells. We compared the super-resolution confocal microscopy images we collected with scanning electron microscopy (SEM) images and confirmed that the WGA- and phalloidin-stained fiber cells match the physical features previously found in SEM experiments. We also found that F-actin was enriched along the membrane in cortical fibers. Surprisingly, F-actin forms a reticulated network in the cytoplasm of nuclear fibers and is no longer strongly present along the cell membrane.

Conclusions: Our data shows that the F-actin network is dramatically reorganized during fiber cell maturation and compaction at the center of the lens. We detail a new method to stain lens nuclear fiber cells and demonstrate that complex membrane interdigitations can be faithfully preserved and imaged with super-resolution confocal microscopy. This novel method will allow further elucidation of the mechanisms that drive changes in fiber cell interdigitations by combining morphological and protein localization information. Potentially, these protocols can be used to study fiber cells from other animal species.

Localization of AQP5 water channels to the broadside gap junction plaques that mediate the outflow of water from the nucleus of rodent lenses

Rosica S. Petrova[1], Sebastien Sachse-Nodder[1], Caterina Sellitto[3], Thomas White[3], Kevin L. Schey[2], Paul J. Donaldson[1]

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Purpose: To compare the distributions of the water channels AQP5 and AQP0 in the water outflow pathway of the lens and to determine whether AQP5 colocalizes with gap junction plaques in wild type (WT), Cx50 and Cx46 knock out (KO) and Cx46 knock in (KI) lenses.

Methods: Eyes from WT rats and mice or Cx50 KO, Cx46 KO and Cx4 6KI mice were fixed in situ. Equatorial sections were double immunolabel with antibodies against AQP5, AQP0, Cx50 and Cx46 proteins and the membrane marker wheat germ agglutinin (WGA). Sections were imaged using a Zeiss 800 microscope. The colocalization between the AQPs and Cxs was quantified using the ZEN Blue 3.4 software.

Results: WT sections double labelled with AQP0 and AQP5 revealed that in the outer cortex AQP5 was localized to large broadside plaques that coincided with WGA negative regions, while AQP0 was excluded although its expression closely surrounded the edges of WGA negative holes. Double labelling of AQP5 with Cx50 or Cx46 showed that AQP5 was strongly colocalized to Cx50 and Cx46 broadside plaques. However, in lenses from either Cx50 or Cx46 KO lenses the localization of AQP5 to the large broad side plaques was abolished and AQP5 was more dispersed in discrete puncta around the entire fiber cell membrane. In Cx46 KI lenses AQP5 did not colocalize with Cx46 plaques. In deeper regions of the lens both AQP5 and Cx50 and AQP5 and Cx46, but not AQP0, revealed a dispersal of the plaques which continued into the core.

Conclusions: The observed colocalization of AQP5 to large broadside gap junctions comprised of Cx50 and Cx46 suggests that AQP5 may contribute to the intercellular water flow that generates the hydrostatic pressure gradient that has been shown to be important for the maintenance of the regulation of the refractive properties of the lens.

Funding: NIH grant EY013462.

The effect of fiber cell compaction on the power and optical quality of the mouse lens

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Purpose: Fiber cells are added continuously to the lens surface with important implications for the growth of the lens and its internal optical properties. We examined the role of cell compaction in constraining radial lens growth and establishing the internal gradient of refractive index (GRIN).

Methods: The cross-sectional profiles of fiber cells were visualized using block face imaging and optical properties were quantified by ray tracing. We evaluated the compressive forces generated by lens growth using finite element (FE) modeling and capsular strain was measured by laser bleaching of lectin-stained lenses.

Results: The cross-sectional area of cells at all radial locations declined with time. Compaction was most evident in the center of the lens and, at later stages (3-12 months), at the border of the organelle-free zone (OFZ). At some locations, the degree of cell compaction was consistent with the calculated protein concentration, assuming that volume was lost solely in the form of cytoplasmic water. Elsewhere (e.g., the perinuclear region) cell compaction was insufficient to account for the local protein concentration. Incorporation of compaction data brought the predictions of lens growth models into agreement with empirical observations. Continuous addition of cells is expected to place the capsule under tension, and bleaching measurements indicated capsular strain values of 10-15%. FE analysis suggested that compressive forces generated by continuous addition of cells into a capsule-constrained volume could lead to compaction of cells at the OFZ boundary, provided the cells soften during the organelle loss process.

Conclusions: Fiber cell compaction helps explain how i) the aging lens fits comfortably within the eye and ii) the concentration of cytoplasmic protein increases in post-synthetic cells. Although volume loss likely contributes to GRIN formation, it is insufficient to account for the high index values observed in some regions of the lens.

Grb2 functions in synergy with Frs2 and Shp2 in FGF-induced lens development

Qian Wang[1], Xin Zhang[1]

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Purpose: Fibroblast Growth Factor (FGF) signaling plays a pivotal role in early lens development. The propagation of FGF signaling is achieved through phosphorylation of adaptor protein Frs2, which subsequently recruits Grb2 and Shp2 to activate mitogen-activated protein kinases (MAPK) pathway. Notably, Frs2 mutants that lack the Grb2 binding site only exhibit a mild effect on MAPK signaling, suggesting the involvement of Shp2 in Grb2 recruitment and subsequent function. Therefore, this study focused on how Shp2 regulates Grb2 function in FGF-induced lens development.

Methods: Conditional knockout mouse models were generated using lens-specific cre Le-cre. Germline mutations were generated by homologous recombination. Embryos were harvested and sectioned for immunostaining.

Results: We first examined the role of Grb2 in lens development. We found that genetic ablation of Grb2 results in the loss of MAPK signaling and a complete arrest of lens vesicle development. This deficiency manifests as reduced cell proliferation and increased apoptosis in the lens epithelium, along with severe disruption of lens fiber differentiation. Therefore, Grb2 is essential for FGF signaling and lens development.

Next, we investigated how Shp2 regulates Grb2 function in the lens. Interestingly, point mutations in Shp2 that disrupt the Grb2 binding has no impact on MAPK activity, suggesting that Shp2-Grb2 binding is not essential to activate MAPK signaling. Similarly, altering Grb2 phosphorylation status, which is controlled by Shp2 phosphatase activity, did not affect MAPK signaling either, suggesting that dephosphorylation of Grb2 by Shp2 is not crucial for MAPK activation. Nevertheless, genetic interaction experiments reveal a synergistic effect among Grb2, Frs2 and Shp2 in lens development.

Conclusions: Grb2 is indispensable in FGF-induced lens development. While the direct Grb2-Shp2 interaction is dispensable for MAPK signaling, a synergistic collaboration among the Grb2, Frs2 and Shp2 are essential for FGF induced lens development.

Elucidating the function of the lens fiber cell tricellular junction adherens junctional protein δ-catenin

Timothy F. Plageman Jr.[1], Angad Dhillon[1], Kenneth Herman[1], Electra Coffman[1], and Jessica Martin[1]

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Purpose: Tricellular adherens junctions (tAJs) are an understudied structure of epithelial cells and little is known of their resident proteins in the lens or in any vertebrate tissue. Strikingly, lens fiber cells form numerous, extremely long and regularly spaced tAJs that are thought to facilitate lens function. Thus, identifying the components of tAJs in the lens can lead to a better understanding of both the lens and tAJ function. We have recently determined that □-catenin, the product of the cortical cataract associated gene Ctnnd2, is enriched within the lens fiber cell tricellular junction.

Methods: Lenses from Ctnnd2 mutant animals, acquired from a repository, were histologically analyzed with immunofluorescently (IF) labeled antibodies. A combination of high-resolution confocal microscopy and deconvolution methods were used to image the labeled tissue. Fiber cell lysates were also isolated and subjected to immunoprecipitation utilizing a □-catenin antibody, and analyzed using LC/mass spectroscopy.

Results: δ-catenin, and another tAJ enriched protein ZO-1, localizes specifically to the tricellular junction of lens fibers at the onset of lens fiber cell differentiation at E12.5, throughout the developing and adult lens. While an embryonic phenotype is not observed, lenses lacking functional δ-catenin have disorganized adult lens fiber cells, reductions in cortical transparency, and are mechanically stiffer. Proteomic analysis also indicates a number of proteins fail to associate with ZO-1 in the absence of δ-catenin. Furthermore, loss of δ-catenin also impacts the enrichment of both ZO-1 and afadin to the tricellular junctional complex.

Conclusions: The δ-catenin-associated protein complex is important to the function of the lens by maintaining the molecular complexes supporting cell adhesion at the tricellular junction permitting the maintenance of transparency.

Monday, December 11th

Session 3: Omics Approaches in Lens Research

Organizer: *Marc Kantorow*
Co-Moderator: *Ales Cvekl*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker		Title
1:15 p.m.		**Salil A. Lachke**	Applying iSyTE to uncover regulatory networks in lens development and its associated defects
1: 33 p.m.		**Ales Cvekl**	Hi-C analysis of long-range chromatin contacts and looping between mouse ES cells, lens epithelium and lens fibers
1:51 p.m.		**Shiwali Goyal**	Patterns of Crystallin Gene Expression in Differentiation State Specific Regions of the Embryonic Chicken and Mouse Lens
2:09 p.m.		**Marc Kantorow**	Regulation of lens fiber cell differentiation by hypoxia-regulated epigenetic programming
2:27 p.m.		**Xue-Bin Hu**	MYPT1 Directly Dephosphorylates HMGA1 and Other Targets to Regulate Lens Development

Spatiotemporal transcriptome meta-analysis to uncover regulatory relationships in isolated lens epithelium and fibers from embryonic, juvenile, adult and aged mice

Salil A. Lachke[1,2], Matthieu Duot[1,3], Sanjaya Shrestha[1], Murali Nagulla[1], Hongzhan Huang[2], Luc Paillard[3]

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Purpose: The lens transcriptome has been examined by microarrays and RNA-sequencing (RNA-seq). These omics-based datasets on various lens stages have contributed toward building of the bioinformatics web-resource iSyTE, which has facilitated identification of new genes linked to lens development and cataract pathology. However, presently iSyTE is based primarily on whole lens transcriptome data and thus does not inform on epithelium or fiber-specific gene expression. To address this knowledge-gap, we meta-analyzed transcriptome data from isolated lens epithelium and fibers and integrated this rich information in iSyTE.

Methods: We identified publicly available transcriptome datasets on isolated lens epithelium and fibers at stages representing mouse embryonic day (E) 12.5, E14.5, E16.5, E18.5, postnatal (P) day 0.5, P0, P13, age 1 month, 3 months, 6 months, and 2 years. These were generated on different microarray platforms or by RNA-seq, and were analyzed by appropriate methods.

Results: Epithelium and fiber expression data exhibit stage-specific clustering (embryonic, postnatal, aged) suggesting that meta-analysis worked effectively on these diverse datasets. Across these stages, ~2000 genes were differentially expressed between epithelium and fibers, of which ~4% are linked to cataract in CatMap. Gene ontology identifies “morphogenesis of epithelium” in epithelium-enriched genes and “lens fiber cell differentiation” in fiber-enriched genes. Interestingly, E12.5 epithelium exhibits enrichment of ribonucleoprotein regulatory processes. Whole embryonic-body (WB)-in silico subtraction identifies high-priority epithelium- and fiber-enriched genes. Together these analyses predict key regulatory relationships in epithelium and fibers. Finally, epithelium and fiber expression data is made publicly accessible at iSyTE (https://research.bioinformatics.udel.edu/iSyTE/).

Conclusions: This meta-analysis advances our knowledge on the dynamic transcriptome changes in mouse isolated lens epithelium and fiber cells at stages spanning embryonic and postnatal development, adulthood, and aged tissue. Additionally, this is the first report providing detailed analysis of lens epithelium and fibers at E12.5. Together, these new expansions to iSyTE are expected to further facilitate cataract gene discovery.

Hi-C analysis of long-range chromatin contacts and looping between mouse ES cells, lens epithelium and lens fibers

Ales Cvekl[1], Michael Camerino[1], and William K. Chang[1]

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Purpose: Nuclear organization of interphase chromosomes involves individual chromosome territories, “open” and “closed” chromatin compartments (A/B), topologically associated domains (TADs), and chromatin loops. Cellular differentiation is driven by temporally and spatially coordinated gene expression that requires changes in both global and local subnuclear organization. The unique feature of lens fiber cells is very high level of transcriptional output while the nuclei are undergoing the process of chromatin condensation in preparation for their ultimate denucleation. It is not known how different chromatin compartments regulate differential gene expression between lens epithelium and lens fibers. Three-dimensional (3D) chromatin organization maps of the lens are thus required for understanding of tissue-specific gene regulatory mechanisms at the level of TADs including a full repertoire of chromatin loops.

Methods: Hi-C analyses included mouse newborn (P0.5) microdissected lens epithelium, lens fibers and ES cells. Contact map computation, TAD and loop calling were conducted using open-source Juicer tools. Chromatin landscape of CTCF was determined also in microdissected P0.5 lens chromatins by ChIP-seq. Our earlier RNA-seq, ATAC-seq, ChIP-seq, and WGBS datasets are also included in final models. Expression of CTCF was analyzed during lens embryogenesis by immunofluorescence.

Results: Chromatin organization between mouse ES cells, microdissected newborn lens epithelium and fiber cells were obtained at the 5-10 kb resolution. Quantitative analyses show major differences between number and size of TADs and chromatin loop size between these three cell types. In depth analyses show similarities between lens samples exemplified by overlaps between compartments A and B. Lens epithelium-specific CTCF binding is found in mostly DNA methylated regions while lens fiber-specific and shared bindings occur mostly within unmethylated genomic DNA regions. Major differences in TADs, loops and CTCF binding are illustrated at the ~500 kb Pax6 locus, encoding the critical lens regulatory transcription factor. Additional interesting loci includes clusters of crystallin genes, Sox1, Sox2, and Hif1a loci, also encoding lens regulatory transcription factors.

Conclusions: Our study has generated the first data on nuclear organization and looping in lens epithelium and lens fibers and directly compared these data with ES cells. These findings generate novel mechanistic insights into lens-specific transcriptional gene control and enable studies of non-coding genetic variants linked to cataract and other ocular abnormalities.

Patterns of Crystallin Gene Expression in Differentiation State Specific Regions of the Embryonic Chicken and Mouse Lens

Shiwali Goyal[1], Zhiwei Ma[1], Daniel Chauss[2], Joshua Disatham[2], Xiaodong Jiao[1], Lisa Ann Brennan[2], A. Sue Menko[3], Marc Kantorow[2], and J. Fielding Hejtmancik[1]

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Purpose: Transition from lens epithelial cells to lens fiber cells is accompanied by numerous changes in gene expression critical for lens transparency. We identify expression patterns of highly prevalent genes, especially crystallins, across lens compartments in embryonic day 13 (E13) chicken lenses and E17 and P2 mouse lenses.

Methods: Lenses from E13 chicks were dissected into central epithelial (EC), equatorial epithelial (EQ), cortical fiber (FP), and nuclear fiber (FC) cell compartments and from E17 and P2 C57BL/6J and FvBN mice into epithelia and fibers. Total RNA was prepared, subjected to RNAseq, and grouped by expression patterns.

Results: A total of 77,097 gene-specific transcripts covering 17,450 genes were expressed in embryonic chicks, of which 10,345 differed between two or more lens subregions. Ubiquitous crystallins increased from EC to EQ and were similar in FP and FC. Highly expressed crystallin genes fell into three coordinately expressed groups with $R2 \geq 0.93$: CRYAA, CRYBB2, CRYAB, and CRYBA2; CRYBB1, CRYBA4, CRYGN, ASL1, and ASL; and CRYBB3 and CRYBA1. Highly expressed transcription factors YBX1, YBX3, PNRC1, and BASP1 were coordinately expressed with the second group of crystallins ($R2 > 0.88$). Comparison of the mouse strains revealed higher expression of crystallins, MIP, BFSP1, and BFSP2 in fibers compared to epithelia, similar to patterns in the chick. On comparing C57BL/6J and FvBN strains, the expression of Bfsp2 is significantly higher and Crybb2 is lower in C57BL/6J compared to FvBN.

Conclusions: These data identify three distinct patterns of expression for subsets of crystallin genes, each highly correlated with the expression of specific transcription factors in the chick and mouse. This provides a basis for designing functional experiments pinpointing the mechanisms governing crystallin expression during fiber cell differentiation. Strain-specific differential expression of lens genes in C57BL/6J and FvBN mice provides a tool to identify genetic mechanisms controlling their expression.

Regulation of lens fiber cell differentiation by hypoxia-regulated epigenetic programming

Marc Kantorow[1], Joshua Disatham[1], Lisa Brennan[1]

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Purpose: Our previous studies showed that the hypoxic microenvironment of nascent lens fiber cells drives the expression of genes required for the form and function of mature lens fiber cells through activation of the master regulator of the hypoxic response, transcription factor HIF1a. HIF1a regulates multiple components of the lens fiber cell differentiation program including elimination of non-nuclear organelles. Transcription factor function is dependent on chromatin accessibility and specific epigenetic modifications including histone modifications. Consistently, our previous studies have identified a link between genome-wide chromatin accessibility changes, DNA methylation patterns and HIF1a binding during lens fiber cell differentiation. Here, we tested the hypothesis that lens fiber cell hypoxia regulates genomewide histone modifications to control HIF1a function and potentially that of other transcription factors important for hypoxia-dependent lens fiber cell differentiation.

Methods: Using ex-vivo embryonic chick lens (E13) cultures we examined global histone modifications following exposure of lenses to hypoxia (1% O2). CUT & RUN was employed to map the genomic landscapes of key histone modifications associated with gene activation (H3K4me3, H3K27ac) under hypoxic conditions. Multiomic integration of the CUT n RUN data with corresponding RNA Seq data was conducted to establish the relationship between hypoxia regulation of histone modifications examined and the expression of lens fiber cell-specific genes.

Results: Western blot analysis of histones extracted from lens fibers cells demonstrated a rapid and robust increase in multiple histone modifications that regulate chromatin accessibility and gene activation including H3K4me3, H3K27ac, H3K14ac and H3K9ac. CUT and RUN analysis of lens fiber cells following exposure to hypoxia identified increased H3K4 methylation and increased H3K27 acetylation in the promotor regions of a number of critical lens fiber genes including BFSP1, BFSP2, BNIP3L, BIRC7 and EPHA2. These changes correlated with chromatin de-condensation previously established by ATAC sequencing.

Conclusions: The results provide evidence that epigenetic programming through histone modifications drives lens fiber cell gene expression to achieve mature lens structure and transparency and that the lens epigenetic landscape is dependent on the hypoxic microenvironment of nascent lens fiber cells.

MYPT1 Directly Dephosphorylates HMGA1 and Other Targets to Regulate Lens Development

Xue-Bin Hu[1], David Wan-Cheng Li[1]

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Purpose: MYPT1 is an important regulator of the protein serine/threonine phosphatase-1. We have recently demonstrated that MYPT1 acts as a specific phosphatase to dephosphorylate the methytransferase EZH2 and thus modulate the epithelial mesenchymal transition (EMT) of lens epithelial cells (LECs) in human and mouse [Zhang et al., 2022. Advanced Science]. Here we explored MYPT1 function in regulating lens development.

Methods: CRISPRA/Cas9 technology was used to silence MYPT1 gene in zebrafish, RNAseq was used to analyze transcriptomes in both wild type and mypt1(-/-) zebrafish, phosphor-proteomics was used to identify MYPT1 targets, QRT-PCR and Western blots analysis were used to analyze gene expression, transmission electron microscopy was used to analyze the lens structure.

Results: Knockout of MYPT1 gene retards lens differentiation and also causes microphthalmia in zebrafish. The expression patterns of eye development related genes including major transcription factors were clearly altered in mypt1(-/-) zebrafish, and the downstream differentiating genes including the 3 families of crystallin genes were significantly suppressed in the absence of MYPT1. Moreover, silence of MYPT1 induces significant upregulation of the phosphorylation of the HMGA1 protein. MYPT1 directly interacts with HMGA1 to mediate its dephosphorylation. Silence of HMGA1 gene also leads to abnormality in lens differentiation which is also associated with altered expression patterns in some of the related genes found in *mypt1*$^{-/-}$ zebrafish.

Conclusions: Our results reveal that MYPT1 regulates lens differentiation and pathogenesis through HMGA1 and other targets in the ocular lens.

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Monday, December 11th

Session 4: Function and Homeostasis of Crystallins

Organizer: *Krishna Sharma*
Co-Moderator: *John Clark*

Talks are in the format of 15 minutes with 3 minutes discussion.

Time	Speaker	Title
3:00 p.m.	**John Clark**	The Significance of Crystallins in the Optical Function of the Lens
3:18 p.m.	**Sayan Ghosh**	Exploring the multifaceted roles of βA3/A1-crystallin: from lens structural protein to non-lens functions in the retina
3:36 p.m.	**Hassane Mchaourab**	Transcriptional coupling between Nrf2 and αB-crystallin in the lens and heart of zebrafish under proteostatic stress
3:54 p.m.	**Laxman Mainali**	Association of αA-, αB-, and α-Crystallin with the Model of Human Lens-Lipid Membranes is Modulated by Cholesterol Content in the Membranes
4:12 p.m.	**Krishna Sharma**	Lens Proteostasis Collapse and Cataract: Can it be rescued by improving crystallin chaperone efficiency?

The Significance of Crystallins in the Optical Function of the Lens

John I. Clark[1]

[1]*Departments of Biological Structure and Ophthalmology, University of Washington, Seattle, WA, USA*

Purpose: The unique characteristics of the biological lens are symmetry, refraction and transparency. As epithelial cells elongate, migrate and detach from the posterior capsule, the short-range order of highly concentrated cytoplasmic proteins, largely crystallins and cytoskeleton, limit light scattering and improve image formation. Symmetry and transparency are established to optimize lens optical function.

Methods: Slit lamp analysis can be correlated with electron microscopy to evaluate the progressive changes in differentiating fiber cells during lens development.

Results: Growth shells of elongated cells produce a symmetric lens as the index of refraction, “n”, is increased by the increased expression of crystallin proteins. As lens cells are enriched in cytoskeletal proteins, a scaffold establishes short-range, transparent order and supports symmetric elongation and migration of lens cells in the formation of growth shells. Crystallins enhance solubility, stability, and protective mechanisms against cell aging, necessary for the lens to function as an optical element during the lifetime of an individual. The degeneration of cytoplasmic organelles and the regression of vasculature maximize transmittance for image formation. In the absence of organelles and vasculature, lens fiber metabolism is limited to anaerobic glycolysis creating a protective environment against oxidation, and a microcirculation that accounts for the continuous and symmetric flow of fluid, containing nutrients and ions that maintain the lens. Crystallins are essential for regulation of the extraordinary functional longevity of the optics of the eye.

Conclusions: The regulation of lens cell differentiation crystallins have selective, heterogenous interactions, often enhanced by post-translational modification, with constituents of the cytoplasm and cell membranes.

Exploring the multifaceted roles of βA3/A1-crystallin: from lens structural protein to non-lens functions in the retina

Sayan Ghosh[1], Stacey Hose[1], Sridhar Bammidi[1], Victoria Koontz[1], Mihir Nemani[1], J. Samuel Zigler., Jr.[2], Debasish Sinha[1,2]

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Purpose: βA3/A1-crystallin, a vital lens structural protein, originates from the Cryba1 gene, generating two isoforms (βA3 and βA1) through leaky ribosomal scanning. Recent research has uncovered its presence and importance in various cell types, notably astrocytes and retinal pigment epithelial (RPE) cells within the retina. βA3/A1-crystallin serves as a lysosomal luminal protein in RPE and astrocytes, playing a significant role in regulating lysosomal function and mechanistic target of rapamycin (mTOR) signaling. Our purpose here is to determine if the βA3- and βA1-crystallin isoforms have distinct functions.

Methods: We developed genetically engineered mouse models with Cryba1 gene knockout (globally, KO or RPE-specific, cKO), global βA3-crystallin knockout (KO), and βA1-crystallin knockdown (KD) to investigate the role of these crystallin isoforms. We used RNAseq, metabolomics, and molecular/cell biology techniques to identify key signaling pathways linked to their functions.

Results: Both βA3- and βA1-crystallin regulate mTOR complex 1 (mTORC1) signaling, and subsequent glucose metabolism, in astrocytes and lipid biosynthesis pathway in RPE cells. βA3-crystallin deficiency (βA3 KO mice) leads to compensatory upregulation of βA1-crystallin in both cell types. In astrocytes, βA1-crystallin can translocate to the nucleus, while this phenomenon does not occur in RPE cells. Loss of βA3-crystallin mitigates high glucose-induced mitochondrial abnormalities, inflammation, and oxidative stress in astrocytes, and reduces activation of p-LIPIN/FASN and the PKC/NFκB-dependent inflammatory pathways in RPE cells. Conversely, loss of βA1-crystallin exacerbates these changes, highlighting its critical role in both astrocyte and RPE function.

Conclusions: The leaky ribosomal scanning responsible for producing βA3/A1-crystallin isoforms precedes Cryba1 recruitment by the lens. While these isoforms clearly have distinct functions in RPE and astrocytes (regulating mTORC1 signaling), their relevance in the lens remains uncertain. As structural components in the lens, these isoforms may have interchangeable roles and understanding their functionality irrespective of the cell type could have potential significance for ocular health.

Transcriptional coupling between Nrf2 and αB-crystallin in the lens and heart of zebrafish under proteostatic stress

Jinhee Park[1], Samantha MacGavin[1], Laurie Niederbrach[1], Hassane S. Mchaourab[1]

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Purpose: A coordinated oxidative stress response, partly triggered by the transcription factor Nrf2, protects cells from the continual production of reactive oxygen species. Left unbuffered, reactive oxygen species can lead to protein aggregation which has been implicated in a spectrum of diseases including cataract of the ocular lens and myopathy of the heart. While proteostasis is maintained by diverse families of heat shock proteins, the interplay between the oxidative and proteostatic stress responses in the lens and the heart has not been investigated.

Methods: Multiple zebrafish lines were generated to investigate the effects of Nrf2 compromised function on protein homeostasis in the lens. We screened the progeny of these lines to identify lens defects as well as changes in heart morphology. In parallel we carried out proteomics and transcriptomics to identify changes in protein levels and perturbation of signaling pathways.

Results: Capitalizing on multiple zebrafish lines that have compromised function of Nrf2 and/or the two paralogs of the small heat-shock protein αB-crystallin, we uncovered a transcriptional coupling that leads to a substantial increase in αB-crystallin transcripts in the heart in response to compromised function of Nrf2. In the lens, the concomitant loss of function of Nrf2 and αBa-crystallin leads to upregulation of the cholesterol biosynthesis pathway thus mitigating the phenotypic consequences of the αBa-crystallin knockout. In contrast, abrogation of Nrf2 function accentuates the penetrance of the heart edema phenotype characteristic of embryos of αB-crystallin knockout lines. Multiple molecular pathways, some implicated in cardiomyopathy, are revealed from transcriptome profiling thus identifying novel targets for further investigation.

Conclusions: Together our transcriptome/phenotypic analysis establishes an intersection between the oxidative stress and chaperone responses in the lens and the heart.

Association of αA-, αB-, and α-Crystallin with the Model of Human Lens-Lipid Membranes is Modulated by Cholesterol Content in the Membranes

Laxman Mainali[1,2], Raju Timsina[1], Geraline Trossi-Torres[2], Preston Hazen[2], Nawal K. Khadka[1], Navdeep Kalkat[2]

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Purpose: α-Crystallin (αABc) is a major protein comprising of two types of subunits, αA-crystallin (αAc) and αB-crystallin (αBc), in roughly a 3:1 ratio of αAc to αBc and works as a molecular chaperone. With age and cataract formation, the concentration of αABc in the eye lens cytoplasm decreases with a corresponding increase in the membrane-bound αABc, accompanied by increased light scattering and cataract formation. This study investigates the role of cholesterol (Chol) and Chol bilayer domains (CBDs) in the binding of αAc, αBc, and αABc to the Chol/model of human lens-lipid (MHLL) membranes.

Methods: The electron paramagnetic resonance spin-labeling method was used to estimate the membrane surface occupied by αAc, αBc, and αABc, binding affinity (Ka) of αAc, αBc, and αABc to Chol/MHLL membranes, and the mobility, order, and hydrophobicity near the headgroup regions of the membranes with αAc, αBc, and αABc binding.

Results: The maximum percentage of membrane surface occupied (MMSO) by αAc, αBc, and αABc and Ka of αAc, αBc, and αABc to Chol/MHLL membranes at a mixing ratio of 0 followed the trends: MMSO (αAc) > MMSO (αBc) ≈ MMSO (αABc) and Ka (αBc) ≈ Ka (αABc) > Ka (αAc) in which MMSO and Ka are close to zero at mixing ratio of 1.5. The binding of αAc, αBc, and αABc to Chol/MHLL membranes modulates mobility and hydrophobicity near the headgroup regions of the membranes.

Conclusions: Our results show that αAc, αBc, and αABc binds differently with Chol/MHLL membranes at mixing ratio 0 and 0.5, decreasing the mobility and increasing hydrophobicity near the headgroup regions of the membrane. No binding of αAc, αBc, and αABc to Chol/MHLL membranes at a mixing ratio of 1.5 indicates that high Chol and CBDs inhibit the binding of αAc, αBc, and αABc to membranes and likely protect against cataract formation.

Lens Proteostasis Collapse and Cataract: Can it be rescued by improving crystallin chaperone efficiency?

Krishna Sharma[1,2], Santhoshkumar Puttur[1]

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Purpose: Normal proteostasis is necessary to keep lens transparency. Dysregulation of proteostasis due to mutations, modifications or reduced clearance of damaged proteins is associated with protein misfolding/unfolding and aggregation as well as in diseases such as cataract, Parkinson, Alzheimer's etc. Cellular chaperones are important components of proteostasis. Therefore, mutations and age-related modifications of α-crystallin affecting the chaperone activity will contribute, at least in part, to the collapse of lens proteostasis. We investigated whether diminished α-crystallin chaperone activity can be recovered or enhanced so that proteostasis can be maintained.

Methods: The crystallins with age-related modification obtained from bovine and human lenses were treated with a reducing agent and assayed for chaperone activity. Cataract causing αAG98R was modified with 50 μM methylglyoxal (MGO) to stabilize the protein and the chaperone activity was evaluated. αB- and αBΔ54-61-crystallin (showing increased chaperone activity) were tested in cell culture systems and C-elegans.

Results: Crystallins from human and bovine lenses showed increased chaperone activity following treatment with reducing agents. MGO treatment stabilized αAG98R and enhanced its chaperone activity. WT and αBΔ54-61-crystallin treatment resulted in 10 to 40 % increase in the life span of C. elegans compared to those untreated. Further, αBΔ54-61-crystallin with increased chaperone activity showed greater lifespan extension than in WT-crystallin treated C. elegans and better protection of ARPE-19 cells under oxidative stress.

Conclusions: In C. elegans, it is shown that increased lifespan is dependent on longer sustenance of proteostasis. Therefore, the results from our studies show that crystallins and crystallin (αBΔ54-61) with increased chaperone activity have a direct impact on proteostasis at cellular and organismal levels. The recovery of chaperone activity in lens crystallin after treatment with reducing agent and stabilization of chaperone activity in a cataract causing αAG98R mutant with MGO treatment suggests that it might be possible to prevent or delay the proteostasis collapse resulting in lens opacity.

Support: R01EY02319

Monday, December 11th

Poster Session

Time	Speaker	Title
4:30 p.m.	**Zhaohua Yu**	Ultrastructural nuclear 3Dmorphometry of lens epithelium in the central zone
	Electra Coffman	Arvcf Stabilizes N-Cadherin and Cytoskeletal Proteins in the Apical Junctional Complex of the Lens Fiber Cell
	Koichiro Mukai	Induced changes in calcification for hydrophilic materials
	Kohei Miyata	Investigation of pupillary response in Synergy® implanted eyes
	Reona Mouri	Investigation of pupillary response in Lentis Comfort® implanted eyes
	Natsuko Maeda	ACOMOREF2® in intraocular lenses eyes
	Tomoko Shiroyama	Backgrounds and post cataract surgery outcomes in patients over ninety years old
	Daisuke Sasaki	The site-specific impact of modifications of aspartyl residues on lens αB-crystallin

Poster 1

Ultrastructural nuclear 3D morphometry of lens epithelium in the central zone

Zhaohua Yu, Jan Bergmanson, Alan Burns, Per Söderberg

[1]Department of Surgical Sciences/Ophthalmology, Uppsala University, Uppsala, Sweden
[2]College of Optometry, University of Houston, Houston, Texas, USA

Purpose: Recent development of serial block-face scanning electron microscopy (SBF-SEM) provides a new possibility to study 3D cell response at subcellular resolution. The current study aimed to establish the 3D nuclear morphometry of lens epithelial cells in the central zone.

Methods: The lenses from Six-week-old female albino Sprague-Dawley rats were extracted. Central zone volumes of epithelium were isolated and fixed (2.5% Glutaraldehyde). The fixed tissue was trimmed into small blocks and embedded in resin for serial sectioning and imaging with SBF-SEM resulting in a stack of images. Section thickness was 200 nm. The field of view was set to 45 µm resulting in a pixel resolution of 14.6 nm. In each image, the epithelial nuclei were segmented. Considering the section thickness, the 3D volume of the nuclei were reconstructed. Finally, morphometric parameters of the nucleus were determined.

Results: A 95 % confidence interval (CI) for the mean nuclear volume of epithelial cells in the central zone was shown to be 223±15 µm3. After fitting each nucleus to an ellipsoid, the semi-major axis, semi intermediate axis and semi-minor axis, respectively, were determined at 95 % CI to be 6.7±0.9 µm, 4.7±0.4 µm and 1.7±0.1 µm. The semi-major and semi intermediate axes were approximately oriented parallel to the capsule, with the semi-minor axis aligned to the sagittal axis. Finger-shaped extrusions and intrusions were observed along the nuclear membrane.

Conclusions: SBF-SEM reveals 3D nuclear morphological features of lens epithelial cells. In the central zone, nuclei are tri-axial ellipsoids, elongated along the lens capsule, with volumes of a similar magnitude. Nuclear membrane extrusions and intrusions observed may indicate exo- and endonucleotic exchanges between the cytoplasm and the nucleus.

Poster 2

Arvcf Stabilizes N-Cadherin and Cytoskeletal Proteins in the Apical Junctional Complex of the Lens Fiber Cell

Electra Coffman[1], Jessica Martin[1], Timothy F. Plageman Jr.[1]

[1]College of Optometry, Ohio State University, Columbus, OH, USA

Purpose: The stability of the cytoskeletal elements such as actin and intermediate filaments are likely playing a critical role in preventing microarchitectural breakdown and maintaining lens transparency. Our mouse model deficient in the p120-subfamily catenin gene, Arvcf, causes remarkable lens fiber disorganization and premature cortical cataracts after 6 months. Both proteomic screening and immunofluorescence of lens fibers reveal that N-Cadherin and its binding partners significantly decrease in expression in the Arvcf knockout mice. We show that the loss of lens fiber structure in our mouse model is likely caused by a destabilization of actin-associated proteins such as N-Cadherin and intermediate filament proteins in the apical junctional complex.

Methods: Arvcf-deficient mice were created by insertion of a lacZ/Neomycin cassette and are referred to as ArvcfKO. ArvcfKO lens fiber cell tissue lysate was used for co-immunoprecipitation for western blotting and liquid chromatography-mass spectrometry (LC-MS) while whole lens fiber cells were used for confocal fluorescent microscopy.

Results: Confocal immunofluorescence (IF), LC-MS, and validating western blots reveal that N-Cadherin and its binding partners β-catenin and α-N-Catenin are significantly downregulated in the absence of ARVCF. IF data indicate a shift in endocytic protein localization in lens fiber cells. LC-MS also shows that ARVCF interacts with the spectrin-ankyrin protein complex and intermediate filaments such as vimentin.

Conclusions: These results illustrate that ARVCF stabilizes N-cadherin and its binding partners by modulating endocytosis. ARVCF also associates with spectrins and lens-specific intermediate filaments to support lens fiber organization. Overall, ARVCF displays prominent levels of control over several cytoskeletal elements to maintain the apical junctional complex and thus cell adhesion in lens fiber cells.

Poster 3

Induced changes in calcification for hydrophilic materials

Koichiro Mukai[1], Hidetoshi Iwamoto[1], Hiroyuki Matsushima[1], Mayumi Nagata[1], Tadashi Senoo[1]

[1]Department of Ophthalmology, Dokkyo Medical University, Tochigi, Japan

Purpose: Calcification is known to be a postoperative complication of hydrophilic intraocular lens (IOL). We succeeded in inducing calcification by intramuscular implanting IOL white rabbits for a long term (6 months) previously, but it took a long time to obtain the results. In this study, we conducted in vitro induction of calcification focusing on contact lenses (CL), which made by hydrophilic material same as the IOL.

Method: Medalist® Plus (Bausch & Lomb: MC38%), True Eye® (Johnson & Johnson: MC46%) and 1-Day Acuvue® Moist (Johnson & Johnson: MC58%) of different moisture content (MC) were prepared. The CLs were immersed in simulated body fluid (SBF) and shaken at a constant temperature of 36.5℃. Each CL was removed from the SBF for 2 weeks, 1 and 2 months. After immersion, the CLs were washed and the calcium deposited on the CL is qualitative assayed using von Kossa stained.

Results: Two weeks after immersion, all CLs had no calcifications. After 1-month, slight calcification was observed in the MC58%CL. After 2 months, wide areas of calcifications were observed in both the MC46% and the MC58%CL. The degree of calcification was higher in the MC58%CL.

Conclusion: Calcification could be induced in a short-term using the hydrophilic material CL in vitro. The SBF immersion method is one of the useful methods to evaluate the degree of calcification in hydrophilic materials.

Poster 4

Investigation of pupillary response in Synergy® implanted eyes

Kohei Miyata[1], Reona Mouri[1], Norihiro Watanabe[1], Natsuko Shima[1], Shinichiro Kobayakawa

[1]Department of Ophthalmology Nippon Medical School Musashikosugi Hospital, Kanagawa, Japan

Purpose: To examine the correlation between postoperative visual acuity, contrast sensitivity, and the dynamic pupillary parameters in multifocal IOLs, Synergy® (Johnson and Johnson Vision, NJ, U.S.A) implanted eyes.

Method: Synergy® was implanted in 78 eyes of 53 patients at Nippon Medical School Musashi-kosugi Hospital between June 2021 and August 2023. Dynamic pupillary parameters were examined preoperatively and after one month postoperatively including maximum (initial, INIT), minimum (end, END) pupillary diameter (mm), percentage of pupillary constriction (DELTA, %), latency from a light stimulus to constriction (LAT, sec), average constriction velocity (ACV, mm/sec), average dilation velocity (ADV, mm/sec), maximum constriction velocity (MCV, mm/sec), and time at 75% recovery (T75, sec). Distance (D), intermediate (I), and near (N) visual acuity (VA), and contrast sensitivity were also examined after one month postoperatively.

Results: The patient age was 54.1±14.2 years old, with a median age of 57 years old. The INIT, DELTA, ACV, MCV, and ADV parameters decreased postoperatively than those preoperative data ($p<0.01$). There were negative correlations between the N-VA and DELTA, ACV, and MCV parameters ($p<0.01$). There was positive correlation between the contrast sensitivity under glare conditions at 1.6 degrees and the INIT or END parameters ($p<0.01$).

Conclusion: Several dynamic pupillary parameters decreased at one month after cataract surgery. In Synergy® implanted eyes, there were correlations between several parameters and N-VA or contrast sensitivity.

Poster 5

Investigation of pupillary response in Lentis Comfort® implanted eyes

Reona Mouri[1], Kohei Miyata[1], Norihiro Watanabe[1], Natsuko Maeda[1], Shinichiro Kobayakawa[1]

[1]Department of Ophthalmology Nippon Medical School Musashikosugi Hospital, Kanagawa, Japan

Purpose: To examine the correlation between postoperative visual acuity and the dynamic pupillary parameters in bifocal intraocular lens (IOL), Lentis Comfort® (Teleon Surgical B.V., Nederland) implanted eyes.

Method: Lentis Comfort® was implanted in 97 eyes of 58 patients at Nippon Medical School Musashi-kosugi Hospital between June 2020 and August 2023. Dynamic pupillary parameters were examined after one month postoperatively including maximum (initial, INIT) or minimum (end, END) pupillary diameter (mm), percentage of pupillary constriction (DELTA, %), latency from a light stimulus to constriction (LAT, sec), average constriction velocity (ACV, mm/sec), average dilation velocity (ADV, mm/sec), maximum constriction velocity (MCV, mm/sec), and time at 75% recovery (T75, sec). Distance (D), intermediate (I), and near (N) visual acuity (VA) were also examined after one month postoperatively.

Results: The patient age was 70.5±9.1 years old, with a median age of 72 years old. It exhibited significant correlations between the END, DELTA, or ACV parameters and the I-VA ($p<0.05$). Also, it exhibited a significant correlation between the patient age and the ADV parameter ($p<0.05$).

Conclusion: In Lentis Comfort® implanted eyes, there were correlations between several parameters and I-VA (intermediate visual acuity). Dynamic pupillary parameter in IOL implanted eyes are affected by aging.

Poster 6

ACOMOREF2® in intraocular lenses eyes

Natsuko Maeda[1], Reona Mouri[1], Norihiro Watanabe[1], Kohei Miyata[1], Shinichiro Kobayakawa[1]

[1]Department of Ophthalmology Nippon Medical School Musashikosugi Hospital, Kanagawa, Japan

Purpose: The ACOMOREF2® (Righton, Tokyo, Japan) is the world first multifunctional autorefractometer including accommodation analysis device. Accommodative micro fluctuation value is possible to analyze, also High Frequency Components (HFC, 1.3 to 2.3Hz) which is suggested the tension for ciliary muscle, is possible to extract from the data. We investigated the accommodation in intraocular lenses (IOLs) eyes using by ACOMOREF2®.

Method: Twenty-four volunteer eyes, 29 monofocal IOLs eyes (group M) and 25 multifocal IOLs eyes (group MF) were analyzed. The HFC in IOLs eyes were measured before (day 0), the day after (day 1), and 30 days after (day 30) surgery.

Results: The volunteer was 42.5 ± 9.0 years old, the accommodative ability was 1.26 ± 1.0 diopter, the HFC for far vision was 56.4 ± 5.2 Hz, and the HFC for near vision was 62.0 ± 7.4 Hz. The group M was 73.4 ± 10.1 years old, for group MF was 59.6 ± 12.6 years old ($p<0.05$). The average HFC for group M was 62.4 ± 9.3, 63.8 ± 8.9, and 60.7 ± 6.8 Hz on day 0, 1, and 30 respectively. The average HFC for group MF was 64.0 ± 9.7, 65.7 ± 10.1, and 61.1 ± 7.7 Hz on day 0, 1, and 30 respectively. The average HFC for group M were decreased between on day1 to day30 ($p<0.05$). The average HFC for group MF were also decreased between on day0 to day30 and on day1 to day30 ($p<0.05$). The average HFC were no differences between the group M and MF. The HFC for near vision in group MF had a tendency to increase.

Conclusion: The average HFC were no differences depending on the IOLs, decreased after surgery at both IOLs. The HFC for near vision after surgery may have differences by IOL types.

Poster 7

Backgrounds and post cataract surgery outcomes in patients over ninety years old

Tomoko Shiroyama[1], Hiroyuki Matsushima[1], Mayumi Nagata[1], Haruka Matsumoto[1], Tadashi Senoo[1]

[1]Department of Ophthalmology, Dokkyo Medical University Hospital, Tochigi, Japan

Purpose: The aging population is increasing worldwide. Japan has entered into an era of super-aging society, which lead to a rise in the total number of cataract surgeries. We conducted a study to examine the backgrounds and post-cataract surgery outcomes of patients over 90 years old at Dokkyo Medical University Hospital, Japan.

Method: We categorized patients who underwent phacoemulsification and intraocular lens implantation from May 2018 to September 2022 at our institution into five groups according to their age: Group 1 (under 60 years old, 50 eyes), Group 2 (60s, 51 eyes), Group 3 (70s, 50 eyes), Group 4 (80s, 50 eyes), and Group 5 (over 90 years old, 50 eyes). We retrospectively assessed preoperative and postoperative visual acuity, intraocular pressure, corneal endothelial cell loss rate, nuclear hardness (Emery-Little classification), preoperative risk factors, and numbers of medical histories.

Results: Group 5 showed statistically low preoperative and postoperative visual acuity compared to all other groups ($p<0.01$). There were no differences in preoperative and postoperative intraocular pressure. Corneal endothelial cell loss rate was statistically significant in Group 5 compared to Group 1, 2, and 3 ($p<0.01$). In terms of patient backgrounds, Group 5 showed statistically higher rate ($p<0.01$) of nuclear hardness (Emery-Little classification grade 3 or above), and preoperative risk factors such as pseudoexofoliation, dementia, and poor mydriasis (30%, 24%, 14% respectively). There were no differences in the number of medical histories.

Conclusion: Patients aged 90 and above showed low visual acuity both preoperative and postoperatively, combined with preoperative risk factors making cataract surgery more challenging. Since the number of cataract surgery of elderly patients is expected to increase, surgeons need to be cautious about intraoperative risks.

Poster 8

The site-specific impact of modifications of aspartyl residues on lens αB-crystallin

Daisuke Sasaki[1], Takumi Takata[2]

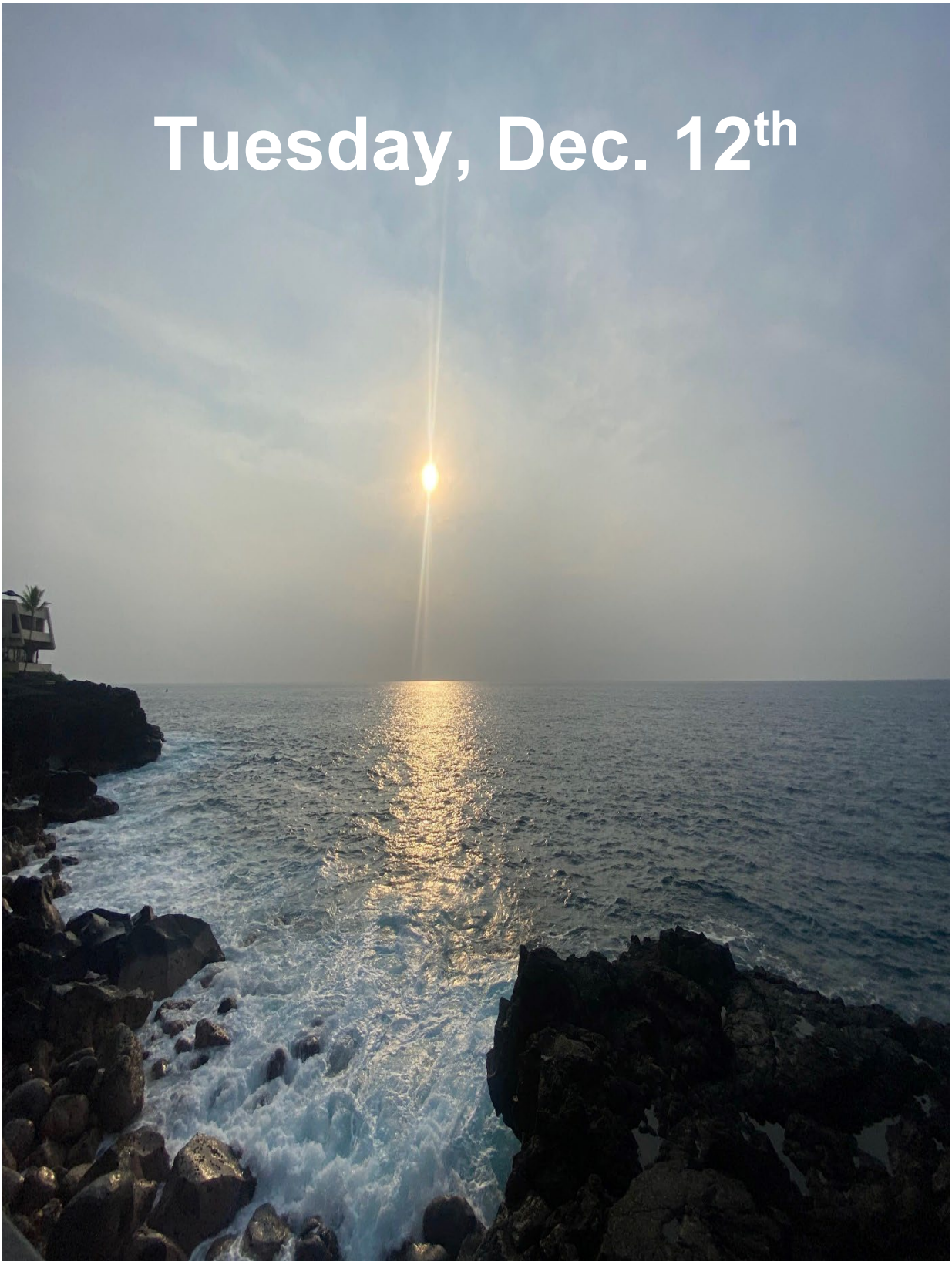
[1]*Department of Chemistry, Graduate School of Science, Kyoto University, Kyoto, Japan*
[2]*Institute for Integrated Radiation and Nuclear Science, Kyoto University, Osaka, Japan*

Purpose: Many of aspartyl residues (Asp) in lens crystallin undergo a spontaneous chemical reaction into isomerized Asp (iso Asp) in aged lenses. We have previously reported isomerization sites at Asp36, Asp62 and Asp96 in αB-crystallin (αB-Cry) However, its contributions on the structure, stability and function of αB-Cry are not well understood. Therefore, the purpose of this study is to estimate the impacts of modifications at each Asp by comparing with various mutants.

Methods: Asp36, Asp62 and Asp96 of human αB-Cry were replaced by various amino acids (Gly, Ala, ILe, Ser, Glu and Lys) by site-directed mutagenesis. All proteins were recombinantly expressed in *E. coli*, and purified by successive chromatography. Their secondary structures, heat stability, oligomer sizes, hydrophobicity and chaperone functions were investigated.

Results: The hydrophobic mutations at Asp96 altered the size of the αB-Cry oligomer. Subtle aggregations were formed in those hydrophobic mutations at Asp96 during purification. Bis-ANS assay showed differences in surface hydrophobicity. In many experiments, mutants replaced with glutamate showed results similar to wild type. As one of important observations, we identified small size αB-Cry dissociation from oligomer during purification process of all mimics.

Conclusions: The result suggest ed that the hydrophobicity of Asp 96 contribute to intra and inter molecular interactions. Though the effects of isomerization of Asp in αB-Cry have been obscure, these formation at the Asp96 may alter the size of oligomer, and decrease the stability of αB-Cry in aged lens. Our result also implied the presence of abnormal subunit exchanging in αB-Cry homo oligomer. There are still obscured the contribution of iso Asp in those mechanism s , but their chemical and physiological alteration of site specific amino acids must have impact on abnormal lens protein-protein interactions, leading senile cataract formation in aged lens.

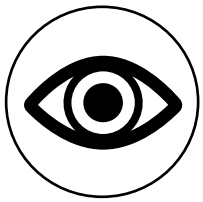

Tuesday, Dec. 12th

Tuesday, December 12th

Session 5: Lens Fiber Differentiation and Autophagy

Organizer: *Amer Riazuddin*
Co-Moderator: *Quili Fu*

Talks are in the format of 15 minutes with 3 minutes discussion.

Time	Speaker		Title
8:30 a.m.		**Amer Riazuddin**	Understanding the role of FYCO1-dependent autophagy for organelle removal during lens fiber cell differentiation
8:48 a.m.		**Velia Fowler**	Lens epithelial cell morphogenesis and hexagonal patterning depend upon nonmuscle myosin IIA (NMIIA) bipolar filament assembly and F-actin distribution
9:06 a.m.		**Sanjaya Shrestha**	Ell2, a conserved transcription regulator, is essential for achieving optimal levels of select epithelial and fiber transcripts, perturbation of which causes lens defects
9:24 a.m.		**Ichiro Masai**	Identification of FGF ligands that differentially control lens growth and lens fiber differentiation in zebrafish

Understanding the role of FYCO1-dependent autophagy for organelle removal during lens fiber cell differentiation

S. Amer Riazuddin[1], Shahid Y. Khan[1], Muhammad Ali Riaz[1], Hira Butt[1], J. Fielding Hejtmancik[2]

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[2]Ophthalmic Genetics and Visual Function Branch, National Eye Institute, National Institutes of Health, Bethesda, MD

Purpose: We previously reported that FYCO1-dependent autophagy, while indispensable for the transparency of the lens, is independent of basal autophagy. Here, we extend our investigation to understand how autophagosomes attached to FYCO1, an adaptor protein, reach the perinuclear area to fuse with lysosomes since FYCO1 facilitates kinesin-dependent anterograde transport.

Methods: We examined the effect of the loss of FYCO1 on the localization of autophagosomes in human lens epithelial (HLE) cells by immunocytochemistry (ICC). We investigated expression of MST1/STK4, a kinase protein reported to phosphorylate LC3B at Thr50 in mouse lens, and phosphorylation of LC3B at Thr50 in mouse lens fiber cells (FC). We employed shRNAs to knock down the expression of MAPK8IP1/JIP1 and RILP, adaptor proteins involved in dynein/dynactin-dependent retrograde movement of autophagosomes and examined the localization of autophagosomes in HLE cells by ICC.

Results: The results of ICC experiments confirmed that in the absence of FYCO1, LC3B/autophagosomes localized close to the perinuclear area in HLE cells. The analysis of our previously published transcriptome and proteome datasets confirmed expression of MST1/STK4 in the developing mouse lens transcriptome and proteome, and the results of the proteome profiling were confirmed by Western blot (WB) analysis. WB analysis using a Thr50-specific antibody demonstrated that LC3B is phosphorylated at Thr50 in mouse lens FC. Finally, ICC analysis confirmed that shRNA-mediated knockdown of MAPK8IP1/JIP1 in HLE cells results in autophagosomes aggregating close to the cell periphery.

Conclusions: These data support the hypothesis that autophagosomes linked to FYCO1 through an FYCO1-LC3B interaction, are released upon phosphorylation of LC3B at Thr50, and are then transported to the perinuclear area by dynein/dynactin-dependent retrograde transport.

Lens epithelial cell morphogenesis and hexagonal patterning depend upon nonmuscle myosin IIA (NMIIA) bipolar filament assembly and F-actin distribution

Velia M. Fowler[1], Sadia T. Islam[1], Justin Parreno[1] and Cathy Cheng[2]

[1]Department of Biological Sciences, University of Delaware, Newark, DE
[2]School of Optometry, Indiana University, Bloomington, IN

Purpose: Ocular lens epithelial cells in the equatorial region undergo a remarkable transformation from randomly packed cells into precisely aligned and hexagon-shaped cells organized into meridional rows, which further differentiate into precisely aligned and hexagonally packed secondary fiber cells. We used genetic knock-in mice with cataract-linked human NMIIA heavy chain (MYH9) mutations to investigate NMIIA function in lens epithelial cell morphogenesis. We showed previously that mice with a NMIIA-E1841K mutation in the rod domain that disrupts bipolar filament assembly have misshapen and misaligned meridional row cells, which differentiate into misaligned fiber cells. To investigate the role of NMIIA contractility in formation of aligned meridional rows, we analyzed actin filament (F-actin) assembly in meridional row cells of NMIIA-E1841K mutant lenses, and in lenses from mice with the NMIIA-R702C mutation in the motor domain, which impairs F-actin sliding but permits tension generation.

Methods: Lens meridional row cell shapes, alignment and F-actin distribution were analyzed by staining whole lenses with fluorescent phalloidin (F-actin) and Hoechst (nuclei), confocal microscopy, and quantitative image analysis (ImageJ).

Results: The misaligned and misshapen meridional row cells in NMIIA-E1841K mutant lenses show increased cortical F-actin along with reorganization of radially-oriented basal stress fibers into aberrant parallel arrays. In contrast, meridional row cells in heterozygous NMIIA-R702C mutant lenses are well-aligned and hexagon shaped with normal F-actin. Ongoing experiments are testing NMIIA-F-actin contractility by immunostaining for phosphorylated myosin light chain, cadherins, catenins and vinculin.

Conclusions: Disruption of bipolar filament assembly in NMIIA-E1841K mutant cells leading to excess cortical F-actin and polarized stress fibers may lead to force imbalances across neighboring cells, with consequent loss of hexagonal cell shape and alignment. The ability of the NMIIA-R702C motor domain to generate tension may be sufficient to maintain normal cortical F-actin and force balances, thereby maintaining hexagonal cell shape and alignments in meridional rows.

Session 5-3

Ell2, a conserved transcription regulator, is essential for achieving optimal levels of select epithelial and fiber transcripts, perturbation of which causes lens defects

Sanjaya K. Shrestha[1], Sandeep Aryal[1], Archana D. Siddam[1], Francisco G. Hernández[1], Salil A. Lachke[1,2]

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[2]Center for Bioinformatics and Computational Biology, University of Delaware, Newark, DE, USA

Purpose: Lens development and homeostasis involves dynamic control over gene expression. While several lens regulatory proteins have been characterized, key questions remain. While it is known that the lens produces abundant levels of specific mRNAs (e.g., crystallins), it is currently unknown whether regulators that control the ubiquitously important transcription "elongation" process, are involved in this process. To address this knowledge-deficit we applied iSyTE to make the new prediction that Ell2 (Elongation factor for RNA polymerase II 2) –which promotes transcription by relieving the "paused" RNA Pol II– has a specific function in regulating key lens genes.

Methods: Ell2 expression was examined by RT-PCR, in situ hybridization, Western blotting and immunofluorescence. Ell2cKO conditional knockout mice were generated using Pax6GFPCre and lens defects were characterized by microscopy and histology. Lens transcriptome was examined by RNA-sequencing (RNA-seq) and differentially expressed genes (DEGs) were validated by RT-qPCR.

Results: iSyTE predicted Ell2 as a high-priority candidate based on its enriched expression in the mouse lens. Ell2 expression was validated in mouse lens embryonic and postnatal development. Ell2 is robustly expressed in fiber cells (FCs) in embryonic and postnatal stages, and was also detected in the lens epithelium postnatally. Light microscopy and histology show that Ell2cKO mice exhibit reduced fully penetrant lens defects starting at age 2 weeks. RNA-seq revealed reduced mRNA levels of a subset of highly expressed FC genes. These include several crystallins as well as other genes important to lens biology/pathology (Birc7, Dnase2b, Mip, etc.). Some epithelial genes (Adamts10, Aqp1, Dmrta2, Mapk14, etc.) were also misexpressed. However, several FCs genes, including key FC TFs (Maf, Prox1, Sox1) were unaltered, suggesting that Ell2 selectively and directly controls lens gene expression.

Conclusions: These findings suggest that the transcription regulator Ell2 is necessary for achieving optimal levels of key transcripts, perturbation of which causes lens defects.

Funding: R01EY021505, R01EY029770.

Identification of FGF ligands that differentially control lens growth and lens fiber differentiation in zebrafish

Ichiro Masai[1]

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Purpose: During lens development, FGF regulates lens epithelial cell proliferation and later lens fiber differentiation. However, among more than 20 FGF ligands, which FGFs are involved in lens development is not fully understood. The aim of this study is to elucidate the spatiotemporal expression of all the zebrafish FGF ligands during lens development and identify which FGFs regulate lens epithelial cell proliferation and fiber differentiation.

Methods: Expression profile of all the FGF ligands was examined by in situ hybridization and RT-PCR. We also inhibited FGF ligands expressed in the ocular tissue, using morpholino antisense.

Results: Among total 27 FGF ligands in zebrafish, only two FGF ligands, FGF3 and FGF8a, are expressed in the developing ocular tissue. fgf3 mRNAs are expressed in the neural retina during the lens vesicle formation stage. After lens fiber differentiation starts at the equator, fgf8a mRNA is expressed in differentiating retinal ganglion cells (RGCs). fgf3 morphants show a smaller lens vesicle through the inhibition of MAPK-dependent cell proliferation and AKT-dependent cell survival, whereas fgf8a morphants show significant delay of lens fiber differentiation at the lens equator. Overexpression of GFP-tagged FGF8a in RGCs induces the accumulation of GFP signals in the posterior lens capsule, indicating that FGF8a secreted form RGCs directly acts on the lens.

Conclusions: In zebrafish, FGF3 and FGF8a differentially regulate distinct steps of lens development, early lens vesicle formation and later fiber differentiation, respectively. Since differentiating RGCs are a source of FGF for lens fiber differentiation, FGF8a may coordinate ocular growth by controlling the lens fiber differentiation rate in response to the level of retinal neurogenic rate.

Tuesday, December 12th

Session 6: Lens Physiology and Channel Proteins

Organizer: *Tom White*
Co-Moderator: *Paul Donaldson*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker		Title
10:30 a.m.		**Tom White**	Probing the role of connexin 50 channels in early postnatal lens physiology using single-cell RNA sequencing
10:48 a.m.		**Paul J. Donaldson**	Physiological modulation of the crystalline lens water transport changes the stiffness of the ex vivo non-decapsulated bovine lenses measured using a lens spin test system
11:06 a.m.		**Nicholas A Delamere**	Activation of lens TRPM3 slows Na,K-ATPase-mediated active transport
11:24 a.m.		**Kevin L. Schey**	Controlling Aquaporin Function in the Lens
11:42 a.m.		**Rob Hufnagel**	SUN1 nullizygosity causes a cataract syndrome LINCing nuclear envelope disorders

Probing the role of connexin 50 channels in early postnatal lens physiology using single-cell RNA sequencing

Thomas W. White[1], Adrienne A. Giannone[2], Barbara Rosati[1,3], David McKinnon[4], Caterina Sellitto[1]

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Purpose: The epithelial monolayer of the ocular lens is a complex cellular network that maintains the overall physiology of the organ. Connexin 50 (Cx50) is the most abundant lens epithelial cell (LEC) connexin during the early postnatal period and has previously been shown to be essential for normal lens growth. Knockout of Cx50 suppresses LEC proliferation during the first postnatal week, causing a life-long reduction in lens size, and ultimately a central cataract. On postnatal day 2 (P2), there is a transitory spike in the proliferation of LECs in wild-type (WT) lenses, which is significantly reduced in Cx50 knockout mice (Cx50 KO).

Methods: To gain insight into the molecular mechanism(s) by which Cx50 mediates LEC proliferation during the early postnatal period, we use single-cell RNA sequencing (scRNA-seq) technology to characterize LEC subpopulations from WT, and Cx50 KO P2 LECs and analyze the transcriptomic impacts of loss of Cx50 function.

Results: Both WT and Cx50 KO cell preparations yielded similar numbers of expressed genes and RNA transcripts, with ≥ 92.5% of isolated cells identified as LECs. Lens epithelial cells could be divided into distinct subpopulations that were classified by specific gene markers. Transcriptional diversity within postnatal LEC subpopulations identified genetic and molecular networks impacted by the deletion of Cx50. Changes were found in the expression of genes associated with lens signaling pathways and ribosomal activtity. We also observed a decrease in the transcription of genes associated with cell proliferation and mitotic processes in Cx50 KO LECs.

Conclusions: These data suggest novel mechanisms and hypotheses for the impact of Cx50 deletion on postnatal LEC proliferation and provide a basis for future experimentation.

Physiological modulation of the crystalline lens water transport changes the stiffness of the ex vivo non-decapsulated bovine lenses measured using a lens spin test system

Paul J. Donaldson[1], Chen Qiu1; Dingchang Shi[1], Yadi Chen[1]; Xingzheng Pan[1], Matthew Reilly[2], Yosuke Nakazawa[3]

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[3]Faculty of Pharmacy, Keio University, Tokyo, Japan

Purpose: To investigate whether changes to lens water content and transport can alter the stiffness of the bovine lens.

Methods: Bovine lenses were removed from the eye with the capsule intact and were placed on the stage of a modified spin test apparatus. Images taken to capture lens geometry before and after the lens was spun at a known speed. Sets of control images were first recorded for lenses incubated in AAH, and then the spin protocol was repeated on the same lenses after they were incubated for up to 4 hours in either hypotonic-AAH, hypertonic-AAH, or AAH+ouabain. Image analysis protocols were used to extract the deformations to lens geometry induced by the different incubation conditions tested and regional changes in shear moduli (stiffness) was estimated using a finite element model developed in COMSOL, a commercial software suite.

Results: Swelling lenses to increase lens water content by exposure to hypotonic-AAH caused the shear modulus to increase and decrease in the outer cortex and core, respectively. In contrast, exposure of lenses to hypertonic-AAH, to reduce lens water content induced a reversable stiffening of the lens. Altering lens water transport by incubating lenses in ouabain-AAH to inhibit the Na+K+ATPase that generates the lens microcirculation, like hypotonic-AAH caused a similar decrease in the shear modulus of the core of the lens, but a similar increase in modulus in the outer cortex was not observed.

Conclusions: These observations show that alterations in water content and transport can induce spatially localized and reversible changes to the lens stiffness. Since lens water content and transport is dynamically regulated, our findings suggest that that lens may also actively regulate its stiffness. This therefore implies that studying the mechanisms that regulate lens stiffness may reveal new targets for pharmaceutical intervention to soften the lens and delay the onset of presbyopia.

***Acknowledgements**: This work was supported by a Health Research Council of New Zealand programme grant.*

Activation of lens TRPM3 slows Na,K-ATPase-mediated active transport

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Purpose: The lens epithelium shows rich expression of a calcium-permeable nonselective cation channel, TRPM3, and it is known that a missense mutation resulting in an isoleucine-to-methionine substitution in TRPM3 (I65M) causes early onset cataract in humans. Little is known about the normal role of lens TRPM3. However, evidence from studies on transgenic mice indicates the same mutation disrupts lens sodium and potassium homeostasis. Here we explore the effect of TRPM3 activation on Na+-K+ transport.

Methods: Cytoplasmic calcium was measured in single cells by the Fura-2 ratiometric method. The rate of rubidium (Rb) uptake was measured as an index of potassium entry.

Results: In cells exposed to the TRPM3 agonist CIM0216 (1 to 50 μM), the rate of Rb uptake was reduced by ~20% in primary cultured lens epithelium from wild-type (WT) mice. A similar response was observed in a human lens epithelium HLE-B3 cell line but a TRPM3 I65M mutant HLE-B3 cell line was an order of magnitude more sensitive to CIM0216. The Rb uptake response to CIM0216 was absent in mouse lens cells that were bathed in calcium-free solution. Consistent with the notion that TRPM3 activation causes calcium entry, the TRPM3 agonist pregnenolone (100μM) caused an increase in cytoplasmic calcium that was inhibited by a TRPM3 antagonist primidone (50 μM). In parallel studies TPM3 activation was found to hyperpolarize lens cells.

Conclusions: Taken together, the findings are consistent with a reduction of Na,K-ATPase activity due to calcium entry that occurs on activation of TRPM3 channels. It has become apparent that the lens has a complex array of ion channels that regulate its ion transporters. Activation of TRPV4 is known to stimulate Na,K-ATPase-mediated active transport. Activation of TRPV1 is known to stimulate NKCC1-mediated transport. Piezo1 also plays a role. Interestingly, lens ion homeostasis is not obviously affected by knockout of either TRPM3, TRPV4 or TRPV1, suggesting there are ways to compensate for the absence of a particular mechanism. Studies are under way to test whether the I65M mutation of TRPM3 results in an alteration in channel function that causes cataract.

Funding: *This work was supported by NIH grants R01EY009532 and R01EY029171*.

Controlling Aquaporin Function in the Lens

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Purpose: To determine how lens aquaporin modifications, localization, protein and lipid interactions affect function.

Methods: Proteomics methods, including crosslinking (XL-MS) and native mass spectrometry (nMS), were used to determine AQP modifications, AQP-protein, and AQP-lipid interactions. Human lenses of varying age and bovine lenses were dissected into cortex and nucleus regions, homogenized, and the urea insoluble fraction (UIF) was isolated. The UIF was digested with trypsin and analyzed by LC-MS/MS to assess age-related AQP modifications. For XL-MS, crosslinking reagents were added to bovine lens membranes followed standard proteomic analysis. For native mass spectrometry, both purified AQP0 and crude lens membranes were solubilized detergent to determine AQP0 modifications and non-covalent lipid binding. Light scattering experiments were conducted to determine water permeability in lens vesicles produced from different lens regions and in human lens epithelial cells expressing only AQP1, AQP5, or both. Immunohistochemical analysis of AQP0 and AQP5 was done to assess AQP localization.

Results: Proteomic analysis revealed extensive age-related modifications to human AQP0 including truncation, phosphorylation, deamidation, crosslinking and covalent lipid modification. AQP5 was truncated and phosphorylated in aged human lenses. XL-MS identified new sites of AQP0 interactions with crystallins, connexins, filensin, phakinin, and vimentin indicating that both N- and C-terminal regions of AQP0 are involved. Native MS analysis showed that multiple lipids non-covalently bind AQP0, potentially affecting its permeability. While AQP0 localization is predominantly in fiber cell membranes throughout the lens, AQP5 maintains a cytoplasmic localization until late in fiber cell maturation. Functional assays are in progress to assess AQP permeability in lens fiber cell vesicles and in lens epithelial cells.

Conclusions: New AQP-protein and new AQP0-lipid interactions have been discovered and these, along with post-translational modifications and cellular localization, are predicted to affect AQP permeability and ultimately may influence the lens microcirculation system.

SUN1 nullizygosity causes a cataract syndrome LINCing nuclear envelope disorders

Robert B. Hufnagel[1,2], Sairah Yousaf[2], Jerry Lee[2], Jimmy Liu[2], Arnaud Giese[3], Zubair M. Ahmed[3], Brian P. Brooks[2]

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Purpose: To describe a novel syndrome of cataract, microcephaly, and muscular dystrophy due to biallelic loss-of-function variants in *SUN1*. Dysfunction of inner nuclear envelope proteins such as SUN1 result in laminopathies and Emery-Dreifuss-type muscular dystrophies, while disorders of the cytoskeletal NESPRIN binding partners in the LINC complex include a variety of neurological and neuromuscular diseases. SUN1 has not been implicated in congenital malformations or cataract pathogenesis previously.

Methods: Exome sequencing identified SUN1 variants in affected individuals. Truncated protein localization and NESPRIN-binding were evaluated by in vitro transfection and immuno-precipitation. Transcript-specific sun1 functions were tested by zebrafish morpholino-rescue strategies.

Results: We evaluated a 9-year-old male born to consanguineous parents with dense congenital cataracts, hearing loss, microcephaly, seizures, and muscular dystrophy, Emery-Dreifuss type. Analysis of whole exome sequencing revealed a homozygous nonsense mutation in *SUN1*. Analysis of exome data from nine unrelated probands revealed biallelic nonsense, frameshift, and canonical splice site SUN1 variants. We hypothesized that truncation of SUN1 results in loss of nuclear envelope anchoring. To test this, SUN1 full-length transcript, a secondary shorter transcript, and mutant transcripts were misexpressed in HEK cells *in vitro*. Only full-length SUN1 localized to the nuclear envelope, while short and truncated forms remained in the cytoplasm or endoplasmic reticulum. Immunoprecipitation assays to determine nesprin-binding properties revealed that only full length SUN1 was capable of binding. Finally, to investigate the developmental brain and eye phenotypes, a novel sun1 splicing morpholino was designed and verified. Morphant fish have significant microcephaly and microphthalmia, which is rescued by co-injection of human full-length *SUN1* mRNA.

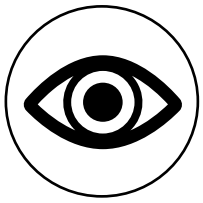
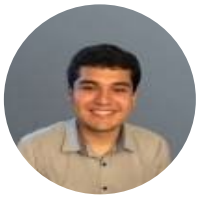
Conclusions: These findings support a novel SUN1-associated eye-ear-muscle-brain syndrome and implicate a novel link between nuclear envelope dysfunction and neurogenesis of the inner ear, eye, and brain.

Tuesday, December 12th

Session 7: Physiological Optics and Biomechanics of the Lens

Organizer: *Matthew Reilly*
Co-Moderator: *Bianca Maceo Heilman*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker		Title
1:15 p.m.		**Matthew A. Reilly**	A Computational Study of the Geometric Optomechanics of the Aging Human Lens
1:33 p.m.		**Bianca Maceo Heilman**	Average refractive index of human and non-human primate lenses: age and accommodation dependence
1:51 p.m.		**Susana Marcos**	Crystalline lens optical, geometrical and mechanical properties from OCT quantitative imaging in vivo
2:09 p.m.		**Pooja Rathaur**	Composition and Structural Organization of the Human Ciliary Zonule
2:27 p.m.		**Christian Zevallos-Delgado**	Crystalline lens biomechanics

A Computational Study of the Geometric Optomechanics of the Aging Human Lens

Matthew A. Reilly[1,2], Myles Cline[1]

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[2]*Department of Ophthalmology & Visual Science, The Ohio State University, Columbus, Ohio, USA*

Purpose: The optical power of the fully accommodated lens declines through adulthood due to growth, remodeling, and changes in material properties [1]. In this study, I summarize our work to date on how evolving lens geometry and gradient refractive index combine to give the aging human lens its accommodated power.

Methods: A finite element model of the young (20yo) adult human lens was constructed in COMSOL Multiphysics (COMSOL, Inc.). The model included continually varying mechanical and optical properties to describe the optical and biomechanical property gradients within the lens. The lens capsule was represented as a membrane having spatially varying thickness. Lens growth was simulated using a hygroscopic swelling model, resulting in increased lens volume. Disaccommodation (lens stretching) was simulated by applying spatially varying force vectors to the region of the zonular insertions. Stiffening was simulated by increasing material properties in the disaccommodated state. Accommodation was then simulated by relieving these zonular forces. Ray tracing was used to characterize the focal length of the lens before and after stretching at each increment of growth.

Results: The young lens was able to alter its focal length significantly in response to small stretching forces. The “older” model lens was unable to alter its focal length under the same forces. This was true whether its volume or its mechanical properties were increased – it was not necessary to increase both.

Conclusions: Growth of the lens without stiffening is sufficient to cause a loss of accommodation. Significant stiffening of the lens can also prevent it from altering its optical power under the influence of zonular tension.

References: *[1] Reilly, M.A., A quantitative geometric mechanics lens model: Insights into the mechanisms of accommodation and presbyopia. Vision Res. 2014;103:20-31*.

Average refractive index of human and non-human primate lenses: age and accommodation dependence

Bianca Maceo Heilman[1,2], Jean-Marie Parel[1,2,3], Fabrice Manns[1,2]

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Purpose: To measure the average group refractive index of human, monkey and baboon lenses and quantify any changes with age and simulated accommodation.

Methods: 24 lenses from 20 cynomolgus monkeys (Ages: 3-14 years), 13 lenses from 9 hamadryas baboon (Two age groups: 2-5 years and 21-28 years), and 13 lenses from 11 human (Ages: 20–78 years) were isolated, placed on a rubber O-ring, submerged in Dulbecco's Modified Eagle's Medium, and imaged with a time-domain optical coherence tomography (TD-OCT) system. Additionally, 33 lenses from 29 cynomolgus monkeys (Ages: 4–18 years) were mounted into a lens stretcher and TD-OCT images were acquired of lenses in the unstretched and fully stretched position. The axial shift of the tissue chamber wall from the images was used to calculate the average group refractive index (n_{avg}) of the lens. (Uhlhorn et al., 2008) n_{avg} was plotted versus age for the various species and the measurements with stretching were compared.

Results: For isolated lenses, n_{avg} was 1.417±0.003 in monkeys and appeared to slightly decrease with age (p=0.06). n_{avg} was 1.418±0.002 in the young baboons, 1.413±0.002 in the older baboons, and decreased with age (p=0.003). n_{avg} was 1.412±0.004 in humans and decreased with age (p=0.03). n_{avg} was 1.417±0.002 for the unstretched (accommodated) monkey lens and 1.416±0.002 for the fully stretched (relaxed) lens. There was no statistically significant change in n_{avg} with stretching (p=0.10) or age (p=0.17).

Conclusions: The monkey lens n_{avg} remained constant with accommodation and did not show a significant age-dependence. The baboon and human lenses n_{avg} decreased with age. An age-related decrease in the average refractive index agrees with an increase in the central free water content of the lens with age.

Support: *National Institutes of Health Center Grant P30EY14801; the Florida Lions Eye Bank and Beauty of Sight Foundation; the Henri and Flore Lesieur Foundation (JMP).*

Crystalline lens optical, geometrical and mechanical properties from OCT quantitative imaging in vivo

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Purpose: The crystalline lens contributes to the retinal image quality. It reshapes to accommodate, undergoes changes with age and may play a role in emetropization and myopia development. Understanding lens optical, geometrical and mechanical properties sheds light into the mechanisms of these processes and help improving treatments.

Methods: The eye's anterior segment in 3D was imaged in 3D with Swept Source OCTs (custom instrument and Zeiss IOLMaster700). Custom routines were used to correct fan, optical and motion distortions, and an eigenlens basis to describe the full lens shape, beyond the pupillary axis. Studies on three cohorts are presented: (1) 19 young (28.6±4.4yr) accommodating (0-6 D) subjects; (2) 21 young (27.4±4.5yr) myopic/emmetropic (-6.25-+0.5D) subjects; (3) 42 older (>55yr) subjects pre-cataract surgery. We estimated: Lens anterior and posterior radii of curvature (Ra, Rp), volume (VOL), surface area (SA), equatorial lens diameter (DIA) and position (EPP) and lens thickness (LT). For (1), lens mechanical properties (nucleus En and cortex Ec shear moduli) were obtained through FEM and inverse optimization.

Results: With accommodation (1) lens steepened by -0.60(Ra) and -0.22(Rp) mm/D; LSA and DIA decreased by -2.8mm2/D and –0.138mm/D; and LT increased by 0.069mm/D. En and Ec increased by 0.27 ± 0.02 kPa/yr. With myopia (2) lens flattened by 0.76(Ra) and 0.18(Rp) mm/mm; LSA and DIA increased by 0.82mm2/mm and 0.15mm/mm; and LT decreased by -0.12 mm/mm of axial elongation. With aging (3) lens steepened by -0.057(Ra) and -0.050(Rp) mm/year; and LT increased by 0.029 mm/yr.

Conclusions: Despite large intersubject variability, lens parameters appear correlated with accommodation and aging, in agreement with Helmholtz theory. Lens changes in myopia are consistent with lens flattening and equatorial expansion leading to decrease in power. Quantification of the lens properties will lead to treatment customization in presbyopia, myopia and cataract.

Composition and Structural Organization of the Human Ciliary Zonule

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Purpose: The ciliary zonule is a system of extracellular fibers that centers the lens on the optical axis. Previously, we examined the structure and composition of the relatively simple zonules of mice and bovine eyes. In contrast to those species, the young human eye is capable of >10 diopters of accommodation. This additional functionality appears to be reflected in the presence of a more complex zonular apparatus. Here, we developed methods to visualize the human zonule three dimensionally in a relatively undisturbed state and to measure the biomechanical properties of individual zonular fibers.

Methods: Human eye globes were obtained from a local eye bank. The zonular fibers were labeled with fluorescein-conjugated lens culinaris agglutinin, while the surface of the lens and ciliary processes were visualized using fluorescent phalloidin. Long working distance objective lenses were used to image the zonular fibers through the vitreous humor. The tensile strength of individual zonular fibers was measured using a novel technique in which individual immobilized fibers were grasped by micromanipulators and subsequently stretched using a force transducer probe.

Results: Human zonular fibers originated in the pars plana region of the ciliary epithelium. Distinct sets of fibers could be distinguished, corresponding to the vitreous and pars plana zonule. We also identified circular fibers in some but not all eyes. They were firmly attached to the anterior hyaloid membrane. High resolution reconstructions identified a series of capsular inclusions beneath zonular anchorage points on the lens surface. Biomechanical measurements suggest that the fibers can stretch 3-4 times their original length before breaking and possess similar tensile strength to that of other species.

Conclusions: We described the three-dimensional organization of the human zonular apparatus. In conjunction with single fiber biomechanical analysis, we anticipate this approach will provide an improved understanding of the accommodative mechanism.

Crystalline lens biomechanics

Christian Zevallos-Delgado[1], Pavel Nikitin[1], Taye Mekonenn[1], Manmohan Singh[1], Salavat R. Aglyamov[2], Kirill V. Larin[1]

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[2]Department of Mechanical Engineering, University of Houston, Houston, TX, USA

Purpose: The crystalline lens plays a vital role in vision, and its complex structure provides accommodative ability. The loss of function, problems with the accommodation, and diseases such as cataracts directly affect vision health. In addition to its structure, the biomechanical properties of the crystalline lens are also affected by these aberrant modifications. Further, the viscoelastic properties of the lens are affected by factors such as age, accommodation, and structural modifications. Here, we utilize Optical Coherence Elastography (OCE), to assess the biomechanical properties of the crystalline lens.

Methods: Crystalline lenses from different animal models (rabbit and porcine) were used to measure the biomechanical modifications due cataracts induced by external agents (H_2O_2 and cold) and removal of the capsule. The biomechanical modifications were assessed using OCE and Acoustic Radiation Force (ARF) as an excitation source.

Results: The biomechanical properties of the lens suffer dramatic modifications due to the changes in age. In addition, the cataracts induced severe modifications in the lens structure and stiffness, showing that oxidative damage from H_2O_2 increased the stiffness of the lens. Furthermore, our results showed that removal of the crystalline lens capsule greatly reduced the lens elasticity from 8.14 ± 1.10 kPa to 3.10 ± 0.43 kPa.

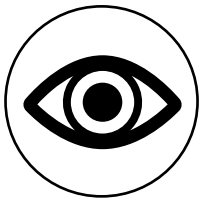
Conclusions: OCE was able to quantify the changes in the crystalline lens due to oxidative damage, cold cataract, and removal of the capsule. These results show that OCE could be useful for clinical applications of disease detection and therapy guidance for a variety of lenticular diseases.

Tuesday, December 12th

Session 8: Biochemistry and Biophysics of Crystallins

Organizer: *Eugene Serebryany*
Co-Moderator: *Ariel Alperstein*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker		Title
3:00 p.m.		**Eugene Serebryany**	The benefits and drawbacks of high Cys content in gamma-crystallins
3:18 p.m.		**Jiayue Sun**	Analysis of the Susceptible Tryptophan Residues on the Greek Key Motifs of βB2-Crystallin
3:36 p.m.		**Liliana Quintanar**	Metal Induced Aggregation of Human Lens Crystallins: The Bioinorganic Chemistry of Cataract Disease
3:54 p.m.		**Ariel Alperstein**	Towards two-dimensional infrared microscopy of crystallin protein protective chaperone activity

The benefits and drawbacks of high Cys content in gamma-crystallins

Eugene Serebryany[1]

[1]*Department of Physiology and Biophysics and the Laufer Center for Physical and Quantitative Biology, Stony Brook University, Stony Brook, NY, USA*

Purpose: As the lens loses its redox homeostasis with age, non-native disulfides begin forming in lens crystallins. The γ-crystallins, by far the most Cysteine-rich class, are particularly prone to disulfide-driven misfolding and subsequent aggregation, leading to loss of transparency. If non-native disulfides are the Achilles' heel of γ-crystallins, why has evolution not replaced their Cys residues with less reactive amino acids, like those in the Cysteine-poor β-crystallins?

Methods: Several multi-Cys variants of human γD-crystallin were generated: variant "allCS" wherein all six native Cys residues were replaced with Ser residues (a total change of only six atoms in the protein); variant "NCS," where only the four Cys residues in the N-terminal domain were replaced with Ser; and variant "NCA/T," where the four N-terminal Cys were replaced with Ala or Thr residues instead. Differential scanning calorimetry, turbidimetry, chromatography, and differential scanning fluorometry were used to evaluate the stability, aggregation propensity, oligomeric state and apparent hydrophobicity, and misfolding propensity of these variants compared to the WT and to the previously well-characterized aggregation-prone variant W42Q.

Results: The allCS variant was greatly destabilized relative to WT and significantly aggregation-prone – yet, the aggregation propensity of the NCS variant exceeded that of allCS, even in reducing buffers, whereas NCA/T showed high stability and little aggregation. Unexpectedly, the enhanced NCS aggregation was entirely attributable to a disulfide-crosslinked dimer, while the monomers remained soluble. This aggregation was correlated with formation of a non-native state observed even in the WT protein, and it was inhibited by the native lens chemical chaperone myo-inositol.

Conclusions: It is possible to generate an aggregation-resistant, Cysteine-less γ-crystallin. However, the Cys-rich sequence may be an evolutionary trap because single-nucleotide changes at most Cys residues are likely to be deleterious. The aggregation mechanism of NCS dimers may offer insights into age-related crystallin aggregation mechanisms.

Analysis of the Susceptible Tryptophan Residues on the Greek Key Motifs of βB2-Crystallin

Jiayue Sun[1], Takumi Takata[2]

[1]*Department of Chemistry, Graduate School of Science, Kyoto University, Kyoto, Japan*
[2]*Institute for Integrated Radiation and Nuclear Science, Kyoto University, Osaka, Japan*

Purpose: The modifications of amino-acids in lens proteins caused by environmental stress and diseases are correlated with cataracts. Conserved tryptophan (Trp: W) residues are known for maintaining lens protein structure and stability in γ-crystallins, however, their roles in β-crystallins are elusive. Accumulated aging-related oxidation of Trp which breaks down Trp into kynurenine (Kyn), is one potential reason for cataract formation. In this study, we investigate how the Trp residues on the Greek Key Motifs contribute to the βB2-crystallin structure and stability.

Methods: Among the five Trp on βB2-crystallin, W59/W82/W85/W151 are located on the Greek Key Motifs while W196 is lied on the C-terminal tail of the βB2-crystallin structure. Due to the structural similarity between Kyn and phenylalanine (Phe: F), these five Trp were replaced by Phe respectively to simulate the oxidation effect of Trp in βB2-crystallin. The protein secondary structure, heat stability, hydrophobicity, folding pathway and oligomeric state were evaluated. Additionally, we studied the chaperone effect for abnormal state of βB2-crystallins using αA-crystallins.

Results: W59F had a slightly different secondary structure than the wild type and other mutants. The mimics also decreased the heat stability of βB2-crystallin respectively. W59F exposed more hydrophobicity than the wild type and other mimics. Except for W151F which only formed dimer, all mimics formed both dimer and tetramer. The folding pathway of W151F was also different from the other mutants and the wild type. 5 (αA): 1 (βB2) ratio was needed for αA-crystallin to suppress the βB2-crystallin aggregation induced by heat.

Conclusions: Our results are consistent with previous studies that W59 and W151 are critical to βB2-crystallin structure and stability. These two positions are almost superimposed on the βB2 crystal structure, but their impacts to βB2-crystallin are different. Our results implies that the oxidative modification of Trp unstabilizes βB2-crystallin, leading to lens dysfunction and cataract formation.

Metal Induced Aggregation of Human Lens Crystallins: The Bioinorganic Chemistry of Cataract Disease

Liliana Quintanar[1,2]

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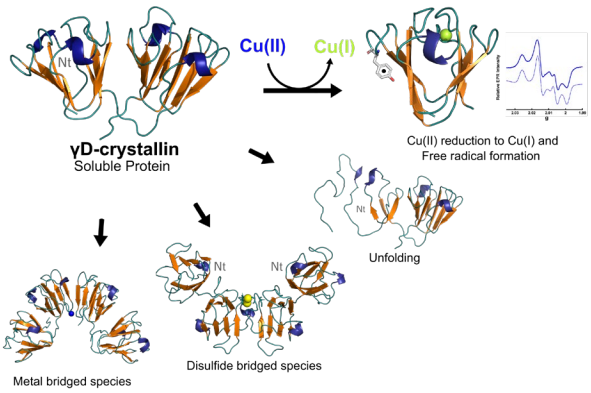
[2]*Center for Research in Aging, Cinvestav, Mexico City, Mexico*

Purpose: Metal ions have been implicated in several degenerative diseases associated to the deposition of protein aggregates, including cataract disease. A principal aim of our research is to understand the mechanisms of metal-induced aggregation of human lens β/γ-crystallins.

Methods: Aggregation of β/γ-crystallins is studied by turbidity assays and aggregates are analyzed by SDS-PAGE analysis and electron microscopy. The impact of metal ions in the thermal stability of β/γ-crystallins is studied by circular dichroism (CD) and differential scanning calorimetry (DSC); while metal binding affinities are measured by isothermic titration calorimetry (ITC). Metal binding to β/γ-crystallins is studied by a wide range of spectroscopic tools, including CD, electron paramagnetic resonance (EPR) and X-ray absorption spectroscopy (XAS).

Results: γD-, γC-, γS-crystallins and βB2-crystallin are sensitive to copper- and zinc- induced aggregation.[1,2] Copper ions activate different site-specific mechanisms, including: protein unfolding, metal bridging, disulfide bridging, and copper redox processes that lead to formation of free radical species (see Figure).[1,3] The role of Cys residues in this interesting redox chemistry will be discussed. The case of copper-induced aggregation of γ-crystallins will be contrasted to that of zinc-induced aggregation.[4]

Conclusions: Essential metal ions induce crystallin aggregation and may be implicated in cataract disease. Understanding how metal ions impact protein stability and aggregation of human lens β/γ-crystallins provides further insights into the biological inorganic chemistry of cataract disease.



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Towards two-dimensional infrared microscopy of crystallin protein protective chaperone activity

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[1]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE, USA

Purpose: Chaperone proteins, including αB-crystallin, are found throughout the body and normally serve to sequester misfolded fragments and prevent further aggregation. Age-related cataract disease as well as cardiomyopathy are believed to be partially caused by a saturation of the chaperone proteins present, resulting in a buildup of damaged and/or misfolded proteins. Here, we investigated how chaperone proteins impact secondary structure in both the lens tissue and with amyloidogenic peptides relevant to heart disease. We developed methods for imaging that will be used for future microscopy of additional crystallin chaperone-protein interactions.

Methods: Two-dimensional infrared spectroscopy and microscopy performed on human lens tissue (with and without cataracts), mice lens tissue (with and without R120G), and protein solution experiments. Additional techniques included FTIR, ThT fluorescence assays, and TEM imaging.

Results: A review of previous 2DIR imaging of cataract lens tissues reveals amyloid β-sheet secondary structure. The high concentration of αB-crystallin serves to resist structural damage. Additionally, αB-R120G mutant mice lenses were imaged to assess their protein structural similarities to human age-related cataract lenses.

In our new work, we measured desmin fragments under aggregation conditions with and without isotope labeling to determine oligomer and amyloid β-sheet secondary structures. Isotope labeling revealed kinetics of a particularly amyloidogenic sequence. We then measured these fragments in the presence of chaperone proteins found in the heart to determine their effect on the aggregation kinetics. Both αB-crystallin and heat shock protein 27 changed aggregation kinetics.

Conclusions: Overall, previous work establishes a new imaging protocol for assessing secondary structure in lens tissue. Our new results build off this work by leading towards an understanding of client protein-chaperone protein interactions, and secondary structure changes. These results will inform future imaging work on aggregate heterogeneity.


Wednesday, Dec. 13th

Wednesday, December 13th

Session 9: Lens Regeneration and Cross-talk

Organizer: *Katia Del-Rio Tsonis*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker		Title
8:30 a.m.		**Jared A Tangeman**	Integrated single-cell multiomics delineates the gene regulatory networks guiding early lens development
8:48 a.m.		**Kelly Tseng**	Regenerating During Development: Understanding Vertebrate Eye Regrowth Using the Frog Embryo
9:06 a.m.		**Konstantinos Sousounis**	DNA Damage Response during Salamander Lens Regeneration
9:24 a.m.		**Katia Del Rio-Tsonis**	A Lens Regeneration Paradigm
9:42 a.m.		**Sepideh Cheheltani**	Effect of Cap2 on the regulation of actin cytoskeleton and fiber cell organization in the lens

Integrated single-cell multiomics delineates the gene regulatory networks guiding early lens development

Jared A Tangeman[1,2], Sofia Rebull[1], Erika Grajales-Esquivel[1], Jacob M Weaver[1,2], Stacy Bendezu-Sayas[1,2], Michael L Robinson[1,2], Salil A Lachke[3,4], Katia Del Rio-Tsonis[1,2]

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Purpose: Lens fiber cell differentiation represents a classic model for investigating how gene regulatory networks facilitate cell fate transitions. Unbiased, high-throughput assays have been leveraged to capture coarse bulk readouts across various modalities of gene regulation in the lens, although it remains a challenge to unify these data into cohesive regulatory networks. Recent advances in single-cell, multiomic technologies newly enable the interrogation of lens development and fiber cell differentiation at unprecedented scale and resolution.

Methods: Single-cell RNA and ATAC-seq was applied across stages of avian lens development, from the early optic cup stage (embryonic day 3) through early stages of fiber cell differentiation (embryonic day 7). In parallel, CUT&RUN-seq was applied to catalog the lens histone landscape and the distribution of select DNA-binding proteins. Data related to gene expression, transcription factor binding profiles, chromatin landscape, and histone modifications were integrated into a unified analysis. Expression patterns were spatially contextualized using fluorescent *in-situ* hybridization and immunohistochemistry.

Results: We present key transcription factors and associated cis-regulatory elements that guide the early bifurcation of cornea and lens lineages. By day 4 of chicken development, a continuum of epithelial to fiber cells emerge, which facilitates temporal modeling of gene expression and chromatin accessibility changes during fiber cell differentiation. Regulatory targets were comprehensively identified for the fiber-cell differentiation effector MAF. Further, polycomb repressive complex 2 (PRC2) was found to be temporally regulated throughout fiber cell differentiation and functions to repress master-regulator transcription factors associated with nonlens ocular cell types.

Conclusions: Single-cell profiling enables the precise capture of gene regulatory networks guiding early avian lens development. Furthermore, a comprehensive catalog of transcription factor dynamics was generated, including for the cataract-linked gene MAF. Finally, PRC2 is newly identified as a sentinel of lens cell identity.

Regenerating During Development: Understanding Vertebrate Eye Regrowth Using the Frog Embryo

Kelly Tseng[1]

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Purpose: Mammals have a limited ability to regrow organs whereas animals such as frogs can restore lost structures. The clawed frog, Xenopus laevis, is a well-characterized model for both animal development and regeneration studies, especially for the eye. Individual Xenopus eye tissues such as the retina and lens can undergo regeneration. Here, we investigate the ability of frog embryos to regrow the eye.

Methods: Eye and lens tissues (~85%) were ablated surgically and the regrowth process was defined using cellular and molecular methodologies.

Results: Tailbud embryos readily regrew eyes after ablation. Within five days, the regrown eye reached a similar size the contralateral control eye and overall development was normal. The regrown eye contained the expected structures including the lens and retina, connected to the brain, and was functional. Lens and retinal regrowth occurred concurrently. During regrowth, proliferation of retinal progenitor cells was extended by one day with a delay in retinal layer formation, which was restored by three days. Regenerative retinogenesis was consistent with that observed for eye development. Thus, differentiation and patterning during regrowth is similar to endogenous eye development. Using this model, the role of known mechanisms in eye development versus regrowth was assessed. Loss-of-function studies indicated that Pax6 is required for both eye development and regrowth whereas apoptosis is only required for regrowth. Apoptosis is also a required mechanism for appendage regeneration, suggesting that there are conserved pathways for initiating regrowth.

Conclusions: These results showed that frog embryos re-initiate lens and retina development after ablation and that both regenerative and developmental mechanisms are required. This study highlights the eye regrowth model as a robust platform to systematically and efficiently define the pathways required for coordinated regeneration. The identification of mechanisms that can coordinate multi-tissue regeneration may lead to discovery of targets that can be translated into therapies.

DNA Damage Response during Salamander Lens Regeneration

Konstantinos Sousounis[1]

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Purpose: Salamanders regenerate most body parts. The eye lens, for instance, can be repeatedly regenerated with astonishing cellular and molecular fidelity. How do salamander cells regulate DNA damage and genotoxic stress while allow multiple rounds of cell cycle entry? Genotoxic stress during regeneration may occur by increased levels of reactive oxygen species, errors during DNA replication, unsuccessful mitosis, and shortening of telomeres. To study the relationship between DNA damage signaling and regeneration, the DNA repair gene eyes absent 2 (eya2) was targeted. By blocking one of the mechanisms that cells use to normally regulate DNA damage, allowed the development and study of a salamander model with elevated genotoxic stress. Identifying how salamander cells maintain genomic integrity to sustain high levels of proliferation may unlock ways to boost human stem cell functions for regenerative applications.

Methods: Eya2 knockout and eya2-cre lineage trace salamanders were generated and studied using molecular and biochemical applications that detect genotoxic stress like COMET assays, gamma-H2AX immunohistochemistry, and cell cycle analysis. Eya2 and Chek1 were also pharmacologically inhibited.

Results: Eya2 is expressed in multiple eye tissues during development including the lens and retina. Ablation of Eya2 increases genotoxic stress, slows cell cycle, and reduces regeneration capacity in salamanders. Elevated levels of gamma-H2AX foci in the nucleus and G2/M arrest indicate that salamander cells require eya2 to prevent mitotic catastrophe. Pharmacological inhibition of DNA damage signaling also decreased regeneration capacity.

Conclusions: The ability of salamanders to repeatedly regenerate tissues is supported by a robust mechanism that alleviates genotoxic stress.

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A Lens Regeneration Paradigm

Katia Del Rio-Tsonis[1], Jared A. Tangeman[1], George Tsissios[1], Sophie Ratvasky[1], J. Raul Perez-Estrada[1], and Anthony Sallese[1]

[1]Department of Biology Miami University and Center for Visual Sciences at Miami University, Oxford, OH, USA

Purpose: Newts have the intrinsic capacity to regenerate their lens at any stage of their lives via the transdifferentiation of iris pigment epithelial cells. This ability is restricted to the dorsal, but not the ventral, iris. We have centered our research on understanding this restricted regenerative competence through extensive gene expression profiling and functional studies.

Methods: We performed RNAseq/scRNAseq and spatial transcriptomics to delineate regional patterns in gene expression across the irises of two newt species, the Eastern newt (Notophthalmus viridescens) and Iberian Ribbed newt (Pleurodeles waltl). Innate immune cells and pathways identified via RNAseq were disrupted via injection of inhibitors into the vitreous cavity following lentectomy, and the effect on regenerative outcome was assessed via optical coherence tomography and histology.

Results: Our data points towards a highly conserved patterning axis associated with vertebrate eye development that is maintained across the newt iris into adulthood. Interestingly, our data suggests that this axis was co-opted to dictate regenerative competence in newts. In response to lentectomy, a number of BMP signaling effectors act as an injury-responsive “switch” to restrict regeneration to the dorsal iris. We found evidence that this signaling axis acts in tandem with the Ephrin signaling axis to maintain iris polarity, and that pharmacologic disruption of this axis is sufficient to confer regenerative competence to ventral iris pigment epithelial cells. Finally, we delve into the immunological requirements for newt lens regeneration, and present evidence that innate immune responses are closely modulated to facilitate the regenerative process.

Conclusions: Our results demonstrate that a highly-conserved patterning axis that governs vertebrate eye development is present in the adult newt eye, and which appears to have been co-opted to modulate regenerative competence within the newt iris. In addition, we show that macrophages are essential for a scar-free lens regenerative response.

Effect of Cap2 on the regulation of actin cytoskeleton and fiber cell organization in the lens

Sepideh Cheheltani[1], Velia M. Fowler[1]

[1]*Department of Biological Sciences, University of Delaware, Newark, Delaware, USA*

Purpose: Cyclase Associated Protein 2 (Cap2) is a highly conserved actin-binding protein that plays a critical role in regulating the assembly and disassembly of actin filaments. Cap2 facilitates the dissociation of actin monomers from the pointed ends of ADF/cofilin-bound actin filaments. The actin monomers can subsequently reassemble into actin filaments, contributing to the formation of various filamentous actin (F-actin) networks in conjunction with other actin-crosslinking proteins. Lens fiber cells contain diverse F-actin networks that are dynamically rearranged during fiber cell differentiation and maturation, and precise control of these network dynamics is essential for maintaining normal lens biomechanical properties. To elucidate the function of Cap2 in relation to other actin-associated proteins and its impact on lens biomechanics, we conducted comprehensive analyses of the lens phenotype at both cellular and tissue levels in lens-specific Cap2-/- mice.

Methods: The lens-specific Cap2-/- mouse model was created using T(Cryaa-cre)10Mlr mice. Western blotting, immunostaining, confocal microscopy, and mechanical testing were used to investigate the effect of Cap2 deficiency in mouse lenses.

Results: Cap2-deficient lenses exhibit normal size, shape, and transparency. However, they are significantly stiffer under compression and demonstrated enhanced recovery after the removal of a load, in contrast to wild-type lenses. Immunostaining of equatorial sections of Cap2-deficient lenses revealed an increase in fiber cell disorder and misalignments. Notably, although the overall expression of actin and the proportion of F-actin to globular (G)-actin remained unchanged in the absence of Cap2, immunofluorescence staining of single fiber cells revealed an apparent increase in number and size of puncta associated with the actin-crosslinking protein, plastin-3 (aka T-plastin, or fimbrin), where they were associated with fiber cell protrusion and paddle domains.

Conclusions: Deletion of Cap2 in the lens significantly increases lens stiffness, which may be attributed to increased plastin-3 cross-linking of F-actin into compact bundles within mature lens fiber cells.

Wednesday, December 13th

Session 10: Lens Fibrosis and EMT

Organizer: *Janice Walker*
Co-Moderator: *Eri Kubo*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker	Title
10:30 a.m.	 **Janice L. Walker**	A role for UTX/KDM6B lysine demethylase enzymes in driving the progression of the fibrotic response to lens injury
10:48 a.m.	**Eri Kubo**	Interaction between periostin and decorin to induce epithelial-mesenchymal transition in the lens
11:06 a.m.	**Bo Ma**	Connexin 50 Inhibits Lens Epithelial Cells Migration and Epithelial-Mesenchymal Transition
11:24 a.m.	 **Suhotro Gorai**	Web-based tool for visualizing the global injury response in lens epithelial cells

A role for UTX/KDM6B lysine demethylase enzymes in driving the progression of the fibrotic response to lens injury

Janice L. Walker[1,2], Alexander Mazo[3], Svetlana Petruk[3], Andrzej Fertala[4] and Morgan D. Basta[5]

[1]Department of Pathology and Genomic Medicine, Anatomy and Cell Biology, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, USA
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[3]Department of Biochemistry and Molecular Biology, Sidney Kimmel Medical College, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, USA
[4]Department of Orthopedic Surgery, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, USA
[5]Department of Dermatology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Purpose: A negative outcome of cataract surgery wounding is fibrosis, characterized by the persistent accumulation of alpha smooth muscle actin (αSMA)-expressing myofibroblasts, which act as disease culprits due to their pervasive ability to produce copious amounts of extracellular matrix (ECM). There is a need to better understand the molecular underpinnings driving the persistent fibrotic phenotype to lens injury. Here, we sought to determine whether H3K27-specific demethylase (KDM) enzymes, ubiquitously transcribed tetratricopeptide repeat on chromosome X (UTX) and lysine demethylase 6B (KDM6B), which we previously showed are required to decondense chromatin to drive precursor cell transition to myofibroblasts and profibrotic gene expression to initiate a lens fibrotic response, also have a key role in the maintenance of the pro-fibrotic transcriptome and the persistence of the αSMA myofibroblast phenotype for the progression of the lens fibrosis.

Methods: Using an ex vivo post-cataract surgery model that recapitulates the major features of the lens fibrotic disease, posterior capsule opacification, we determined whether UTX/KDM6B demethylase activity is required for the progression of fibrosis. Explants were treated with a vehicle, or an UTX/KDM6B KDM inhibitor, GSK-J4, after a pathological fibrotic phenotype had already been acquired. The impact of treatment on the progression of fibrosis was evaluated by western blot analysis and immunofluorescence, followed by confocal analysis. Myofibroblast proliferation was assessed by EdU.

Results: Inhibiting UTX/KDM6B demethylase activity with GSK-J4 led to a downregulation of the cell cycle and fibrotic ECM genes. UTX/KDM6B demethylase activity was required for increased αSMA expression, expansion of myofibroblast populations, and the continued accumulation of fibrotic matrices, Fibronectin EDA and Collagen I, associated with a progressive and persistent fibrotic phenotype.

Conclusions: Our work shows the therapeutic potential of inhibiting the lysine demethylase activity of UTX/KDM6B to condense chromatin structure, downregulate pro-fibrotic and cell cycle genes, and interrupt the progression of lens fibrosis.

Interaction between periostin and decorin to induce epithelial-mesenchymal transition in the lens

Eri Kubo[1], Teppei Shibata[1], Hidetoshi Ishida[1], Ryo Sakasai[2], Yasuo Yoshitomi[3], Hideto Yonekura[3], Kuniyoshi Iwabuchi[2], Dhrendra P Singh[4], Hiroshi Sasaki[1]

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[3]Department of Biochemistry II, Kanazawa Medical University, Ishikawa, Japan
[4]Department of Ophthalmology, University of Nebraska Medical Center, Omaha, NE, USA

Purpose: Periostin (Postn) and decorin (Dcn) are components of the extracellular matrix that influence the progression of various cancers, skin wound healing, and intraocular proliferative diseases. In this study, we will clarify the role of Postn in posterior capsule opacification (PCO) after cataract surgery, epithelial-mesenchymal transition (EMT), migration in lens epithelial cells (LECs), and the interaction between Postin and Dcn.

Methods: All animal and recombinant DNA experiments were approved by the Kanazawa Medical University Ethics Committee. The rat PCO model was generated using 12-week-old Sprague-Dawley rats. Using Postn knockdown Mouse LEC (Postn KD MLECs) and Postn overexpressing MLECs (Postn OE MLECs), effects of addition of TGFβ1, and FGF2 on the expression of Postn, Dcn and α smooth muscle actin (αSMA) mRNA were analyzed by RT-qPCR and a cell migration assay was performed.

Results: Postn mRNA expression significantly increased in PCO tissues at 1 and 2 weeks after ECLE. Expression of Postn mRNA decreased after the addition of FGF2 and increased with TGFβ1. Dcn mRNA expression was upregulated in the Postn KD MLECs group compared with that in the Cont group. FGF2 treatment significantly increased Dcn mRNA expression in the Cont group, which was even more pronounced in Postn KD MLECs. In Postn KD MLECs, TGFβ treatment did not decrease Dcn expression. αSMA mRNA expression was increased by TGFβ treatment, but its increase was significantly suppressed in Postn KD MLECs. In Postn OE MLECs, cell migration was promoted, whereas in Postn KD MLECs, it was inhibited.

Conclusions: Postn may induce EMT and fibrosis. Suppression of Postn promotes Dcn expression and may suppress EMT. In other words, the suppression of Postn or administration of Dcn may be a potential therapeutic agent for ocular fibroproliferative diseases, including PCO.

Connexin 50 Inhibits Lens Epithelial Cells Migration and Epithelial-Mesenchymal Transition

Bo Ma[1], Zhen Li[1], Guangyan Wang[1], Sumin Gu[1] and Jean X. Jiang[1]

[1]Department of Biochemistry and Structural Biology, University of Texas Health Science Center, San Antonio, TX, USA

Purpose: Our previous results have showed that connexin (Cx) 50 plays an important role in cell-cell adhesion, a process known to inhibit cell migration and epithelial-mesenchymal transition (EMT). The purpose of the current study is to test the hypothesis that the cell adhesive function of Cx50 prevents lens epithelial cell migration and EMT, thereby reducing the occurrence of posterior capsule opacification (PCO) development.

Methods: The primary embryonic chick lens cells (CLCs) and the embryonic chick lens ex vivo mimicking wound repair model were infected with RCAS(A) retrovirus containing the vehicle, Cx50WT, Cx50 adhesion-impaired E2 domain mutants: G186S and W188P. These cultured cells were treated with 10 ng/mL TGF-β2 and western blotting and immunofluorescence were used to assess the expression of protein markers associated with cell adhesion, proliferation, migration and EMT. Additionally, an extracapsular lens extraction (ECLE) study was performed in WT and Cx50KO mice. The isolated eyeballs were processed for immunofluorescence to determine the expression of EMT, ECM, and Macrophages markers.

Results: TGF-β2 treatment decreased the expression of Cx50 and E-cadherin while increasing the expression of α-SMA and fibronectin in the CLCs. These effects were suppressed by the overexpression of Cx50WT. Moreover, in the ex vivo model, lens epithelial cells (LECs) infected with Cx50WT exhibited significantly reduced migration and EMT compared to vehicle. However, Cx50 E2 domain mutants, G186S and W188P, significantly increased the expression of α-SMA and fibronectin in CLCs and promoted cell migration in the ex vivo model. Additionally, Cx50 was found to be co-localized with β-catenin and E-cadherin on the surface of LECs and prevented loss of TGF-β2-induced E-cadherin and nuclear translocation of β-catenin. Furthermore, the expression of EMT and macrophages markers in Cx50KO mice are significantly higher than that in WT mice.

Conclusions: The results suggest that Cx50 could effectively suppress the migration of LECs, inhibit EMT, and prevent PCO development possibly through its interaction with β-catenin and E-cadherin. The adhesive function of the Cx50E2 domain plays a critical role in inhibiting the EMT process.

Web-based tool for visualizing the global injury response in lens epithelial cells

Suhotro Gorai[1], Adam P. Faranda[1], Melinda K. Duncan[2]

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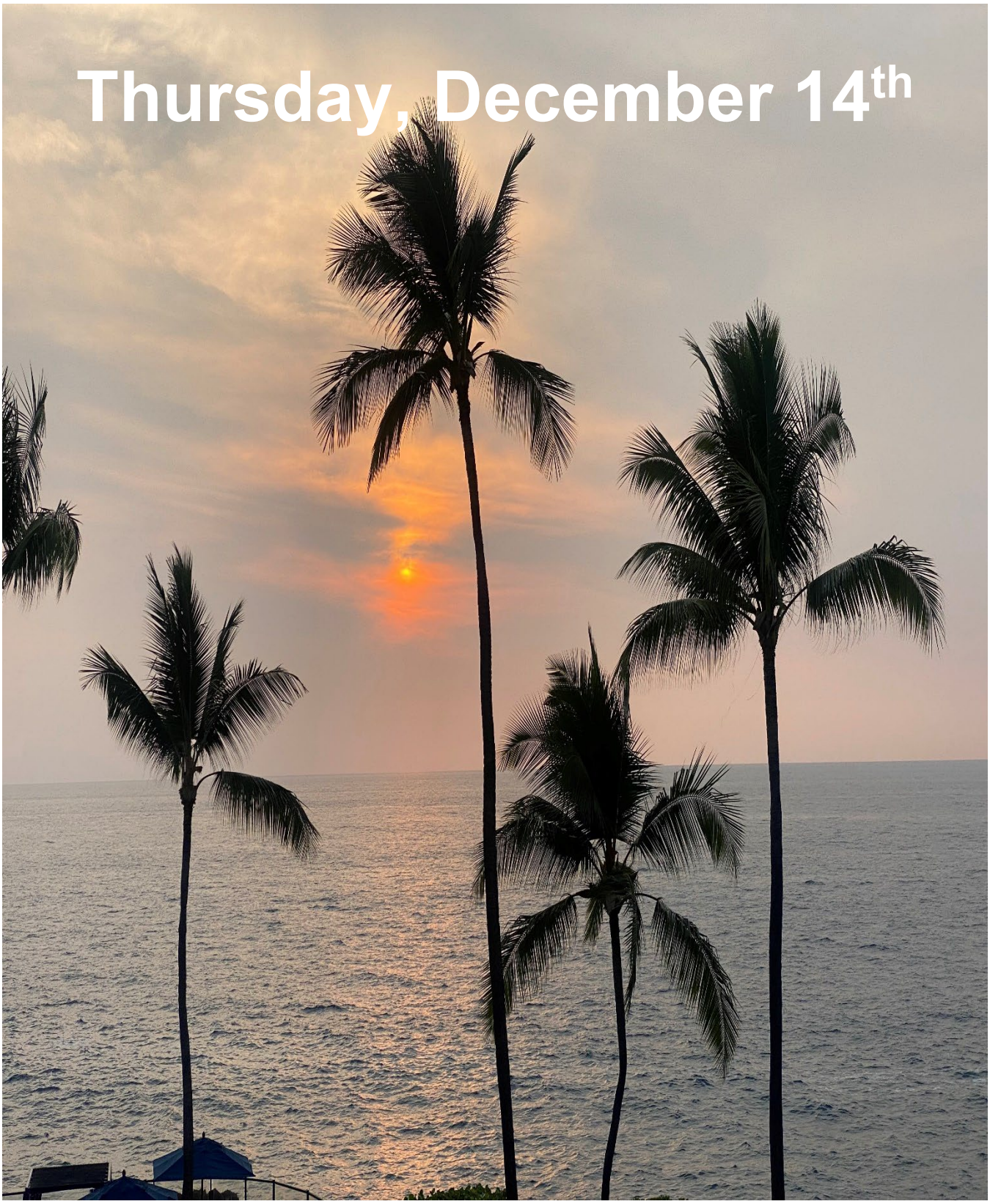
Purpose: Cataract surgery involves lens fiber cell removal followed by intraocular lens implantation. However, the lens epithelial cells (LECs) remaining behind post-surgery undergo an injury response which can compromise long term visual outcomes. In some cases, this causes a re-clouding of the visual axis which is known as posterior capsular opacification (PCO). Our lab has generated numerous RNA-seq datasets that reveal the time course of transcriptomic changes occurring in LECs in response to surgery. However, these large datasets are cumbersome to query simultaneously limiting their utility in hypothesis generation. Here we describe a user-friendly and interactive data visualization online tool for the lens injury response time series (LIRTS).

Methods: Existing RNA-seq datasets from wildtype mouse LECs at 0h, 6h, 24h, 48h, 72h and 120h post injury were reanalyzed using a standard pipeline to obtain fragments per kilobase million (FPKM) values for all genes using R. This data is being queried and visualized using a framework consisting of ReactJS, NodeJS, PlotlyJS and Typescript. The resulting web-based visualization tool is hosted on an in-house server at University of Delaware.

Results: The LIRTS tool allows users without bioinformatics experience to visualize the expression time course for any gene of interest during the LEC wound healing response via either a normalized boxplot, a scatterplot or an overlay of scatterplot on boxplot expressing FPKM values obtained for each analyzed sample. The graphs are downloadable in SVG format for use in publications.

Conclusions: This visualization tool has already contributed to numerous ongoing studies by helping in prediction of genes involved in the lens injury response driving PCO leading to both a recent publication and grant funding. This tool will aid researchers interested in the pathogenesis of PCO, and fibrotic diseases in general, understand the dynamics of gene expression during injury induced epithelial to mesenchymal transition of LECs.


Thursday, December 14th

Thursday, December 14th

Session 11: Lens Genetics and Cataract

Organizer: *Elena Semina*
Co-Moderator: *Fielding Hejtmancik*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker	Title
8:30 a.m.	 **Fielding Hejtmancik**	The c.119-123dup5bp mutation in human γC-crystallin, destabilizes the protein and activates the unfolded protein response to cause highly variable cataracts
8:48 a.m.	 **Hélène Choquet**	Transcriptome-wide association study based on 54 tissues identifies novel candidate susceptibility genes for cataract
9:06 a.m.	 **Elena V Semina**	Dido1 is involved in vertebrate lens development, with a possible role in human disease
9:24 a.m.	**Jia Ling Fu**	Sumoylation of HSF4 at K293 by SUMO2/3 Attenuates Its Transcription Activity and Modulates the Resistance Against Stress-Induced Cataractogenesis
9:42 a.m.	 **Johanna L Jones**	Analysis of isolated congenital cataract genes in Australian families

The c.119-123dup5bp mutation in human γC-crystallin, destabilizes the protein and activates the unfolded protein response to cause highly variable cataracts

J. Fielding Hejtmancik[1], Venkata Pulla Rao Vendra[1], Christian Ostrowski[1], Zhiwei Ma[1], Mohd Hussain Shah[1]

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Purpose: Ordered cellular architecture and high concentrations of stable crystallins are required for the lens to maintain transparency and the high refractive index necessary to focus the light on the retina. Mutations in crystallin genes are known to affect their folding, compactness, stability and solubility thereby causing congenital cataracts. Here we investigated the molecular mechanism of cataractogenesis of the CRYGC c.119-123dupGCGGC (p.Cys42AlafsX63) (CRYGC5bpd) mutation

Methods: Lenses were extracted from wild type and transgenic mice carrying the CRYGC5bpdup minigene and RNA was isolated and converted into cDNA. Expression of genes in the unfolded protein response (UPR) pathways was estimated by qRT-PCR and RNA seq.

Results: P3W Transgenic mice exhibited phenotypic diversity with a dimorphic population of severe and clear lenses. PCA of RNA seq data showed clustering of wild-type and clear CRYGC5bpd lens groups separate from severe CRYGC5bpd lenses. Transgenic mice did not show IRE1α dependent activation (Xbp1 splicing) but showed differential upregulation in Master regulators (GRP78) and downstream targets in the PERK-dependent UPR pathway including ATF4, eIF2A, CHOP and GADD34. Genes in the mitochondrial UPR pathway including ATF4, ATF5, CHOP, and C/EBPbeta were also elevated. ER chaperones (GRP94, P58IPK and ERP72) and Pro-survival genes (Trib3 and Irs2) also showed higher levels of expression compared to mutant clear and wild-type lenses

Conclusions: High levels of CRYGC5bpd transgene expression in severely affected lenses induces ER stress through the PERK-dependent and mitochondrial pathways causing apoptosis and congenital nuclear cataracts. This effect is correlated to CRYGC5bpd transgene expression so that lower expression levels result lower levels of UPR activation with delayed onset of cataract, offering insight into cataract pathogenic pathways in humans.

Transcriptome-wide association study based on 54 tissues identifies novel candidate susceptibility genes for cataract

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Purpose: Cataract is the leading cause of blindness among the elderly worldwide. Twin and family studies support an important role for genetic factors in cataract susceptibility with heritability estimates up to 58%. Our recent large and multiethnic genome-wide association study (GWAS) meta-analysis identified 55 genetic loci for cataract. However, how the majority of these loci impact cataract etiology is unknown. To identify novel genes associated with cataract and examine the impact of their expression, we conducted a transcriptome-wide association study (TWAS).

Methods: We performed tissue-specific and multi-tissue TWAS analyses to assess associations between imputed gene expression from 54 tissues (including 49 from the Genotype Tissue Expression (GTEx) Project v8) with cataract using FUSION software. Meta-analyzed GWAS summary statistics from 59,944 cataract cases and 478,571 controls, all of European ancestry and from two cohorts (GERA and UK Biobank) were used. Finally, we examined the expression of the novel genes in lens tissues using the iSyTE database.

Results: Across tissue-specific and multi-tissue analyses, we identified 46 genes for which genetically predicted gene expression was associated with cataract after correcting for multiple testing. Interestingly, 23.9% of the identified genes were found in the 11q13.3 genomic region which was previously identified in our GWAS. Of these 46 genes, 5 (GSTM1, GSTM2, GSTM4, INSRR, and CEP95) did not overlap with known cataract-associated loci identified from GWAS. Tissue-specific analysis identified 202 gene-tissue pairs and brain tissues represented the highest proportion of the Bonferroni-significant gene-tissue pairs (21.8%), followed by gastrointestinal (21.3%), and cardiovascular tissues (18.8%). Gstm1, Gstm2, Gstm4 and Cep95 were robustly expressed in iSyTE lens data.

Conclusions: Our TWAS reports novel genes for cataract susceptibility, including three members of the glutathione S-transferase (GST) supergene family. Our findings also highlight the fact that expression of genes associated with cataract susceptibility is not necessarily restricted to lens tissue.

Session 11-3

Dido1 is involved in vertebrate lens development, with a possible role in human disease

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Purpose: A family with a history of dominant ocular phenotype involving early onset cataract, lattice degeneration and retinal detachment enrolled into the research study to identify the genetic cause for the observed phenotype.

Methods: Human DNA samples were analyzed by exome sequencing. Expression studies in zebrafish were performed by in situ hybridization with dido1-specific RNAscope® probes. The mutant line sa14468 carrying dido1 premature termination allele was obtained from ZIRC and characterized by gross morphology, histology and immunohistochemistry.

Results: A novel missense variant p.Ala1425Val in DIDO1 co-segregated with the cataract phenotype in all seven affected individuals. An additional intronic VCAN:c.4004-5T>C variant was present in six individuals affected with lattice degeneration/retinal detachment and cataract but absent in the individual affected with cataract only. Zebrafish dido1 showed robust expression during eye development. Zebrafish dido1^{sa14468} homozygotes demonstrated variable pigmentation defects, early onset cataract and juvenile lethality.

Conclusions: VCAN encodes extracellular matrix protein isoforms that regulate matrix assembly, cell adhesion, migration, differentiation, survival, and proliferation. Variants in VCAN cause dominant Wagner vitreoretinopathy, characterized by abnormal vitreous, chorioretinal atrophy with loss of the retinal pigment epithelium, lattice degeneration, presenile cataracts, myopia, and retinal detachment. The identified intronic VCAN variant is classified as likely pathogenic for the observed phenotype. DIDO1 encodes a Death InDucer-Obliterator 1, a BMP-specific Smad-regulated target gene. Previous studies demonstrated a role for Dido1 in promoting the attachment, migration, invasion and apoptosis resistance of melanoma cells. The presented zebrafish data implicate dido1 in lens development. While the identified DIDO1 allele represents a variant of uncertain significance, it may be contributing to the cataract phenotype in the affected family.

Sumoylation of HSF4 at K293 by SUMO2/3 Attenuates Its Transcription Activity and Modulates the Resistance Against Stress-Induced Cataractogenesis

Jia-Ling Fu[1], David W. Li[1]

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Purpose: Heat shock factor 4 is an important transcription factor for the maintenance of lens transparency, and the dysfunction of HSF4 is associated with congenital cataract. In this study, we analyzed the sumoylation regulation on HSF4 function.

Methods: K293R mice were generated using CRISPR/Cas9 technology. Both wildtype and HSF4 K293R mice were used as testing systems. Co-IP and GST pull down assays were used to determine the sumoylation of HSF4 at specific residue. EMSA and Dual luciferase reporter gene assay, and ChIP were used to detect its transcriptional activities and control of downstream genes. Western blot and WES were used to detect the protein expression levels of HSF4 and its downstream target proteins. Immunofluorescence was used to analyze the localization of HSF4 and its downstream target proteins.

Results: Co-IP and GST pull down assays demonstrated that HSF4 is sumoylated by sumo2/3 conjugation at the K293 site in mouse lens and human lens epithelial cells. EMSA and dual luciferase reporter gene assay and ChIP assay revealed that sumoylation of HSF4 by SUMO2/3 attenuates its transcriptional activity. The K293R mouse lens displayed differential susceptibility to stress-induced apoptosis and cataractogenesis.

Conclusions: Our results suggest that SUMO2/3-mediated congjugation of HSF4 at K293 significantly attenuates its transcriptional activity and also modulates the resistant ability against stress-induced apoptosis and cataractogenesis.

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Analysis of isolated congenital cataract genes in Australian families

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Purpose: The aim of this study is to identify disease-causing variants in Australian families with isolated pediatric or congenital cataract (CC).

Methods: Probands from 95 Australian families were screened for rare, coding variants in 63 known isolated CC genes using whole-genome sequencing and segregation in the family was confirmed with Sanger sequencing. The ACMG-AMP guidelines were used to classify the pathogenicity of segregating variants. Extended gene lists and non-coding or structural variants were assessed in unsolved families.

Results: Pathogenic or likely pathogenic variants were identified in 16 families. Novel variants were identified in genes CRYBB1, CRYBB2, BFSP1, LONP1, GJA8 and PITX3 and previously reported variants in CRYAA, CRYBB2, COL4A1, GJA3, HSF4 and NHS. Thirty families only had variants of uncertain significance (VUS). Novel VUS in CRYBB1, CRYBB3, BFSP2, GJA8, HSF4 and MIP almost reach a likely pathogenic classification but require more evidence. A large intronic deletion in NHS was identified in one family and evaluation of non-coding and structural variants is ongoing in the remaining 46 unsolved families.

Conclusions: A molecular diagnosis has been achieved for 16 families using a standard cataract gene panel. This included a de novo NHS p.Arg248Ter variant in a female who can now be accurately counselled on the risk of having a son with Nance-Horan Syndrome. Many VUS were identified with at least six being the likely cause of disease, but cannot be returned to patients without further evidence. This highlights the need for establishing robust pipelines for functional assessment of variants and classification criteria specific to CC genes to improve diagnostic rates. The genome sequencing data is enabling assessment of novel genes, non-coding regions and structural variants in the unsolved families. These findings expand our current understanding of known congenital cataract genes and progress us towards better genetic testing outcomes for patients.

Thursday, December 14th

Session 12: Biology of the Aging Lens and Redox Biology

Organizer: *Xingjun Fan*
Co-Moderator: *Rachel Martin*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker	Title
10:30 a.m.	 **Marjorie Lou**	The Ying-Yang effect, or the damage and benefit of reactive oxygen species (ROS)to the lens – A review
10:48 a.m.	 **Rachel Martin**	From calcium storage to vision, by way of biomineralization: Tunicate βγ-crystallin
11:06 a.m.	 **Julie Lim**	Interactions between the lens and ocular humors: does the lens contribute to the maintenance of high levels of ascorbic acid in the vitreous?
11:24 a.m.	 **Lee Goldstein**	Tracking molecular aging and hyperglycemia in murine and human lenses by in vivo quasi-elastic light scattering spectroscopy
11:42 a.m.	 **Xingjun Fan**	Deficiency in glutathione peroxidase 4 (GPX4) results in abnormal lens development and newborn cataract

The Ying-Yang effect, or the damage and benefit of reactive oxygen species (ROS) to the lens – A review

Marjorie F. Lou[1,2]

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Purpose: Reactive oxygen species (ROS) are known to be toxic and harmful byproducts of living in an aerobic environment. These species include superoxide anion (O_2-), hydroxyl radical (OH−) and hydrogen peroxide (H_2O_2). The long-lived H_2O_2 is freely diffusible and can be made internally through some enzyme systems or externally from ultraviolet light (UV) or ionizing radiation. The lens is rich in antioxidants, such as glutathione (GSH), plus other detoxification enzyme systems to protect it from oxidative damage, but the prolonged exposure to ROS during the long lifespan is considered as the leading cause for senile cataract formation. On the other hand, ROS have also been shown to be beneficial to the lens, as they are essential elements in mediating growth factor's mitogenic function during cell proliferation.

Methods: Protein thiol oxidation or protein thiolation (protein-SS-glutathione or PSSG, protein-SS-protein or PSSP) has been the main focus by demonstrating its early oxidative damage that can lead to protein aggregation and cataractous formation. In vivo and in vitro animal models with H_2O_2 or UV exposure were used to examine the levels of protein thiolation, GSH and the activity of dethiolation enzyme, such as thioltransferase (glutaredoxin). Clear human lenses of 1st to 7th decades as well as cataractous lenses with various severity were studies. The beneficial aspect of ROS was examined with human lens epithelial cells (HLE B3) for cell growth, and cell migration using various concentrations of H_2O_2.

Results: Animal models clearly showed that only oxidation could cause protein thiolation. ROS induced early stage PSSG formation not only caused lens crystallin to unfold, but also modified the protein into PSSP, with eventual protein aggregation. Human lenses showed elevated levels of PSSG and PSSP that were inversely related to the decreased GSH level and thioltransferase activity during aging, especially over 60-70 age groups. In cataratous lenses, PSSG level was also inversely related to that of GSH content and the antioxidant enzyme activities in the same lens. The benefit of ROS was demonstrated that H_2O_2 was not only essential in the growth factor cell signaling event but also at certain low levels it could mimic growth factor to promote proliferation in lens epithelial cells and other physiological functions, such as cell migration.

Conclusions: This Ying-Yang aspect of H_2O_2 indicates the importance of redox regulation and balance for the general health of cells and tissues, and certainly to the lens.

From calcium storage to vision, by way of biomineralization: Tunicate βγ-crystallin

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Purpose: The ubiquitous vertebrate βγ-crystallins are thought to have evolved from a Ca^{2+}-binding ancestor, similar to the extant microbial crystallins. The βγ-crystallin from the tunicate Ciona intestinalis represents a transitional protein with both functions, as it is expressed in both the palps and the visual organ. Studying the structure, divalent cation binding, and optical properties of this protein can provide insight into the evolution of modern vertebrate lens proteins.

Methods: Binding of recombinantly produced tunicate βγ-crystallin to Ca^{2+} and other divalent metal ions was measured using isothermal titration calorimetry. NMR spectroscopy was used to investigate detailed interactions between the proteins and the metal ion at the residue level. Optical spectroscopy provides information about the biophysical characteristics of the cation-bound and apo states.

Results: Tunicate βγ-crystallin strongly binds to Ca^{2+}, and addition of the metal ion greatly increases its stability. Using NMR spectroscopy, we have identified the specific residues responsible for the binding and investigated the protein's dynamics.

Conclusions:

These data provide insight into how Ca^{2+}-binding in tunicate βγ-crystallin stabilizes the protein. Comparisons with human β- and γ-crystallins suggest alternative mechanisms for stabilization in the mammalian crystallins, which lack these Ca^{2+}-binding sites.

Interactions between the lens and ocular humors: does the lens contribute to the maintenance of high levels of ascorbic acid in the vitreous?

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Purpose: In previous work, we have shown that the lens is capable of exporting the antioxidant glutathione (GSH) into the humors and potentially protecting neighbouring tissues from oxidative stress. In this study, we have examined whether the lens can also act as a source of ascorbic acid (AsA) particularly to the vitreous humor, to maintain low levels of oxygen in the back of the eye.

Methods: To determine the contribution of the lens as a potential source of AsA to the vitreous humour, vitreous was collected from patients undergoing vitrectomy who either had a natural clear lens (n=10) or an artificial intraocular implant (IOL) (n=10) and AsA and oxidised ascorbic acid (DHA) measured using a biochemical assay. To determine whether lenses could directly export AsA, human donor lenses of varying ages (n=15 donors) were cultured in artificial aqueous humour (AAH) for one hour under hypoxic conditions and AsA and DHA measured. Human lenses (n=6 donors) were also cultured in compartmentalised chambers to determine whether efflux of Asa and/or DHA could occur at the anterior or posterior surface.

Results: Analysis of vitreous from patients undergoing vitrectomy revealed that AsA levels were decreased in the IOL compared to the natural lens group suggesting that the lens may contribute to AsA levels in the vitreous. Cultured human donor lenses were also shown to release both AsA and DHA with efflux occurring at both surfaces, although efflux was in the µM range.

Conclusions: The levels of AsA exported by the human lens are relatively low and are unlikely to responsible for sustaining the high levels of AsA known to exist in the vitreous. However, AsA exported by the lens may help to provide a localised supply of antioxidant protection at the interface between the lens and vitreous to minimise oxygen exposure to the lens.

Tracking molecular aging and hyperglycemia in murine and human lenses by in vivo quasi-elastic light scattering spectroscopy

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Purpose: The absence of clinical tools to evaluate individual variation in the pace of aging represents a major impediment to understanding aging and maximizing health throughout life. The lens is an ideal tissue for quantitative assessment of molecular aging in vivo. Long-lived proteins in lens fiber cells are expressed during fetal life, do not undergo turnover, accumulate molecular alterations throughout life, and are optically accessible in vivo. This study is designed to longitudinally measure both age- and glycemia-dependent signals in vivo in lenses of control C57BL/6 mice and in mice with chemically-induced diabetes, in addition to cross-sectional clinical validation in humans. We hypothesized that cumulative alterations in lens proteins may constitute an *in vivo* biomarker of molecular aging, and that the rate of these changes will be accelerated by chronic diabetic hyperglycemia.

Methods: Quasi-elastic light scattering (QLS) was used to measure: (i) longitudinal age-dependent changes *in vivo* in lenses of unanesthetized C57BL/6 mice aged 4–18 months, (ii) longitudinal glycemia-dependent changes in lenses of unanesthetized C57BL/6 mice aged 4–10 months treated with streptozotocin (STZ), a model of type-1 diabetes, and (iii) cross-sectional analysis of human subjects with type-1 diabetes compared with age-matched controls.

Results: Our results indicated that QLS metrics can be acquired noninvasively in unanesthetized mice and human subjects with diabetes. Both static and dynamic light scattering were significantly altered in lenses of mice with diabetes compared to controls, with some of those changes recapitulated in aged mice and human subjects with diabetes.

Conclusions: Our findings demonstrate that QLS analysis of lens proteins provides a practical technique for noninvasive assessment of molecular aging and long-term glycemic status *in vivo*.

Deficiency in glutathione peroxidase 4 (GPX4) results in abnormal lens development and newborn cataract

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Purpose: Both lens crystallins and lipids are known for their longevity and minimal turnover rates, particularly within the core region. These enduring proteins and lipids are susceptible to accumulating damage over time, which is regarded as the central pathogenic mechanism underlying age-related cataract development. It has been observed that lipid peroxidation increases with age in both human and mouse lenses, yet the precise contribution of lipid peroxidation to cataractogenesis remains a subject of debate. In our previous study (published in Free Radical Biology and Medicine, 2021, Volume 167, Pages 94-108), we provided evidence that aging lens epithelial cells are susceptible to a specific form of cell death known as ferroptosis. Building upon this research, we embarked on an investigation to elucidate the role of lipid peroxidation in the formation of cataracts, with a particular focus on the key detoxification enzyme glutathione peroxidase 4 (GPX4).

Methods: Mouse lens cataracts were meticulously examined using slit lamp and darkfield microscopy techniques. Lens epithelial cell counts were conducted through a series of hematoxylin and eosin (HE) stained sections. Both apoptosis and ferroptosis markers were assessed through Western blot analysis and immunostaining procedures. To investigate potential rescue mechanisms, we administered intraperitoneal injections of Liproxstatin-1 to Gpx4 knockout (KO) mice. Gpx4 was selectively knocked out using CRISPR-Cas9 technology in human lens epithelial cells (FHL124). We assessed cell viability through a CCK8 assay, and lipid peroxidation levels were quantified using C11-Bodipy 581/591 fluorescence techniques.

Results: In vitro investigations revealed that FHL124 cells lacking Gpx4 (Gpx4 KO) cannot survive without the presence of a ferroptosis inhibitor, Liproxstatin-1. However, when both cytosolic and mitochondrial forms of Gpx4 were overexpressed in Gpx4 KO LECs, cell death was rescued.

Ex vivo experiments demonstrated that inhibiting GPX4 activity in cultured mouse lenses resulted in lens opacity and cellular demise. In vivo studies corroborated these findings, as Gpx4 deletion led to the development of newborn cataracts and microphthalmia. Furthermore, our comprehensive analysis indicated that markers associated with ferroptosis, rather than apoptosis, exhibited a significant increase in the Gpx4 KO lens.

Conclusions: Our studies demonstrate that Gpx4 protects the lens against lipid oxidation; loss of Gpx4 causes mouse lens cataracts. Targeting lipid peroxidation is a possible way to prevent cataracts.

Thursday, December 14th

Session 13: Lens Pathology and Regulatory Pathways

Organizer: *Vasanth Rao*
Co-Moderator: *Melinda Duncan*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker	Title
1:15 p.m.	 **Alan Shiels**	A TRPM3 mutation disturbs cation homeostasis and gene expression in the lens
1:33 p.m.	 **David Li**	A Chaperone System Guards Normal Lens Development and Prevents Cataractogenesis
1:51 p.m.	 **Peter Huynh**	Eph and ephrin gene expression changes in the ocular lens of aging and knockout mice
2:09 p.m.	 **Melinda Duncan**	The molecular basis of aniridic cataract
2:27 p.m.	 **Vasanth Rao**	Regulatory Mechanisms of Myosin II Activity and Their Influence on Lens Function

A TRPM3 mutation disturbs cation homeostasis and gene expression in the lens

Alan Shiels[1], Thomas M. Bennett[1], Philip A. Ruzycki[1], Zhaohua Guo[2], Yu-Qing Cao[2], Mohammad Shahidullah[3], Nicholas A. Delamere[3], Yuefang Zhou[1]

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Purpose: Transient receptor potential cation channel, subfamily M (melastatin), member 3 (TRPM3) has been shown to function as a Ca^{2+} channel when activated by the neurosteroid pregnenolone sulfate (PS). A missense mutation resulting in an isoleucine-to-methionine substitution (p.I65M) in TRPM3 has been shown to underlie an inherited form of early-onset cataract in humans and mice. Here we model the pathogenic effects of this TRPM3 mutation in the mouse lens and human cell-lines.

Methods: In situ hybridization and immunofluorescence confocal microscopy were used to localize gene expression in the eye. CRISPR gene-editing technology was used to generate knock-in mutant mice and human lens epithelial (HLE-B3) cell-lines. Lens cation levels, cytosolic Ca2+ levels, protein kinase levels, and gene expression levels were determined by atomic absorption spectroscopy, plasmid transfection and fluorescence imaging techniques, immunoblotting, and RNA-sequencing, respectively.

Results: TRPM3 is strongly expressed in the lens epithelium and other non-pigmented and pigmented ocular epithelia. TRPM3-mutant lenses displayed a Na+:K+ imbalance, increased water content, increased Ca2+ levels, and like TRPM3-mutant HLE-B3 cell-lines, exhibited elevated phosphorylation of mitogen-activated protein kinase 1 (MAPK1/ERK2/p42) and MAPK3/ERK1/p44 compared to wild-type. Mutant TRPM3-M65 channels displayed an altered dose response to PS activation and an increased sensitivity to extracellular Ca2+ compared to wild-type TRPM3-I65 channels when over-expressed in HEK-293T cells. TRPM3-mutant lenses shared dysregulation of genes involved in membrane secretion, transport, and repair when compared to wild-type. By contrast, TRPM3-deficient lenses did not display any of the pathophysiological changes observed in TRPM3-mutant lenses.

Conclusions: Collectively, our data suggest that TRPM3-related early-onset cataract results from a deleterious gain-of-function rather than a loss-of-function mechanism in the lens.

A Chaperone System Guards Normal Lens Development and Prevents Cataractogenesis

David Wan-Cheng Li[1], Jia-Ling Fu[1], Shu-Yu Zheng[1], Yan Wang[1], Xue-Bin Hu[1], Yuan Xiao[1], Lan Zhang[1], Jing-Miao Wang[1], Yu-Wen Gan[1] and Xing-Miao Liang[1]

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Purpose: Cataract is derived from protein aggregation due to genetic mutations, stress, and aging. The damaged protein aggregates are bound by α-crystallins, the small heat shock proteins which were shown to possess chaperone activities initially demonstrated by Dr. Horwitz's laboratory in 1992 and subsequently by many others. Lack of ATP binding and hydrolysis ability limit their functions as chaperones and could not recycle the bound proteins into cytoplasm. Thus, maintaining lens transparency requires functions of true chaperone proteins. Here, I will discuss systematically the new chaperone system in the ocular lens.

Methods: Human lens capsular epithelia, lens epithelial cell lines, zebrafish and mice were used as testing systems. QRT-PCR and Wes were used to analyze mRNA and protein expression. Morppholino Oligos and CRISPR/Cas9 technology were used to silence gene expression in ex vivo and in vivo.

Results: HSP90, HSP70, HSP60 and HSP40 in normal and cataract human lenses, and in young and aged mouse lenses were analyzed, and it is found that HSP90β is a dominantly expressed heat shock protein in lens. From transparent human lens to cataract patients, also from young to aged mouse lens, HSP90β is significantly downregulated. Silence of HSP90β expression in lens epithelial cells of zebrafish caused significant in vivo apoptosis followed by cataractogenesis.

Conclusions: HSP90β acts as an important chaperone to guard normal lens development and prevent cataractogenesis. (Supported by NSFC grants #82271071, #81970787, #81770910, and NSFG joing grant #2019B1515120014), and the Fundamental Funds, 3030901010111 of the State Key Laboratory of Ophthalmology of Zhongshan Ophthalmic Center).

Eph and ephrin gene expression changes in the ocular lens of aging and knockout mice

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Purpose: Eph-ephrin signaling has been characterized as a major contributor to maintaining lens transparency. Human mutations in the EphA2 receptor and ephrin-A5 ligand have been linked to age-related cataracts. In mice, loss of *Epha2* leads to cortical or nuclear cataracts, while deletion of *Efna5* (ephrin-A5) manifests as anterior polar cataracts. Notably, EphA2 and ephrin-A5 do not appear to be an exclusive ligand-receptor pair in the lens. However, characterizing functional binding partners has been a formidable endeavor due to the presence of 12 of 14 known Ephs and 8 of 8 known ephrins in the lens. Here, we identify differentially-expressed Eph and ephrin genes in wild-type (control) and knockout (KO) lenses in young and aged mice as putative leads in normal lens aging and cataract formation.

Methods: Lenses from wild-type, *Epha2* KO, or *Efna5* KO mice were harvested at young adult (6-9 weeks) and aged (7-8 months) stages. Lenses were separated into epithelial and fiber cells, and gene expression changes were examined using real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR).

Results: qRT-PCR quantification of Eph and ephrin genes reveal transcript expression level changes resulting from *Epha2* or *Efna5* KOs that selectively change with age. For example, *Epha1* is highly upregulated in the epithelial cells of aged lenses, regardless of genotype. Meanwhile, *Efna4* (ephrin-A4) is upregulated in the fiber cells of aged *Epha2* KO mice, but not in *Efna5* KO mice. Overall, we identified permutations of gene expression changes that are cell-type specific, exclusive to either *Epha2* or *Efna5* KOs, and some that change with age regardless of genotype.

Conclusions: Ephs and ephrins maintain lens transparency through diverse signaling mechanisms that change as a mouse ages. Expression changes with age ostensibly indicate a temporally-restricted cross-talk between various Eph-ephrin pairings that warrant further study into the mechanisms that result in cataract formation.

The molecular basis of aniridic cataract

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Purpose: Heterozygous Pax6 mutations in humans cause Aniridia; a disorder which impairs vision at birth due to foveal hypoplasia and iris malformations. Later, humans with aniridia develop juvenile onset cortical and/or anterior subcapsular cataracts (ASC) although their molecular etiology is unknown. This investigation establishes a mouse model of aniridic cataract and uses it to investigate the molecular mechanisms underlying this condition.

Methods: Lens/corneal diameter ratios were determined in human patients with aniridia and compared to those with albinism. The lens phenotype of Pax6+/^{tm1Pgr} mice (Sey) was evaluated over their first year of life by dark field imaging and hematoxylin/eosin staining. RNAseq was used to determine how the ocular lens transcriptome is affected in young adult mice haploinsufficient for Pax6, and bioinformatic analyses performed.

Results: Aniridic lenses have a significant reduction in lens diameter compared to controls similar to Sey mice. Over 1000 genes are differentially expressed (DEGs) in 20 week old Sey LECs, and over 400 DEGs were detected in Sey LFCs in contrast to prior reports that found newborn Sey lenses to exhibit very few DEGs. Of these, nearly 200 of these DEGs were previously experimentally verified as Pax6 targets in the lens by Chip-seq (PMID: 23342162). The DEGs found in both cell types were significantly enriched for genes involved in WNT and Tgfβ signaling as well as ion homeostasis. In Sey LECs, the upregulated DEGs included several pro-inflammatory genes and known fibrotic markers.

Conclusions: The aniridic lens is reduced in size compared to lenses from patients with albinism, similar to adult mice with Pax6 mutations. Sey mice develop lens abnormalities with age. Sey lens cells have molecular changes consistent with several well established cataractogenic mechanisms including inflammation, pro-fibrotic changes, and alterations in ion homeostasis. This demonstrates that the correct Pax6 dosage is critical for adult lens homeostasis.

Regulatory Mechanisms of Myosin II Activity and their Influence on Lens Function

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Purpose: Nonmuscle myosin II (NMII), an actin-binding motor protein, plays a vital role in lens morphogenesis, growth, mechanics, and function. Mutations in NMIIA lead to cataract development in humans, yet little is known about the regulation of NMII activity in the lens. In this study, we investigate the expression and distribution profiles of various proteins involved in the regulation of NMII activity in the mouse lens and their roles in lens growth and function.

Methods: We utilized various approaches, including RNAseq, biochemical analyses, immunofluorescence imaging, pharmacological agents, siRNA, and gene-targeted mouse models, to determine the expression and distribution profiles of proteins involved in the regulation of NMII activity and their potential impact on lens function.

Results: Our findings reveal that NMIIA is distributed in both the lens epithelium and fibers, whereas NMIIB is predominantly concentrated in the epithelium. Phosphorylated myosin light chain, and myosin light chain kinase are robustly distributed in the lens fibers. Interestingly, LIMCH1, a regulator of myosin light chain (MLC) phosphorylation, and calponin-3, a regulator of myosin II activity, exhibit discrete distribution patterns in the epithelium. Conversely, S100A4, a regulator of myosin II assembly, is discretely distributed in the fibers. The lens also expresses Rho kinases-1 & 2 and ZIPK. Additionally, myosin phosphatase Rho-interacting protein (MPRIP), a regulator of myosin phosphatase, is expressed at relatively high levels in the mouse lens. Inhibition of MLCK kinase induces nuclear cataracts, while the absence of S100A4 leads to late-onset cataracts in association with decreased NMIIA phosphorylation. In contrast, Rho kinase inhibition has a milder impact on lens function.

Conclusions: Our findings suggest that the lens is equipped with both shared and distinct molecular mechanisms that regulate myosin II activity in the epithelium and fibers. Dysregulation of these regulatory mechanisms appears to affect lens architecture and function.

Thursday, December 14th

Session 14: Lens Clinical Studies and Treatment

Organizer: *Hiroshi Sasaki*
Co-Moderator: *Hiroyuki Matsushima*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker	Title
3:00 p.m.	**Natsuko Hatsusaka**	The risk of developing pterygium and various cataract types due to ocular UV exposure during childhood
3:18 p.m.	**Norihiro Watanabe**	The microstructure for zonular fibers under ocular inflammation
3:36 p.m.	**Shinichiro Kobayakawa**	
3:54 p.m.	**Hiroyuki Matsushima**	Current Status of Intraocular Lens Long-Term Stability
4:12 p.m.	**Mayumi Nagata**	Differences in Intraocular Inflammation in the Anterior chamber Depending on the Position of Intraocular Lens

The risk of developing pterygium and various cataract types due to ocular UV exposure during childhood

Natsuko Hatsusaka[1], Eri Kubo[1], Hiroshi Sasaki[1]

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Purpose: This epidemiologic study of adults on Iriomote Island, Okinawa Prefecture, which has the highest UV intensity in Japan (194 J/m2), examined the relationships between cumulative ocular UV exposure from childhood to present and the risk of pterygium and cataract development.

Methods: This study surveyed 279 adults aged ≥40 years (mean age: 55.7±10.4) in 2019. Among the enrolled individuals, half were native to Okinawa, while the rest had moved to Okinawa as adults. Information was collected via questionnaires on participants residences from childhood to present (including childhood, elementary/middle school years, high school years, ages 18-29, 30-39, and 40 years to present age), time spent outdoors during each period, and whether they used glasses or hats. Cumulative ocular UV exposure (COUV) was calculated from the information collected.

Pterygium and cataract were graded by one physician using a slit-lamp microscope. Cataracts were categorized into cortical, nuclear, posterior subcapsular (PSC), retrodot (RD), and watercleft (WC). For cortical and WC cataracts, opacity within 3 mm of pupil center was determined. Associations between COUV and the risk of developing pterygium and cataract were examined using logistic regression adjusting for age and sex.

Results: Pterygium prevalence was significantly higher among Okinawan natives than among adult immigrants (41.1% vs. 8.2%; $p<0.001$). The risk of pterygium development was 6.15 times higher in natives than in immigrants. The respective risks of nuclear and RD were 8.67 and 3.05 times higher, respectively, in natives compared to immigrants. The risk of WC risk was lower (0.17) in natives than immigrants, and no significant association was found between cortical and PSC and place of origin.

Conclusions: Ocular UV exposure in adulthood was not significantly different between natives and immigrants, implying that childhood UV exposure has a greater impact on ocular health. Childhood exposure may be a risk factor for pterygium, and nuclear and RD.

The microstructure for zonular fibers under ocular inflammation

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Purpose: The weakness for zonular fibers makes complications in cataract surgery. Ocular inflammation is well known to cause the weakened zonular fibers, those details were unclear because of microstructure. We investigated the microstructure for zonular fibers using low-vacuum scanning electron microscopy (LV-SEM) in a rat cornea alkali burn model.

Methods: Rats were divided into two groups: a control group and an alkali injury group. Seven days after injury, the eyes were enucleated, the anterior segment was dissected and embedded in gelatin after immunofluorescence (IF), observations were performed.

Moreover, the gelatin specimens were embedded in paraffin, observed by LV-SEM, and performed quantitative reverse transcription polymerase chain reaction (RT-qPCR).

Results: There were significant decreased in the fluorescence-positive area, also observed those infiltrated cells onto zonular fibers, in the injured group. The most of infiltrated cells were matrix metalloproteinase 2 (MMP2)-expressed macrophages, MMP2 expression were increased in RT-qPCR. The color density and thickness for zonular fibers were significantly reduced in the injured group observed by LV-SEM. The fibrillar girdle was also identified and attached to the posterior capsule branch in LV-SEM images. The fibrillar girdle was significantly disrupted in the injured group.

Conclusions: It was possible to observe the effects for zonular fiber, Zinn's zonule by anterior ocular inflammation. Those zonular fibers became thinner by inflammation, macrophages infiltrated onto zonular fibers, and the fibrillar girdle was disrupted.

[Abstract Title]

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Purpose: Late postoperative spontaneous IOL dislocation is a serious complication of cataract surgery, results from zonular apparatus capsular bag (ZACB) insufficiency. The possible main causes of ZACB are trauma (surgical or non-surgical), zonular deficiency, capsular deficiency, chronic inflammation, and atopy.

We experienced unique characteristics late postoperative IOL dislocation cases with spontaneous posterior capsule rupture in the past couple of years. In this study, we reported the clinical features and outcome of such cases.

Methods: Seven IOL subluxation or dislocation cases by spontaneous posterior capsule rupture were applicable. Those cases were investigated a case summary, including the patient's age, sex, associated general medical and ocular conditions, medications, IOL implantation and explantation date, signs and symptoms leading to the explantation procedure, and results of the secondary surgery.

Results: The average age was 52 years (29-83 years), 6 men and 1 woman. Five (4 men and 1 woman) patients had atopic dermatitis. Those explanted IOLs had been implanted for 13.8 years, 4 cases were dislocated (dropped) into the vitreous and 3 cases subluxated into the anterior chamber. Damaged posterior capsules were transparent in all cases.

The type of explanted IOLs were 6 acrylic 1P IOLs and 1 PMMA IOL. Sulcus fixation was performed because of residual anterior capsule in 5 cases, and 2 cases were performed intrascleral fixation. In 2 of the 5 cases with sulcus fixation, Both were atopy, the residual capsular bag including the anterior capsule completely dislocated into the vitreous cavity shortly after surgery. Soemmering rings were explanted in all intrascleral fixation cases.

Conclusions: We reported on the seven spontaneous posterior capsule rupture cases. The majority cases of those were atopic dermatitis, suggesting the course of posterior capsule rupture and complete dislocation of the lens capsule. The posterior capsule was transparent, and Soemmering rings were present in all cases.

Current Status of Intraocular Lens Long-Term Stability

Hiroyuki Matsushima[1], Koichiro Mukai[1], Mayumi Nagata[1], Tadashi Senoo[1]

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Purpose: Intraocular lenses (IOLs) in ophthalmology have made remarkable progress. Currently, hydrophobic and hydrophilic acrylics, which are soft materials compatible with small incision cataract surgery, are the mainstream IOL materials. In this study, the stabilities of materials were evaluated using rabbit eyes.

Methods: Eyes of 8 weeks albino rabbits (about 2kg) had phacoemulsification. After that, different types of hydrophobic acrylic IOLs SN60WF Q code (Alcon), SN60WF A code (Alcon), CC60WF (Alcon), ZCB00 (AMO), YP2.2 (Kowa), XY1 (HOYA) and hydrophilic IOL LS-313 MF-15 (Santen) were implanted. After 6 months post operation, the IOLs were extracted and cleaned to remove attachment proteins. The surface and inner light scatterings of the IOLs were imaged and analyzed with an anterior segment analyzer.

Results: The CC60WF, ZCB00V, YP2.2 and XY1 developed a few glistening but no light scatterings. The Q and A code developed same levels of glistening and sub-surface nano glistenings (SSNGs). The LS-313 developed light calcifications.

Conclusions: The long periods implantation in rabbits are useful model to evaluate stabilizations of IOL materials. The resent IOLs are designed to keep transparent conditions.

Differences in Intraocular Inflammation in the Anterior chamber Depending on the Position of Intraocular Lens

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[1]Department of Ophthalmology, Dokkyo Medical University, Tochigi, Japan

Purpose: Differences in anterior chamber inflammation induced by IOL shape and fixation position were experimentally investigated.

Methods: Twelve 15-week-old Dutch colored rabbits underwent lens reconstruction using PN6AS (3-piece IOL, Kowa) and YP2.2 (single-piece IOL, Kowa) IOLs. The subjects were divided into two groups: one group with PN6AS in one eye and YP2.2 in the other eye fixed in the capsular bag (“In the bag group”, 12 eyes), and the other group with the same IOL fixed in the sulcus (sulcus group, 12 eyes).

After general anesthesia (Ketamine, Xylazine), twelve 15-week-old dutch rabbits were performed cataract surgery after 2.4mm corneal incision. Three-piece IOLs (PN6AS, Kowa) and single-piece IOLs (YP2.2, Kowa) were randomly implanted in the lens capsule (Capsule group) or ciliary sulcus (Sulcus group). The FLP (Laser Flare cell Photometry) value were measured using a flare cell meter (FM700, Kowa) at preoperative, 3 days, 1 week, and 2 weeks postoperatively.

Results: The FLP values (3 days, 1 week, and 2 weeks postoperatively) were 68.00±71.46, 65.89±52.49, and 16.93±5.85 in the Capsule group and 142.21±146.09, 95.43±99.18, and 19.57±8.54 in the Sulcus group. The FLP values tended to be higher in the extracapsular fixation group. Among the Sulcus group, the FLP values were 111.17±159.11, 66.02±28.70, and 15.32±5.95 for the eyes implanted PN6AS, and 172.25±139.01, 124.85±136.90, and 23.82±9.04 for the eyes implanted YP2.2.

Conclusions: Single-piece in the sulcus fixation tends to induce intraocular inflammation. A three-piece IOL should be selected in cases requiring sulcus fixation.

The Kinoshita Lecture

Fielding J. Hejtmancik, MD, PhD


Friday, December 15th

Friday, December 15th

Session 15: Pharmacological Approaches to Lens Disease

Organizer: *Vincent Monnier*
Co-Moderator: *Barbara Pierscionek*

Talks are in the format of 15 minutes presentation with 3 minutes discussion.

Time	Speaker		Title
8:30 a.m.		**Barbara Pierscionek**	Functional approaches to investigating novel anti-cataract therapies
8:48 a.m.		**Gus Grey**	Ion, pharmaceutical and nutrient transport in the lens resolved with imaging mass spectrometry
9:06 a.m.		**Vincent M Monnier**	Is the prevention of γ-crystallin aggregation with small molecules a valid strategy for cataract prevention?
9:24 a.m.		**Juliet Moncaster**	Microtubule-Associated Protein Tau (MAPT) in mouse non-transgenic and transgenic Alzheimer's Disease lenses
9:42 a.m.		**Peter F. Kador**	Pharmaceutical Prevention of Sugar Cataracts

Functional approaches to investigating novel anti-cataract therapies

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Purpose: Potential alternatives to surgery for the treatment of cataract have been proposed in the literature. To present and discuss these alternatives, particularly nanoceria and oxysterol, as well as methods for investigating functional parameters.

Methods: Investigations were conducted on a) nanoceria applied to cultured human lens epithelial cells to determine anti-oxidant and anti-glycation properties; b) oxysterol: VP1-001 (25-hydroxycholesterol) in C57BL/6J mice with αB-crystallin (Cryab-R120G) or αA-crystallin (Cryaa-R49C) knock-in mutations. Analysis of the latter included measurement of transparency using slit-lamp biomicroscopy and refractive index using X-Talbot interferometry at the SPring-8 synchrotron.

Results: The current findings indicate potential modalities for protecting against the processes that are considered to lead to opacification. The results on murine eyes show promising differences in lens functional parameters: transparency and refractive index after treatment with oxysterol. However, these are not evident in all eyes tested.

Conclusions: There may be viable alternatives to cataract surgery but these require further investigation of lens function and its maintenance post-treatment.

References

[1] Hanafy, B.I., et al. Ethylene glycol coated nanoceria protects against oxidative stress in human lens epithelium. RSC Advances, 9, 16596-16605 (2019)
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[3] Wang K et al. Oxysterol Compounds in mouse mutant αA- and αB-crystallin lenses can improve the optical properties of the lens. Investigative Ophthalmology and Visual Science, 63(5):15 (2022)

Ion, pharmaceutical and nutrient transport in the lens resolved with imaging mass spectrometry

Angus C. Grey[1], Dingchang Shi[1], Melody Tang[1], Luis Knight[1], Yann Tenier[1], George Guo[1], Paul J. Donaldson[1]

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Purpose: To deliver novel therapeutics to the lens, a better understanding of its transport properties at the whole tissue level is required. This study aimed to develop and utilise imaging mass spectrometry techniques to visualise ion and small molecule transport in the ocular lens.

Methods: IMS (imaging mass spectrometry) was applied to the normal bovine lens and rat eye. MALDI (matrix-assisted laser desorption/ionisation) was used to detect nutrients and pharmaceuticals, while LA-ICP (laser ablation inductively coupled plasma) ionisation was used for elemental analysis. Cryosections from bovine lenses organ cultured in artificial aqueous humour containing either isotopically-labelled glucose, rat eyes incubated in pharmaceuticals (0-24hrs), or Rubidium as a replacement for Potassium (0-2hrs) were analysed by IMS at 150□m spatial resolution. A novel data analysis pipeline was developed to present kinetic IMS data and automatically identify metabolic pathways that were active in the lens.

Results: In normal bovine lenses, ion uptake was predominantly at the lens equator, as indicated by an abundance of Rubidium signal in this region after 2h of incubation. Similarly, isotopically-labelled glucose uptake was predominantly in the peripheral lens epithelium and lens equator, while its metabolism to several isotopically-labelled metabolites was detected predominantly in the cortex. Kinetic IMS images showed spatially-distinct accumulation of isotopically-labelled metabolites in the lens cortex. In contrast, the patterns of pharmaceutical uptake and metabolism were different to ion and nutrient uptake, and appeared related to their lipophilicity.

Conclusions: The delivery of ions and metabolism of endogenous and exogenous molecules to the ocular lens can be mapped over time with IMS.

Is the prevention of γ-crystallin aggregation with small molecules a valid strategy for cataract prevention?

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Purpose: We recently identified six small molecules from a Microsource Discovery library (N=2650) with ability to block thermal aggregation of human recombinant γ-crystallins (Islam et al JBC 2022). These were selected based on a screen that included both an oxidation step with H2O2 and a thermal stress. Results showed that closantel (Durg C, antihelmintic drug) and gambogic acid (Drug G,an Asian tree bark extract drug) most potently inhibited thermal aggregation of native hγD and deamidated hgS, and bovine gamma crystallins. However, these inhibitors had either no or only partial activity at high concentrations against hγD mutants W43R, R58H and R14C. In contrast, both drugs C and G quite effectively inhibited thermal aggregation of OPJ mouse mutant hγD F9S leading us to choose this strain for in vivo testing.

Methods: Mice were started on drug or vehicle administration after weaning at 21 days of age, Testing groups included diet control, diet with drug C (n = 52 lens), topical control, topical drug C, topical drug G (50-60 days n=14 lens, 85-91 days n=80 lens, 100-110 days n=6 lens). The mice were sacrificed at approximately 60 days of age. The mice on topical drug were sacrificed in one of three age groups: 50-60 days, 85-91 days, or 100-110 days. Quantitation of opacity was achieved with Image J software and expressed as % surface opacity.

Results: Drug C in the diet was no better than control at lowering opacity. At 50-60 days, 70% of control lenses had no opacity while 70% of topically treated lenses with Drug G had cataract. At 85-91 days topically applied Drug G was no better than control, but Drug C had more mice with lower opacity than control (p=0.011). When opacity was ranked as continuing variable at day 85-91, Drug opacity was 5-16% lower in the Drug C than control group (p=0.01) receiving vehicle only.

Conclusions: These results show that neither Drugs C nor G can block the progression of cataract in the OPJ mice. However, long-term follow-up of the mice reveals that total opacification is reduced in presence of Drug C suggesting presence of anti-aggregation activity especially in the outermost cortical layers of the lens.

Microtubule-Associated Protein Tau (MAPT) in mouse non-transgenic and transgenic Alzheimer's Disease lenses

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Purpose: We previously discovered that Aβ accumulates in the cortical/supranuclear region of the lens in people with Alzheimer's Disease (AD) (Goldstein et al., 2003) and Down Syndrome (DS; (Moncaster et al., 2010). We also demonstrated Aβ in the Tg2576 APP Swedish mutation AD mouse model (Moncaster et al., 2022). Another protein that is involved in AD is Microtubule-Associated Protein Tau (MAPT). Tau is expressed in the brain and becomes hyperphosphorylated in AD eventually forming neurofibrillary tangles. Tau has previously been reported to be expressed in the mouse lens (Bai et al., 2007; Zhao et al., 2013) but the results vary depending on the mouse model. Here we investigated whether tau protein was expressed in non-transgenic mouse lenses and in a transgenic tau mutant (P301S) and whether there was a cataract phenotype.

Methods: P301S tau mutant transgenic and non-transgenic mice were bred and maintained at Boston University. Breeder mice were purchased from Jackson Laboratories, Bar Harbor, ME. Male and Female transgenic and non-transgenic mice were sacrificed throughout their lifespan at ages 3-12 months. Mice were perfused with phosphate buffer saline, lenses were isolated and then imaged under two different sources of light using a D70 digital Nikon camera and a custom-adapted Zeiss stereophotomicroscope. Lenses were then snap frozen and analyzed by Western Blotting using a panel of tau antibodies.

Results: P301S transgenic and non-transgenic mouse lenses all expressed tau during their lifespan when analyzed by Western blot. P301S transgenic lenses demonstrated an additional higher molecular weight band compared to the non-transgenic mice. No cataract phenotype was observed in transgenic or non-transgenic mouse lenses.

Conclusions: There was no overt difference in lens phenotype between the P301S transgenic and non-transgenic mice. However, the banding pattern observed by Western blot for P301S transgenic mice compared to non-transgenic was different. Our data suggest the P301S tau mutation affects tau processing but does not result in cataract phenotype. Based on our current and previous results, the data suggests that Aβ may play a more significant role than tau in lens pathology in AD

Pharmaceutical Prevention of Sugar Cataracts

Peter F. Kador[1], Karen Blessing[1], Manley Paulos[1], Milton Wyman[1], and Robert Munger[1]

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Purpose: Cataracts are associated with the metabolic disorders of diabetes mellitus (DM) and galactosemia (GAL). While with DM a reduction of insulin results in increased tissue levels of glucose, in GAL a deficiency of the enzyme galactose-1-phosphate uridylyltranserase (GALT) leads to increased tissue levels of galactose. Studies have identified that the lenticular presence of excess glucose or galactose is linked to the development of “sugar” cataracts through the enzymatic reduction of glucose and galactose to their respective sugar alcohols (polyols) sorbitol and galactitol by aldose reductase. Excess glucose /galactose also can modify lens proteins through nonenzymatic glycation and become more susceptible to oxidative stress generated by the induction endoplasmic reticulum (ER) stress in lens epithelial cells. The purpose of this presentation is to demonstrate that inhibiting lens polyol accumulation with inhibition of aldose reductase is the central drug target for preventing the onset and progression of sugar cataracts.

Methods: Experimental studies employing in vitro lens cultures, transgenic diabetic/galactosemic rodents, and clinical studies utilizing galactosemic/diabetic dogs have been utilized.

Results: While polyols can rapidly form and accumulate in the rat lens, iso-osmotic lens culture studies indicate that this polyol accumulation by itself does not initiate the biochemical changes associated with cataract formation. Instead, it is the localized osmotic changes secondary to polyol accumulation that induce the biochemical changes initiating cataract formation. Aldose reductase is primarily localized in the lens epithelium of rat, dog and human lenses and the localized polyol generated osmotic stress in the lens epithelial cells induces ER stress that subsequently leads to oxidative stress through the generation of reactive oxygen species (ROS). Lens proteins also react with excess LENS glucose/galactose via nonenzymatic glycation. While glycated proteins are present in the lenses of all diabetic/galactosemic animals, cataracts only develop when certain lens levels of aldose reductase are present. Antioxidant therapy can delay sugar cataract formation; however, only administration of aldose reductase inhibitors can prevent the onset and progression of these cataracts.

Conclusions: Aldose reductase catalyzes the formation of polyols that induce localized osmotic changes within the lens epithelial cells. By specifically inhibiting polyol formation within the lens with aldose reductase inhibitors, osmotic changes do not occur and there is no induction of ER stress and the generation of ROS. Topical administered aldose reductase inhibitors have been clinically shown to prevent osmotic sugar cataracts independent of blood sugar control for up to 10 years.

PRESENTING AUTHOR INDEX


A hui hou...
Until we meet again...