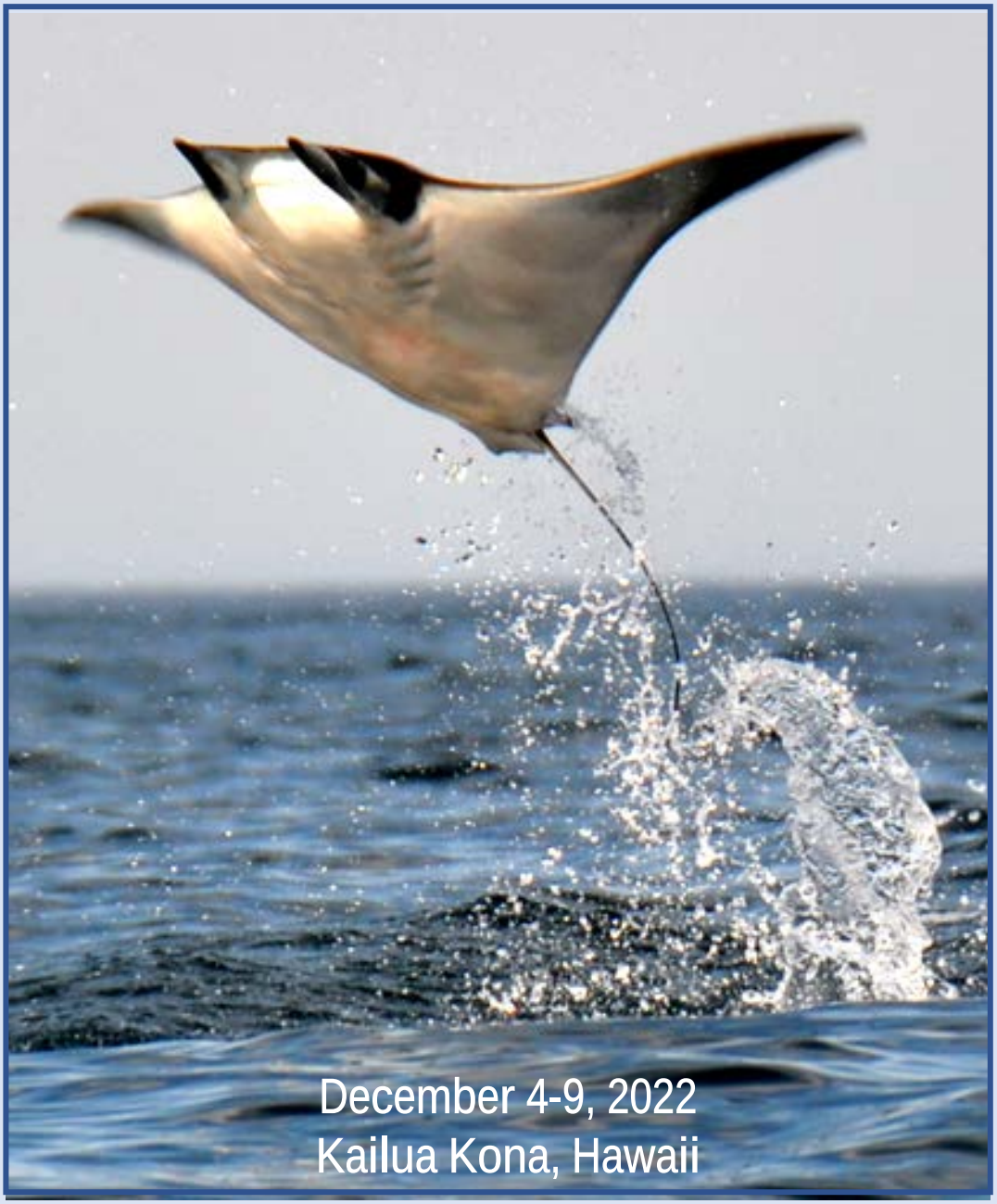
Program & Abstracts

International Conference on the Lens



Captain Cook
Monument
Kealakekua Bay

International Conference on the Lens 2022

Lens Risk, Resilience and Adaption

Organized by Professors:

- **Barbara Pierscionek, Anglia Ruskin University**
- **Roy Quinlan, University of Durham**
- **Michael Wormstone, University of East Anglia**

The London Eye,
London, England

ICL 2022 – MEETING AT A GLANCE

Sunday, December 4th	
3:00 pm	Registration Bay View Grounds
5:00 pm	Welcome Reception at Bay View Grounds
7:00 pm	Opening remarks and presentation of travel awards
7:30 pm	Session 1: The Kinoshita Lectureship
Monday, December 5th	
7:30 am	Registration outside Convention Center
8:45 am	Welcoming Remarks, Convention Center
9:00 am	Session 2: Lens Development
	Break
11:00 am	Session 3: Physiological Optics and Biomechanics of the Lens
	Lunch
2:00 pm	Session 4: Architecture and Ultrastructure of the Lens
	Break
4:00 pm	Session 5: Channels, Barriers, and Fluid Circulation in the Lens
Tuesday, December 6th	
9:00 am	Session 6: Crystallins
	Break
11:00 am	Session 7: Aging Conditions of the Lens
	Lunch
2:00 pm	Session 8: Poster Presentations including 2-Minute Flash Talks
Wednesday, December 7th	
9:00 am	Session 9: Autophagy in Lens Differentiation, Maintenance, and Disease
	Break
11:00 am	Session 10: Redox Biology of the Lens
Thursday, December 8th	
9:00 am	Session 11: Lens Regeneration, and Lentoids
	Break
11:00 am	Session 12: Large Scale Data in Lens Research
	Lunch
2:00 pm	Session 13: Lens Maintenance and Anti-Cataract Strategies
	Break
4:00 pm	Session 14: TGFβ and Lens Fibrosis
Friday, December 9th	
9:00 am	Session 15: Biomechanics and Matrix Biology of the Lens
	Break
11:00 am	Session 16: PCO Mechanisms and Management
	Closing Remarks from Kona 2022 Organizers

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NFER AWARDS FOR KONA 2022

Kinoshita Lectureship

Roy A. Quinlan, Ph.D.
Durham University
Durham, UK

Henry Fukui Award

Petr Sherin, Ph.D.
Imperial College London
London, UK

Alvira and Venkat Reddy Award

Aidan Grosas, Ph.D.
Australian National University
Canberra, Australia

Frederick Bettelheim Award

Santosh Paidi, Ph.D.
University of California at Berkeley
Berkeley, California

Gerald Robison Award

Zongbo Wei, Ph.D.
Medical College of Georgia
Augusta, Georgia

Young Investigator Awards

Lisa Brennan, Ph.D.
Florida Atlantic University
Boca Raton, Florida

Lee Cantrell, B.S.
Vanderbilt University
Nashville, Tennessee

Grace Cooksley, B.Sc.
University of Brighton
Brighton, UK

Matthiew Duot, B.Sc.
Universite de Rennes
Rennes, France

Mary Flokis, B.A.
The University of Sydney
Camperdown, Australia

Rifah Gheyas, M.S.
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Jefferson Medical College
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Portland, Oregon

Chen (Peter) Qiu, Ph.D.
University of Auckland
Auckland, New Zealand

Sanjaya Shrestha, M.S.
University of Delaware
Newark, Delaware

Aftab Taiyab, Ph.D.
McMaster University
Hamilton, Canada

Michael Vu, B.S.
Indiana University at Bloomington
Bloomington, Indiana

Xiaolu Xu, B.S.
University of Delaware
Newark, Delaware

Zhaohua Yu, M.D., Ph.D.
Uppsala University
Uppsala, Sweden

INTERNATIONAL CONFERENCE ON THE LENS

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

Organizing Committee

Scientific

Barbara Pierscionek, Ph.D.
Anglia Ruskin University

Roy Quinlan, Ph.D.
University of Durham

Michael Wormstone, Ph.D.
University of East Anglia

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 Keahou II Convention Center

Sunday, December 4th

3:00 pm **Registration Bay View Grounds**

5:00 pm **Welcome Reception at Bay View Grounds**

7:00 pm - 8:00 pm **Session 1: The Kinoshita Lectureship**

Roy Quinlan: Lifting the lid on the Lens to reveal the secrets of protein chaperones, the cytoskeleton, protein condensation and aging

Monday, December 5th

7:30 am **Registration outside Keahou II**

9:45 am **Welcoming Remarks**

9:00 am **Session 2: Lens Development**

Chairs: Jeff Gross and Qian Wang

1. Kristen Koenig: Differentiation, regulation, and morphogenesis in the anterior segment of the squid
2. Sarah Coomson: The conserved RNA-binding protein Elavl1 regulates the lens developmental transcriptome and its deficiency causes lens defects and early onset cataract
3. Timothy Plagemann: Defining the nature of the lens fiber cell tricellular adherens junctional complex
4. Qian Wang: Novel function of mTOR signaling in lens vesicle formation
5. Jodie Harrington: Human Lens Aquaporin Water-Channels (AQPs 0, 1 and 5) and Crystallin αA (CRYAA) Characterisation Utilising In Vitro Ocular Model Known as SEAM (Self-Formed Ectodermal Autonomous Multizone) from Human Induced Pluripotent Stem Cells (hiPSCs)

10:30 am **Break**

11:00 am **Session 3: Physiological Optics and Biomechanics of the Lens**

Chairs: Ehsan Vagefi and Doug Tobias

1. Ehsan Vaghefi: Computational Model of the in-vivo Human Lens and Related Anterior Eye Conditions
2. Tom Schilling: Differential modulation of water permeability and adhesion by zebrafish Aquaporin 0 orthologs determine lens development and optics
3. Kehao Wang: Aquaporin 0 regulates the optical development in the zebrafish eye lens
4. Douglas Tobias: Ca2+ regulation of aquaporin 0 membrane water permeability: insights from molecular dynamics simulations
5. Alfredo Freites: Membrane composition effects on aquaporin 0 membrane water permeability
6. Lee Goldstein: In vivo quasi-elastic light scattering eye scanner detects molecular aging in humans and mice

Monday, December 5th (Cont'd)

12:30 pm Lunch

2:00 pm **Session 4: Architecture and Ultrastructure of the Lens**

Chairs: Joe Costello and Cathy Cheng

1. Joe Costello: Prosimian Galago (bush baby) monkey employs a unique mitochondrial derived Nuclear Excisosome supported by extensive autophagy
2. Cathy Cheng: Cellular and molecular determinants of lens biomechanical properties
3. Paul Fitzgerald: Deciphering the role of the putative caspase cleavage site in filensin, a component of the lens-specific beaded filament
4. Steve Bassnett: Volume fluctuations in differentiated fiber cells determine lens power and optical quality
5. Eric Beyer: Pathologic calcification contributes to cataracts of different etiologies

3:30 pm Break

4:00 pm **Session 5: Channels, Barriers, and Fluid Circulation in the Lens**

Chairs: Paul Donaldson and Tom White

1. Tom White: Age-dependent changes in the water content and refractive index of the in vivo mouse lens revealed by multi-parametric MRI
2. Nick Delamere: Activation of piez01 increases Na,K-ATPase-mediated ion transport in mouse lens
3. Viviana Berthoud: A γC-crystallin mutant disrupts the lens circulation and causes cataracts through calcium precipitation
4. Peter (Chen) Qui: A new spin on lens biomechanics – the roles of steady state water and intracellular pressure
5. Bianca Maceo Heilman: Ouabain-induced hydrostatic pressure changes influence the shape, optics, and stiffness of the lens during simulated accommodation in a lens stretcher
6. Lee Cantrell: Proteomic measurement of aging influx networks in the lens microcirculatory system

6:00 pm **Luau Dinner and Show**

Tuesday, December 6th

9:00 am **Session 6: Crystallins**

Chairs: Kirsten Lampi and Rachel Martin

1. Steve Reichow: Mechanisms of amorphous and fibrillar aggregation of the α-crystallins visualized by electron microscopy
2. Dorota Skowronska-Krawczyk: The expression of the alpha and beta crystallins upon stress in aged retina
3. Adam Miller: Client-induced expansion, elongation, and co-aggregation of the lens α-crystallins
4. Meghan Alma Rocha: Probing the dynamics of deamidated human γS-crystallin
5. Hassane Mchaourab: Transcriptional coupling between Nrf2 and αB-crystallin in the lens and heart of zebrafish under proteostatic stress
6. Rachel Martin: How crystallin structure impacts refractive index in the lens

Tuesday, December 6th (Cont'd)

10:30 am **Break**

11:00 am **Session 7: Aging Conditions of the Lens**

Chairs: Kevin Schey and Santosh Paidi

1. Kevin Schey: Molecular Aging in the Human Lens
2. John Clark: Establishment and Maintenance of Lens Transparency
3. Petr Sherin: Visualisation of viscosity changes within plasma membranes of eye lens
4. Santosh Kumar Paidi: Illuminating cellular organization of ocular lenses in vivo using adaptive optical two photon fluorescence microscopy
5. Aiden Grosas: Structural, functional, and mechanistic basis for the oligomerisation of the major eye lens protein βB2-crystallin
6. Kirsten Lampi: Deamidation increases the unusual disulfide crosslink between cysteines 24 and 36 in gammaS-crystallin

12:30 pm **Lunch**

2:00 pm **Session 8: Poster Presentations and Flash Talks**

Chairs: Michael Wormstone and Viviana Berthoud

1. Russel McFarland: Disruption of a conserved N-terminal IXI motif of αB-crystallin leads to the formation of dynamic fibrillar aggregates
2. Sanjaya Shrestha: The transcription elongation factor Ell2 is necessary for achieving optimal expression levels of a specific cohort of genes, including several crystallins, in lens development
3. Michael Vu: Mapping the universe of Eph receptor and ephrin ligand transcripts in epithelial and fiber cells of the eye lens
4. Xiaolu Xu: Modeling lens development in Xenopus tropicalis
5. Zhaohua Yu: In vivo Cytochrome C release in the lens epithelial cells after subthreshold exposure to UVR-300 nm

Wednesday, December 7th

9:00 am **Session 9: Autophagy in Lens Differentiation, Maintenance and Disease**

Chairs: Marc Kantorow and Lisa Brennan

1. Fielding Hejtmancik: The role of FYCO1-dependent autophagy in lens fiber cell differentiation and cataractogenesis
2. Rifa Gheyas: Role of PI3K Signaling Inhibition in Inducing Autophagy-Dependent Organelle Elimination
3. Lisa Brennan: Hypoxia-dependent mitophagy regulates formation of the lens OFZ
4. Om Srivastava: Dual function of βA3/A1-crystallin in the lens: Structural protein and as an Autophagy modulator
5. Alan Shiels: A novel role for charged multivesicular-body protein 4b in the lens
6. Marc Kantorow: HIF1 is a master regulator of hypoxia-dependent lens fiber cell gene expression

10:30 am **Break**

Wednesday, December 7th (Cont'd)

11:00 am **Session 10: Redox Biology of the lens**

Chairs: Xingjun Fan and Eugene Serebryany

1. Marjorie Lou: The Ying-Yang effect, or the damage and benefit of reactive oxygen species (ROS) to the lens – A review
2. Hongli Wu: Glutaredoxin Activators with Multiple Antioxidative Activities Protect the Lens from Oxidative Damage
3. Elizabeth Whitcomb: Expression of a mutant ubiquitin in the lens results in cataract and alterations in redox status and amino acid metabolism
4. Eugene Serebryany: Myo-inositol, natively abundant in the human eye lens, acts as a chemical chaperone suppressing redox-dependent γ-crystallin misfolding and aggregation
5. Xingjun Fan: Blocking glutathione synthesis enzyme GCLC truncation delays cataract formation

12:30 pm **Lunch**

Afternoon free

Thursday, December 8th

9:00 am **Session 11: Lens regeneration and lentoids**

Chairs: Michael Wormstone and Kristen Koenig

1. Antonio Carlos Lottelli: A clinical perspective on pediatric cataract treatment
2. Katia Del Rio-Tsonis: Macrophages are essential for newt Lens Regeneration
3. Michael O'Connor: Investigating in vivo human lens regeneration via lens cell transplantation
4. Sofia Moriam: Using stem cell-derived human lens cells to explore responses to BMPs
5. Sadia Islam: NMIIA:F-actin contractility regulates meridional row cell shape transformation & alignment during lens differentiation

10:30 am **Break**

11:00 am **Session 12: Large scale data in lens research**

Chairs: Salil Lachke and Ales Cvekl

1. Ales Cvekl: Lens development and lens fiber cell differentiation: New insights on gene control mechanisms from multi-omics and imaging
2. Hélène Choquet: Genome-wide association study of cataract: identification of risk loci and shared genetics with other common vision disorders
3. Salil Lachke: Applying iSyTE to uncover regulatory networks in lens development and its associated defects
4. Matthieu Duot: Application of a multi-omics approach using iCLIP-seq and RNA-seq to the lens to identify downstream RNA targets of the cataract-linked RNA-binding protein CELF1
5. Adrienne Giannone: Single Cell RNA Sequencing of the Lens Epithelium in Wild Type Postnatal Mice

Thursday, December 8th (Cont'd)

2:00 pm **Session 13: Lens Maintenance and Anti-Cataract Strategies**

Chairs: Juliet Moncaster and Vincent Monnier

1. Vincent Monnier: Novel pharmacological approaches for the delay of cataract surgery based on drug repositioning and artificial intelligence
2. Krishna Sharma: Specific sequences in the N-terminal domain of αB-crystallin control oligomerization and chaperone activity
3. Caili Hao: Lens Gpx4 deficient mice induce lipid peroxidation and cataract
4. Marta Soltesova Prnova: Cemtirestat - novel indole-based bifunctional aldose reductase inhibitor/antioxidant as a promising drug for treatment of diabetic complications
5. Juliet A. Moncaster: Phenotyping of P301S tau mutant Alzheimer's Disease mice lenses

3:30 pm **Break**

4:00 pm **Session 14: TGFb and lens fibrosis**

Chairs: Frank Lovicu and Judy West-Mays

1. Melinda Duncan: Atf4 Regulates Genes Controlling Nutrient Metabolism in the Avascular Lens
2. Aftab Taiyab: Understanding the role of biomechanical signaling during lens fibrosis
3. Mary Flokis: FGF differentially regulates lens epithelial cell behaviour during TGF-β-induced EMT
4. Michael Wormstone: Is EMT a pre-requisite for matrix contraction
5. Zongbo Wei: A Tamoxifen-inducible Cre Knock-in Mouse for Lens-specific Gene Targeting

6:00 pm **Cocktails - location to be announced**

7:00 pm **Banquet**

Friday, December 9th

9:00 am **Session 15: Lens matrix biology**

Chairs: Melinda Duncan and Ram Nagaraj

1. Ram Nagaraj: Role of the lens capsule in lens epithelial cell to mesenchymal transition
2. Matt Reilly: What the Cells See: A Mechanobiological Model of Lens Stretching
3. Leah O'Neill: Mechanisms Regulating Inflammatory and Fibrotic Responses of LECs to Lens Injury
4. Alyssa L. Lie: The mechanics of water circulation in the in vivo accommodating human lens
5. Sepideh Cheheltani: Deletion of Cap2 causes altered F-actin distribution in lens fiber cells, affecting the lens stiffness

10:30 am **Break**

Friday, December 9th (Cont'd)

11:00 am **Session 16: PCO mechanisms and management**

Chairs: Michael Wormstone and Grace Cooksley

1. Aram Saeed: 3D printing of foldable, transparent, implantable intraocular lenses
2. Grace Cooksley: Positive resolution of the wound healing response contributing to posterior capsule opacification development byTi3C2Tx (MXene)
3. Hiro Matsushima: Is it possible to maintain visual function permanently after cataract surgery?
4. Liping Tang: An In Vitro System to Investigate IOL: lens capsule interaction

12:15 pm **Closing Remarks from Kona 2022 Organizers**


SUNDAY

Sunday, December 4th

Session 1: The Kinoshita Lecture

KINOSHITA AWARD LECTURE given by Professor Roy Quinlan

Lifting the lid on the Lens to reveal the secrets of protein chaperones, the cytoskeleton, protein condensation and aging

Roy A Quinlan

Emeritus Professor of Biomedical Sciences, Department of Biosciences, The University of Durham, Durham DH1 3LE, UK

"Vision is the art of seeing what is invisible to others" is a quote from Jonathan Swift, an 18th Century satirist. It applies to every walk of life including, I suggest, lens research. Dr. Jin Kinoshita was one of those individuals who not only had a vision, but who communicated and gave substance to that vision. I am very, very honored to be awarded the Kinoshita Lectureship and to be invited to deliver this award lecture to you. I shall highlight some specific points in my career that have particular significance to me and then I shall pose a few teasers for you to discuss as we socialize afterwards and beyond!

Dr. Kinoshita established the Laboratory of Vision Research in the National Eye Institute (NEI). I did not meet Dr Kinoshita, but I did spend 6 weeks in the lab of another giant in the field and previous recipient of the Kinoshita Lectureship, namely Joram Piatigorsky. He established the Laboratory of Molecular and Developmental Biology in the NEI. The Laboratory of Physical Biology headed by Alasdair Steven was in the basement of the same building. Alasdair worked on Intermediate Filament structure, the subject of my postdoc career. Intermediate Filaments were my ticket into the lens, encouraged by both George Duncan and Joram Piatigorsky who were both gracious and generous in their support.

I will highlight the pivotal role of mentors, encouragers, supporters and critics in my career. I will pay tribute to the many researchers, visitors and fellows who enriched my lab over the past 30-odd years. Their efforts uncovered the partnership of small heat shock proteins and their associated intermediate filaments, crystallin condensation and amyloid, the regulation of AQP0 by the intermediate filament protein BFSP1 and the importance of beaded filaments to emmetropia. My most recent academic adventure has been into the lens literature, under the watchful eye of John Clark. This very quickly made me aware of how myopic my view of the lens had been and how many questions I had conveniently parked "for later". Lens transparency and optical function adapt as it and the eye ages. Any clinical ophthalmologist will happily introduce you to the lens paradox! Talking to mathematicians, philosophers and even theologians quickly exposes the knowledge gaps and yet vision and the lens supports one of the most profitable industries on the planet – gaming!

So now to another teaser and one to broaden our horizons. The lens has either directly or indirectly given Natural Sciences some of its most important discoveries and delivered several Nobel Prizes. Dare I ask where the next Nobel Prize for vision and lens-related research is coming from? That was certainly on the Kinoshita agenda and perhaps it is time to dream again. "The eye sees only what the mind is prepared to comprehend" – R. Davies.


MONDAY

Monday, December 5th

Session 2: Lens Development

Chairs: Jeff Gross and Qian Wang

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

Time	Speaker Coauthors	Title
9:00 - 9:18 am	Kristen Koenig Christina Daly Francesca Napoli	Differentiation, regulation and morphogenesis in the anterior segment of the squid
9:18 - 9:36 am	Sarah Coomson Sandeep Aryal Salil A. Lachke	The conserved RNA-binding protein Elavl1 regulates the lens developmental transcriptome and its deficiency causes lens defects and early onset cataract
9:36 - 9:54 am	Timothy Plagemann	Defining the nature of the lens fiber cell tricellular adherens junctional complex
9:54 - 10:12 am	Qian Wang Hao Wu Xin Zhang	Novel function of mTOR signaling in lens vesicle formation
10:12 - 10:30 am	Jodie Harrington Andrew Quantock Justyn Regini Ryuhei Hayashi Barbara Pierscionek	Human Lens Aquaporin Water-Channels (AQPs 0, 1 and 5) and Crystallin αA (CRYAA) Characterisation Utilising *In Vitro* Ocular Model Known as SEAM (Self-Formed Ectodermal Autonomous Multizone) from Human Induced Pluripotent Stem Cells (hiPSCs)

Differentiation, regulation, and morphogenesis in the anterior segment of the squid

Kristen M. Koenig[1,2], Christina Daly[1,2], Francesca Napoli[1,2]

[1] *John Harvard Distinguished Science Fellowship Program, Harvard University, Cambridge, MA, USA*
[2] *Department of Organismic and Evolutionary Biology, Harvard University; Harvard University, Cambridge, MA, USA*

Purpose: The lens is a requisite innovation for high resolution vision. Coleoid cephalopods, including squid, cuttlefish and octopus, have a single chambered eye, similar to the vertebrate visual system, and have convergently evolved a single refractive lens. The developmental mechanisms underlying lens evolution across species remain largely unknown. Our interest is to better understand lens ontogeny and gene regulation in the squid Doryteuthis pealeii to identify mechanisms important for generating complexity in this tissue.

Methods: We have performed large scale in situ hybridization experiments and scRNA-seq at hatching stage to better define the anatomy and distinguish cell type identity. We have generated an in-depth time course description of lentigenic cell differentiation during lens formation using fluorescent confocal microscopy. In addition, we have performed scATAC-seq to better evaluate key regulators of crystallin expression and lens development.

Results: We have generated a cell type atlas of the cephalopod anterior segment and revealed the complex morphogenesis of the tissue. This includes redefining the lens generating cell population, identifying the time of differentiation as well as diversity of cell types in the tissue. We are also beginning to uncover the transcriptional regulators of the large S-crystallin gene family that are the template for the most abundant proteins in the lens.

Conclusions: This comparative dataset not only provides an unparalleled resource but also gives greater insight into how the cephalopod has gained this novel trait.

The conserved RNA-binding protein Elavl1 regulates the lens developmental transcriptome, and its deficiency causes lens defects and early onset 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: Gene expression regulation at the level of transcription and post-transcription determines the cellular proteome, which impacts tissue development and homeostasis. RNA-binding proteins (RBPs) bind RNA and mediate post-transcriptional control over mRNA splicing, transport, stability and translation into protein. In the lens, while the impact of transcriptional regulation is established, that of post-transcriptional control is only now being understood owing to functional characterization of RBPs (e.g., Celf1, Rbm24, Tdrd7) whose deficiency causes lens defects and/or cataracts. Here, we applied iSyTE to identify and functionally characterize a new RBP Elavl1 (HuR) in lens development.

Methods: Elavl1 expression in embryonic and postnatal mouse lens was examined by RT-PCR, Western Blotting and Immunofluorescence. Pax6GFPCre was used to delete Elavl1 in the lens starting from the lens placode stage in a mouse conditional knockout (Elavl1cKO) model. RNA-sequencing (RNA-seq) was performed on Elavl1cKO lenses to examine the impact of Elavl1 deletion on the lens transcriptome.

Results: Elavl1 is expressed in the lens from early development stages and is present in both epithelium and fiber cells. Elavl1cKO mice exhibit lens defects at embryonic stages and develop early onset cataract. RNA-Seq identifies known lens genes (e.g., Aqp1, Birc7, Caprin2, Crybb3, Cryga, Crygb, Crygd, Crygn, Ell2, Fbn1, Fyco1, Gja1, Hspb1, Palm2, Slc16a12), as well as other novel genes with promising function in the lens, to be misexpressed in Elavl1cKO mice, suggesting their relevance to the observed lens defects.

Conclusion: The conserved RBP Elavl1 is expressed at high levels in lens development. Elavl1 deficiency causes embryonic-onset lens defects and early postnatal cataracts in mice. In addition to the genes known to be relevant to lens biology, Elavl1-deficient lenses exhibit differential expression of several novel candidates. Together, these data indicate that Elavl1 has an important role in controlling gene expression in lens development, disruption of which causes cataract.

Defining the nature of the lens fiber cell tricellular adherens junctional complex

Timothy F. Plageman Jr.[1]

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

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.

Methods: Histological sections of embryonic and adult mouse lenses from control and mutant animals were immunofluorescently (IF) labeled for the detection of various antibodies. A combination of high-resolution confocal microscopy and deconvolution methods were used to image the labeled tissue. Fiber cell lysates were also isolated from two, 28-day old lenses in quadruplicate (8 lenses), subjected to immunoprecipitation utilizing a delta-catenin antibody, and analyzed using LC/mass spectroscopy.

Results: Utilizing a combination of proteomics and a candidate protein approach it was determined that δ-catenin, afadin, ZO-1, and SDK1, are enriched within the tAJs throughout lens development and within adult lens fiber cells. The localization of δ-catenin to the tAJ is notable because mutations in its encoding gene (*CTNND2*) are associated with cortical cataracts. Afadin is absent from the tAJ in embryonic lenses but is recruited to the tAJs during postnatal development. Lens-specific removal of afadin causes minor embryonic lens defects but disrupts the organization of adult lens fiber cells and the localization of δ-catenin and ZO-1 proteins to the tAJ.

Conclusions: The tAJ is structure of the tAJ complex is important to the function of the lens by maintaining its structure and transparency.

Novel function of mTOR signaling in lens vesicle formation

Qian Wang, Hao Wu, Xin Zhang

Department of Ophthalmology, Columbia University, New York, NY, USA

Purpose: The mechanistic target of rapamycin (mTOR) is known as a central regulator of cell growth and metabolism. Dysregulation of mTOR signaling is associated with major diseases such as cancer, diabetes, arthritis, and neurological disorders. In this study, we investigated the role of mTOR signaling in lens development.

Methods: We generated conditional knockout mouse models using *Le-cre*, which is specifically expressed in the lens progenitors. Immunofluorescence was performed to characterize the lens phenotype.

Results: During early lens development, mTOR activity was detected in the invaginating lens pit at E10 and lens vesicle at E11. After E13, mTOR signaling is high in the lens epithelium and the transitional zone, but low in the lens fiber cells. Genetic deletion of Fgfr1/2 results in the reduction of mTOR activity in the lens pit. However, disruption of FGF downstream cascades, PI3K and MAPK, individually did not affect mTOR signaling both *in vivo* and *in vitro*. Only concomitant blocking of PI3K and MAPK cascades downregulated mTOR activity in the lens epithelial cells.

Interestingly, genetic inactivation of mTOR signaling caused lens-corneal attachment at E13, which mimics Peters Anomaly in humans. Further characterization demonstrated that the canonical Wnt signaling was downregulated in the surface ectoderm and the lens epithelium of the mTOR mutant, suggesting that Wnt signaling is a target of mTOR during lens development. Indeed, genetic disruption of canonical Wnt signaling recapitulated the mTOR mutant phenotype of persistent lens stalk.

Conclusion: FGF acts upstream of mTOR in early lens development. PI3K and MAPK function jointly to promote mTOR activity in the lens epithelium. mTOR signaling controls lens-corneal separation process through regulating canonical Wnt signaling.

Human Lens Aquaporin Water-Channels (AQPs 0, 1 and 5) and Crystallin αA (CRYAA) Characterisation Utilising *In Vitro* Ocular Model Known as SEAM (Self-Formed Ectodermal Autonomous Multizone) from Human Induced Pluripotent Stem Cells (hiPSCs)

Jodie Harrington[1,2], Andrew Quantock[3], Justyn Regini[3], Ryuhei Hayashi[2], and Barbara Pierscionek[1]

[1] *Medical Technology Research Centre, Faculty of Health, Education, Medicine & Social Care, Anglia Ruskin University*
[2] *Department of Stem Cells and Applied Medicine, Osaka University*
[3] *School of Optometry and Vision Sciences, Cardiff University*

Purpose: All thirteen (0-12) mammalian aquaporins (AQPs) exist within human eye, mainly acting as water-channels. AQPs are important contributors to lens and cornea transparency and refractive indices. AQP characterization in specific human eye tissues, however, is less established compared to animal species, especially during early development stages. Thus, utilizing SEAM (Self-Formed Ectodermal Autonomous Multizone) that differentiates from human induced pluripotent stem cells (hiPSCs), which partially mimics *in vivo* ocular development, offers a highly-resourceful technology to advance understanding of specialized cellular differentiation, from essential gene expression to protein regulation.

Methods: SEAM is a well-established *in vitro* ocular model, partially mimicking *in vivo* eye development, largely for lineages *e.g.,* retina, lacrimal gland, and corneal sheets [1]. Whilst lens-tissues are of specific interest, as the “refracton” hypothesis proposes [2], both cornea and lens derive from embryogenic ectoderm, whose common biologic role focuses light onto the retina, and therefore judged a single functional refractive unit. To explore these inter-tissue relationships, SEAM was differentiated from hiPSC line 201B7, and showed aquaporin-positive presumed -lens and -retinal tissue cells.

Results: AQP0, AQP1, AQP5 and CRYAA expression significantly increased (8 weeks) and immunofluorescence (19 weeks) identified presumed retina, lens -epithelium and -fiber; founded on highly-characterized SEAM tissue zonular location and cellular morphology. Thus, SEAM well replicated the refracton hypothesis that development of refractive tissues are not distinct, but in concert, inclusive of retinal tissue, and effectively represented a 2D ocular organoid to study aquaporins.

Conclusion: Establishing relevant ocular human AQPs within SEAM, substantiates the superiority of this human *in vitro* hiPSC-ocular model in understanding the development of such specific eye tissues, which can be easily manipulated compared to animal-model studies. Further investigations are necessary to identify the function of AQPs in differentiation of specific lens and corneal lineages, as-well-as their role in maintaining transparency, and potentially malfunction in pathophysiologies.

***References*:**

[1] DOI: 10.1038/s41586-022-04613-4 [2] DOI: 10.1097/00003226-200111000-00015

Monday, December 5th

Session 3: Physiological Optics and Biomechanics of the Lens

Chairs: Ehsan Vagefi and Doug Tobias

Talks are in the format of 12 minutes presentation with 3 minutes discussion

Time	Speaker Co-Authors	Title
11:00 -11:15 am	Ehsan Vaghefi Paul Donaldson Duncan Wu	Computational Model of the in-vivo Human Lens and Related Anterior Eye Conditions
11:15 - 11:30 am	Tom Schilling Irene Vorontsova Olga Safrina Alexander Vallmitjana Belén Torrado Enrico Gratton Leonel S. Malacrida James E. Hall Paul Donaldson	Differential modulation of water permeability and adhesion by zebrafish Aquaporin 0 orthologs determine lens development and optics
11:30 - 11:45 am	Kehao Wang Irene Vorontsova James Hall Barbara Pierscionek	Aquaporin 0 regulates the optical development in the zebrafish eye lens
11:45 - 12:00 pm	Douglas Tobias J. Alfredo Freites Karin L. Németh-Cahalan James E. Hall	Ca2+ regulation of aquaporin 0 membrane water permeability: insights from molecular dynamics simulations
12:00 - 12:15 pm	Alfredo Freites James E. Hall Douglas J. Tobias	Membrane composition effects on aquaporin 0 membrane water permeability
12:15 – 12:30 pm	Lee Goldstein Douglas S. Parsons Srikant Sarangi Olga Minaeva Danielle M. Ledoux Juliet A. Moncaster John I. Clark David G. Hunter	In vivo quasi-elastic light scattering eye scanner detects molecular aging in humans and mice

Computational Model of the in-vivo Human Lens and Related Anterior Eye Conditions

Ehsan Vaghefi[1], Duncan Wu and Paul Donaldson[2]

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Purpose: The lens microcirculation system has always been modelled/ studied in isolation, i.e., the physiological experiments were performed in-vitro, and the computational models did not include the surrounding tissue of the lens.

Methods/Results: In this research – for the first time, we have connected the lens microcirculation system computational model to fluid dynamics of the surrounding tissues in the eye, in order to investigate this system of fluid dynamics in a living eye.

Conclusion: In particular, we have modelled the effect of vitreous humour viscosity change (e.g., vitrectomy surgery) and iris channel size (e.g., glaucoma) on the lens microcirculation system.

Differential modulation of water permeability and adhesion by zebrafish Aquaporin 0 orthologs determine lens development and optics

Thomas Schilling[1], Irene Vorontsova[1,2,] Olga Safrina[1], Alexander Vallmitjana[3], Belén Torrado[3], Enrico Gratton[3], Leonel S. Malacrida[3,4], James E. Hall[5], Paul Donaldson[2]

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Purpose: Aquaporin 0 (AQP0) is the most abundant lens membrane protein, essential for lens development and transparency. Functions for mammalian AQP0 include water transport and adhesion, but these have been difficult to test individually. An ancestral teleost genome duplication generated two zebrafish AQP0 orthologs, *aqp0a* and *aqp0b*, which both permeate water *in vitro*. *aqp0a-/-* mutants develop anterior polar opacities as adults, while *aqp0a-/-*/*aqp0b-/-* double mutants have more severe lens defects, consistent with distinct functions. We have also shown an adhesive role for Aqp0b, but not Aqp0a.

Methods: To test roles for Aqp0s in lens water homeostasis we have used a nano-environmentally sensitive sensor (6-acetyl-2-dimethylaminonaphthalene, ACDAN) in larval Aqp0 zebrafish mutants *in vivo.* Spectral phasor analysis of ACDAN fluorescence reveals water dipolar relaxation (DR), a readout for macromolecular crowding directly affected by water homeostasis. We examined localization of the lens nucleus in the optical axis with DIC optics. We also tested the ability of Aqp0 variants in residues implicated in either water transport or adhesive functions to rescue mutant phenotypes.

Results: As lenses matured, DR increased, and the maximum DR signal shifted from nucleus to cortex. This DR shift coincided with fiber cell compaction and crystallin accumulation in the nucleus required for lens optics and emmetropia. In *aqp0a-/-*, but not *aqp0b-/-* lenses, DR was lower in the cortex, suggesting that Aqp0a promotes fluid influx in the deeper lens cortex, whereas Aqp0b facilitates fluid efflux. Overexpression of a water-deficient variant of a killifish aquaporin 0 (MIPfun) partially rescued larval water homeostasis in *aqp0a-*/- mutants. Wild-type MIPfun or an adhesion-deficient MIPfun variant partially rescued nucleus centralization in mutants.

Conclusion: Our results suggest that the two zebrafish Aqp0 orthologues play spatially distinct roles in lens water permeability that are required for its normal development and optics, while adhesive functions appear to be less essential.

Aquaporin 0 regulates the optical development in the zebrafish eye lens

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Purpose: The eye lens has a gradient of refractive index (GRIN) increasing progressively from the periphery to the center that is attributed to its inner cytosolic protein concentrations. The water transport and adhesion are regulated by membrane proteins of lens fiber cells called Aquaporins which can also interact with cytoskeletal proteins [1]. This study aims to investigate how Aquaporins contribute to the optical development in the lens.

Methods: Loss-of-function deletions of *aqp0a* and/or *aqp0b* in zebrafish were generated using CRISPR/Cas9 gene editing. Lenses of single *aqp0a*$^{-/-}$ mutants, single *aqp0b*$^{-/-}$ mutants, and double *aqp0a*$^{-/-}$*/aqp0b*$^{-/-}$ mutants from larval to elderly adult stages were measured using X-ray Talbot interferometry at SPring8 in Japan. The three-dimensional GRIN profiles in two orthogonal cross-sectional planes of each lens were analyzed and compared with *in vivo* images and previous results obtained from wild-type lenses [2].

Results: For single *aqp0a*$^{-/-}$ mutants, the GRIN profiles tended to show asymmetrical shape with the central plateau regions shifted anteriorly. For single *aqp0b*$^{-/-}$ mutants, GRIN profiles throughout development are smooth and symmetric but spoke opacities appeared in extremely old samples. For double *aqp0a*$^{-/-}$*/aqp0b*$^{-/-}$ mutants, GRIN profiles showed lower magnitude as well as dips in the central plateau region [3].

Conclusions: Results from our study suggest that the two types of Aquaporin proteins, Aqp0a and Aqp0b, have region-specific functions in the lens: the former one has dominant functions in the periphery, regulating centralization of the GRIN plateau, and this function cannot be compensated for by the latter. For the formation of the plateau as well as for the GRIN to reach its maximum magnitude in mature lenses in the lens center, either Aqp0a or Aqp0b is required.

References*[1] Lo WK, Biswas SK, Brako L, Shiels A, Gu S, Jiang JX. Aquaporin-0 targets interlocking domains to control the integrity and transparency of the eye lens. Invest Ophthalmol Vis Sci. 2014;55(3):1202–1212.[2] Wang K, Vorontsova I, Hoshino M, et al. Optical development in the zebrafish eye lens. FASEB J. 2020;34(4):5552–5562.[3] Wang K, Vorontsova I, Hoshino M, et al. Aquaporins Have Regional Functions in Development of Refractive Index in the Zebrafish Eye Lens. Invest Ophthalmol Vis Sci. 2021;62(3):23*

Ca2+ regulation of aquaporin 0 membrane water permeability: insights from molecular dynamics simulations

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Purpose: Aquaporin 0 (AQP0), the most abundant membrane protein in the lens, is a key player in maintaining the circulation of water that is essential for lens homeostasis. AQP0 water permeability is regulated by Ca2+ ions, pH, and membrane composition, which depend on position within the lens. Experiments have established that Ca2+ regulation of AQP0 is mediated through the binding of calmodulin (CaM) to AQP0. We constructed atomically detailed models of the AQP0-CaM complex to investigate the mechanism of Ca2+ regulation of AQP0 water permeability using molecular dynamics (MD) simulations.

Methods: We performed atomistic MD simulations on the μs timescale of AQP1, AQP0, and the AQP0-CaM complex in a lipid bilayer under an osmotic gradient. We used a graph-based network analysis to elucidate how Ca2+-mediated CaM binding to AQP0 affects water connectivity networks within the water conduction gates in the AQP0 water-conducting pore, and why the water permeability of AQP0 is much lower than that of AQP1.

Results: The relative single-channel water osmotic permeability coefficients (pf) calculated from our simulations are in good agreement with experiment. The network analysis revealed that the pf of AQP1 (vs. AQP0) is relatively high because of a much higher proportion of single-file water states and the presence of only a single gate (CS-I) on the extracellular side, that AQP0 has a much higher (vs. AQP1) proportion of blockages at CS-I and two other gates (CS-II and Y23) that are unique to AQP0, and the reduced pf of AQP0-CaM is due to higher proportions of blockages at CS-I and involving CS-I/Y23 and CS-II.

Conclusion: Our experimentally validated MD simulations have provided atomically detailed insights into the relatively slow water conduction of AQP0 due to the multiple gates in its water conducting pore, and how Ca+2, via CaM binding, reduces AQP0 water permeability in the lens.

Membrane composition effects on aquaporin 0 membrane water permeability

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Purpose: The lipid composition of lens fiber cell plasma membrane is characterized by the high content ratios of cholesterol to phospholipids and sphingomyelins (SMs) to glycerolipids (GL), both of which vary between nuclear and cortical fiber cells and as a function of aging and cataract. Osmotic permeability measurements of Aquaporin 0 (AQP0) in proteoliposomes have shown that AQP0 single-channel water permeability is sensitive to membrane composition and decreases in the presence of cholesterol (Chol) and SM relative to GL bilayers. However, the molecular mechanisms underlying the regulation of AQP0 water permeability by the membrane environment remain largely unexplored.

Methods: We performed atomistic molecular dynamics simulations on the 10-μs timescale of AQP0 embedded in a lipid bilayer under an explicit osmotic gradient. We simulated three different lipid bilayer compositions: phosphatidylcholine (PC) with 25 mol% phosphatidylglycerol (PG), PC with 40 mol% Chol and 20 mol% PG, and sphingomyelin (SM), with 40 mol% Chol and 20 mol% PG.

Results: The relative single-channel water osmotic permeability coefficients (pf) calculated from our simulations are in good agreement with experiment, following the trend of pf reduction with increasing membrane deformation due to hydrophobic mismatch. We observed segregation of PG at the protein-lipid interface as annular lipids, while Chol is largely excluded from the interface. The addition of Chol to the PC:PG system increases pore closure by Y149 at constriction site II (CS-II) on the intracellular side. In contrast, we find increased pore closure by R187 at CS-I on the extracellular side in the SM:PG:Chol system relative to the PC:PG system.

Conclusion: Our simulations suggest that, through distinct modes of coupling between the protein and the lipid bilayer, the presence of SM and/or Chol in the AQP0 membrane environment modulate water permeability by altering the dynamics of the single-file water chain.

In vivo quasi-elastic light scattering eye scanner detects molecular aging in humans and mice

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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 the biology of aging, assessing risk for aging-related diseases, and maximizing health throughout life ("healthspan"). The lens is an ideal tissue for quantitative assessment of molecular aging *in vivo*. We sought to measure aging-dependent signals *in vivo* in lenses of healthy human subjects and wild-type C57BL/6 mice. We hypothesize that cumulative alterations in long-lived lens proteins represent *in vivo* biomarkers of molecular aging *in vivo*.

Methods: We used *in vivo* quasi-elastic light scattering (QLS) to measure aging-dependent changes in clear lenses of 34 healthy humans without history of eye disease (18 males, 16 females; ages 5–61 years) and longitudinally in unanesthetized C57BL/6 mice with clear lenses (ages 4–16 months). We also examined time- and oxidation-dependent effects on QLS signals in a closed system of water-soluble human lens protein extract (hLPE) during long-term (~1 year) incubation *in vitro*.

Results: Our results show that aging-related QLS signals in the lens can be acquired noninvasively in human subjects and unanesthetized mice. Age-dependent QLS signal changes detected *in vivo* in humans and mice recapitulated time-dependent changes in hydrodynamic radius, protein polydispersity, and supramolecular order of human lens proteins during long-term incubation (~1 year) and in response to sustained oxidation (~2.5 months) *in vitro*.

Conclusions: Our findings demonstrate that QLS analysis of lens proteins provides a practical quantitative technique for noninvasive assessment of molecular aging *in vivo*.

Monday, December 5th

Session 4: Architecture and Ultrastructure of the Lens

Chairs: Joe Costello and Cathy Cheng

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

Time	Speaker Coauthors	Title
2:00 -2:18 pm	Joe Costello Kurt Gilliland Kevin Schey	Prosimian Galago (bush baby) monkey employs a unique mitochondrial derived Nuclear Excisosome supported by extensive autophagy
2:18 - 2:36 pm	Cathy Cheng Sadia Islam Justin Parreno Velia M. Fowler	Cellular and molecular determinants of lens biomechanical properties
2:36 - 2:54 pm	Paul Fitzgerald Brad Shibata Ning Sun	Deciphering the role of the putative caspase cleavage site in filensin, a component of the lens-specific beaded filament.
2:54 - 3:12 pm	Steve Bassnett Qing Tan Hrvoje Šikić Juan Rodriguez	Volume fluctuations in differentiated fiber cells determine lens power and optical quality
3:12 - 3:30 pm	Eric Beyer Peter J. Minogue Oscar Jara Sarah H. Rodriguez James C. Williams, Jr.	Pathologic calcification contributes to cataracts of different etiologies

Prosimian Galago (bush baby) monkey employs a unique mitochondrial derived Nuclear Excisosome supported by extensive autophagy

Costello, M J.[1], Gilliland, Kurt O.[1], Schey, Kevin L[2]

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[2] *Biochemistry, Vanderbilt University, Nashville, TN, USA*

Purpose: To further characterize the unique Nuclear Excisosome (NE) in Galago (bush baby) monkey lenses that attacks the nuclear envelope during organelle-free zone (OFZ) formation and to elaborate on the enhanced autophagy in the equatorial epithelium that supports the process.

Methods: Adult lenses (ages 2-5, n=4) were processed for Airyscan confocal microscopy or thin section transmission electron microscopy (TEM).

Results: Confocal confirms the beads-on-a-string morphology in the vicinity of the degrading nuclei with the linear rod segments containing the unique four-membrane structure, which is the active segment derived from mitochondria in the equatorial epithelium. New data suggest that the beads are formed by fiber cell plasma membranes into distinctive clusters of dark staining protein aggregates and at least one degradative vesicle with these separate units (up to 11) surrounded by a single membrane that could be an extension of the plasma membrane. The beads are two-membrane structures and have no mitochondrial components, no apparent relation to autophagic vesicles and no direct contact with nuclei. Although their function is unknown, the degradative vesicle components consistently contain multiple lipid bilayers around the perimeter that extend into the interior, suggesting that the beads may be specialized for processing lipids removed from degrading nuclei by the NE. New images are presented to follow the degradation of nuclei by condensation and the formation and maturation of autophagic vesicles in the epithelium and throughout the developing fiber cells up to the OFZ.

Conclusions: NE formation is accompanied by increased mitochondria and autophagy to supply needed components and to degrade non-nuclear organelles during OFZ formation. Massive autophagy leads to unique pathways for return to normal autophagy levels. Energy for all these processes in developing fiber cells is provided by mitochondria attached to the four-membrane NE rod components.

Cellular and molecular determinants of lens biomechanical properties

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Purpose: The fine focusing function of the lens is intimately tied to tissue shape, elasticity, and stiffness. Recent studies have tested the relationship between biomechanical properties of lenses, namely stiffness and resilience/elasticity, to changes in cell cytoskeleton, morphology, and patterning.

Methods: Lenses from wild-type and various knockout (KO) and mutant mice were examined using tissue mechanical testing and electron or confocal microscopy.

Results: Our data from tropomodulin-1 (Tmod1) KO lenses indicated that lens stiffness at low mechanical loads depends on the large paddle interdigitations that form between the short sides of mature lens fiber cells. Loss of Tmod1, an actin-pointed-end-capping protein, caused disruption of the spectrin-actin network in fiber cells leading to changes in interdigitations that form the 3D zipper between fibers. Depletion of tropomyosin 3.5 (Tpm3.5), a F-actin stabilizing protein, resulted in softer lenses with low resilience. Super-resolution confocal microscopy revealed a change in the type of F-actin network in lens fiber cells that is linked to decreased lens stiffness. Disruption of Eph-ephrin signaling or myosin IIA can lead to lens fiber cell shape and packing defects, but these changes in hexagonal cell organization do not affect lens stiffness. Our comprehensive aging study in wild-type mice revealed that lens fibers are abnormally shaped and poorly organized in lenses from very old mice, but there is still an increase in lens stiffness with age. Data from EphA2 KO mice, Tpm3.5 knockdown mice, and very old wild-type mice also indicate that the size and stiffness of the lens nucleus does not affect whole lens biomechanical properties. Surprisingly, loss of EphA2 or ephrin-A5 leads to an increase in lens resilience due to suture patterning and alignment defects.

Conclusions: Together, these studies suggest that hexagonal fiber cell shape and precise alignment are not required for normal lens biomechanical properties. Rather, the F-actin network, interdigitations between lens fibers, and the alignment of the sutures between different shells of differentiating fibers are crucial for determining lens stiffness or resilience. In mouse lenses, the stiffness of the lens nucleus also does not affect the stiffness of the whole lens.

Deciphering the role of the putative caspase cleavage site in filensin, a component of the lens-specific beaded filament

Paul Fitzgerald, Brad Shibata, Ning Sun

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Purpose: Filensin and phakosin are two unusual intermediate filament proteins, expressed only in the lens. They assemble into the Beaded Filament (BF), a fiber cell-specific cytoskeletal element. Western blot analysis reveals that filensin undergoes a cleavage event relatively early in the differentiation process that yields two major fragments. Mass Spectroscopy suggests that the cleavage occurs at a caspase motif. We used a CRISPR gene editing approach to generate mutants to gain insight into the role of this caspase site in BF biology.

Methods: CRISPR was used to generate two mutant strains of mice: 1) a “deletion mutant” with the caspase site deleted. And 2)a truncation mutant with a stop codon immediately after the caspase site. Lenses were analyzed by western blot, transmission and scanning electron and immunofluorescence. Monoclonal antibodies were generated to track the fate of different regions of filensin.

Results: The truncated filensin accumulated in fiber cells, though the subcellular distribution was altered. No BFs were found by TEM. The truncation mutant showed a loss of the highly-ordered alignment of fiber cells in the deeper cortex, as seen in the filensin and phakosin KOs. In contrast, beaded filaments still formed in the caspase site deletion mutant. Western blotting showed that the deletion mutant still underwent progressive cleavage events, but the pattern of fragments differed from that seen in the WT, and fiber cell stacking remained normal. Subcellular distribution of filensin also appeared normal.

Conclusions: The data suggest that the filensin tail domain is essential for BF and confirms that the tail domain appears to be required for the BF association with the plasma membrane usually seen in younger fiber cells. Data from the caspase site deletion mutant show that that specific cleavage event is not required for filament assembly, correct packing of fiber cells, or association of the BF with the plasma membrane of younger fiber cells.

Volume fluctuations in differentiated fiber cells determine lens power and optical quality

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Purpose: We and others have proposed that cell compaction prevents lens overgrowth and contributes to the formation of the refractive index gradient (GRIN). We measured cell volume over time in the mouse lens to test this hypothesis and built an optical model to study refractive development.

Methods: Optimum fixation times were determined in *ROSA^{mTmG}* mice using the loss of intrinsic fluorescence to monitor fixative penetration into the tissue in real-time. Fixed lenses (E16 to 6 months of age) were sequentially bisected in the sagittal and equatorial planes and stained with a cocktail of fluorescent dyes to highlight the cross-sectional profiles of the fiber cells. We used a laser ray tracing technique to visualize lens aberrations and, in conjunction with an optical model, determine the mouse lens GRIN.

Results: Fiber cells at all depths were well preserved, and fixation-induced volume changes were small (<5%). The cross-sectional areas of cells in the various growth shells were measured and used to reconstruct cellular volume changes over time. Significant (1.5- to 4-fold) decreases in cell volume occurred during lens maturation and were especially pronounced in the lens core. In most regions, compaction was complete by three months of age. In lenses from younger (<2 weeks old) mice, cross-sectional areas transiently increased during differentiation. Volume fluctuations in deeper lying cells caused the radial position of a given cell to shift significantly over the course of development.

Conclusion: The measured changes in cell volume are consistent with calculated increases in refractive index and protein content during development. This suggests that volume loss (mainly as water) significantly contributes to GRIN formation in mice. Likewise, the ongoing compaction of cells in the lens interior helps explain why growth models that fail to properly account for this phenomenon tend to overestimate lens size, particularly at later times points.

Pathologic calcification contributes to cataracts of different etiologies

Eric C. Beyer[1], Peter J. Minogue[1], Oscar Jara[1], Sarah H. Rodriguez[2], James C. Williams, Jr.[3], Sharon B. Bledsoe[3], Andre J. Sommer[4], and Viviana M. Berthoud[1]

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Purpose: The present investigations were designed to examine the generality of pathologic mineralization as a mechanism of cataract formation by studying different mouse cataract models and lens material obtained from human cataract surgeries.

Methods: Lenses of homozygous mutant mice from three different lines were studied (Cx46fs380, Cx50D47A, and a mouse expressing a crystallin gene mutation) together with their wild-type counterparts. Human lens material was obtained from cataract surgeries performed on three pediatric patients with cataracts from different causes. Mouse lenses were examined by dark-field microscopy, Alizarin red staining and micro-CT scanning. Insoluble fractions of disrupted mouse or human lenses were stained with Alizarin red. Sections of mouse lenses were stained with Yasue and examined by Attenuated Total Internal Reflection micro–Fourier transform infrared spectroscopy.

Results: Whole-mounts from the mutant mouse lenses stained with Alizarin red (in a similar distribution to the cataracts), whereas staining was not detected in preparations from wild-type lenses. Micro-CT scanning showed mineralized material within the mutant lenses occupying the same region as the cataract. In all of the mouse cataract models, the mineral was identified as apatite by Attenuated Total Internal Reflection micro–Fourier transform infrared spectroscopy. The aspirated material from cataract surgeries contained particles that stained with Alizarin red and had a similar appearance to the insoluble, Alizarin red-stained particles detected in the homogenates of cataractous mouse lenses.

Conclusions: These results demonstrate the formation of calcium-containing precipitates (apatite) in different mouse cataract models and extend the generality of calcium crystal formation to human cataracts. We suggest that pathologic mineralization may have a mechanistic role in formation of cataracts of many etiologies.

Monday, December 5th

Session 5: Channels, Barriers, and Fluid Circulation in the Lens

Chairs: Paul Donaldson and Tom White

Talks are in the format of 12 minutes with 3 minutes discussion

Time	Speaker Coauthors	Title
4:00 - 4:15 pm	Tom White Xingzheng Pan Eric R. Muir Caterina Sellitto Kehao Wang Catherine Cheng Barbara Pierscionek Paul J. Donaldson	Age-dependent changes in the water content and refractive index of the *in vivo* mouse lens revealed by multi-parametric MRI
4:15 - 4:30 pm	Nick Delamere J. Lopez Rosales J. Gao R.T. Mathias M. Shahidullah	Activation of piez01 increases Na,K-ATPase-mediated ion transport in mouse lens.
4:30 - 4:45 pm	Viviana Berthoud Peter J. Minogue Junyuan Gao Richard T. Mathias James C. Williams, Jr. Sharon B. Bledsoe	A γC-crystallin mutant disrupts the lens circulation and causes cataracts through calcium precipitation
4:45 - 5:00 pm	Peter (Chen) Qui Y. Chen X. Pan P.J. Donaldson	A new spin on lens biomechanics – the roles of steady state water and intracellular pressure
5:00 - 5:15 pm	Bianca Maceo Heilman Rebecca Menendez Esdras Arrieta Marco Ruggeri Fabrice Manns Jean-Marie Parel Paul Donaldson	Ouabain-induced hydrostatic pressure changes influence the shape, optics, and stiffness of the lens during simulated accommodation in a lens stretcher
5:15 - 5:30 pm	Lee Cantrell Romell B. Gletten Kevin L. Schey	Proteomic measurement of aging influx networks in the lens microcirculatory system

Age-dependent changes in the water content and refractive index of the *in vivo* mouse lens revealed by multi-parametric MRI

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Purpose: To utilize *in vivo* magnetic resonance imaging (MRI) to investigate how changes in water transport contribute to changes in the gradient of refractive index (GRIN) of the mouse lens as a function of age.

Methods: C57BL/6 wild-type mice between 3 weeks and 12 months of age had their lenses imaged using a 7T pre-clinical MRI scanner (Biospec, Brucker) equipped with a customized circular coil receiver (6mm) placed on the left eye of the mouse. Measurements of lens geometry, and maps of the distribution of T2 (water-bound protein ratios) and T1 (free water content) values were extracted from MRI images. T2 values were converted into refractive index using an age-corrected calibration curve to produce maps of the GRIN across lenses of different ages.

Results: The mouse lens showed two distinct phases of change. The first phase was from 3 weeks to 3 months, where the T2 decreased, the refractive index increased, and the T1, an index of water content, decreased in all lens regions. This was accompanied by increases in the lens thickness, volume, and radii of surface curvatures. The second phase occurred between 6 and 12 months of age, where all physiological, geometrical and optical parameters remained relatively constant, although the lens continued to grow slowly.

Conclusions: In the first 3 months after eye opening, a decrease in the water content of the central lens nucleus drives changes in the lens geometry and GRIN that would be expected to increase the optical power of the mouse lens. Further research into the cellular and molecular mechanisms that regulate this decrease in lens water in the mouse has the potential to shed light on the mechanisms that drive the changes in lens power associated with the process of emmetropization in the developing human lens.

Activation of piez01 increases Na,K-ATPase-mediated ion transport in mouse lens

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Purpose: Lens ion homeostasis depends on Na,K-ATPase and NKCC1. TRPV4 and TRPV1 channels, which are mechanosensitive, appear to regulate the activity of these transporters. Here, we examined another mechanosensitive channel, piezo1, which also is expressed in the lens.

Methods: Rubidium (Rb) uptake rate was measured as an index of inwardly directed potassium transport. Cytoplasmic calcium was measured using Fura-2. Hydrostatic pressure (HP) in cells at the lens surface was determined using a manometer-coupled microelectrode approach.

Results: Rb uptake was increased in intact lenses and cultured lens epithelium exposed to Yoda1, a piezo1 agonist. Most Rb uptake is Na,K-ATPase-dependent although there is a significant NKCC-dependent component. In the presence of ouabain, an Na,K-ATPase inhibitor, Yoda1 did not increase Rb uptake. In contrast, Yoda1 increased Rb uptake similarly in the presence or absence of bumetanide, an NKCC inhibitor. The Rb uptake response to Yoda1 was inhibited by a selective piezo1 antagonist GsMTx4 and also by nonselective stretch-activated channel antagonists, ruthenium red and gadolinium. Yoda1 was observed to cause a pronounced, transient, HP decrease in intact lenses. Yoda1 increased cytoplasmic calcium in isolated cultured lens epithelium. The calcium and Rb uptake responses to Yoda1 were absent in calcium-free bathing solution, consistent with calcium entry when piezo1 is activated.

Conclusion: The findings point to stimulation of Na,K-ATPase, but not NKCC, when piezo1 is activated. Na,K-ATPase is the principal mechanism responsible for ion and water homeostasis in the lens. The functional role of lens piezo1 is a topic of further study.

A γC-crystallin mutant disrupts the lens circulation and causes cataracts through calcium precipitation

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Purpose: In several mouse cataract models (including mice expressing connexin mutants), components of the lens circulation are disrupted leading to accumulation of calcium to levels that may surpass the *Ksp* for some of its salts, causing their precipitation. To examine the generality of these changes, we characterized mice expressing a novel crystallin mutant.

Methods: Genomic sequencing, darkfield and confocal microscopy, immunoblotting, immunofluorescence, electrophysiology, Alizarin red staining, microcomputed tomography (microCT).

Results: We identified the mutation as a 5-bp duplication in the γC-crystallin gene (*Crygc*^{dup}), causing a frameshift that alters the amino acid sequence and destroys the fourth Greek key motif. Homozygous *Crygc*^{dup} mice had severe cataracts at an early age, but heterozygous animals developed small cataracts later in life. Levels of crystallins were decreased in the mutant lenses without major changes in protein solubility or cleavage. Connexin46 and connexin50 levels were substantially reduced in lenses from 30-day old heterozygous and homozygous *Crygc*^{dup} mice. The lens fiber cells showed a scarcity of immunoreactive gap junction punctae, especially towards the center of the lens. Electrophysiological studies demonstrated significant reductions in fiber cell-to-fiber cell gap junction-mediated coupling in heterozygous and homozygous *Crygc*^{dup} lenses. Wholemounts from homozygous lenses stained with Alizarin red, whereas no remarkable staining was detected in wild-type lenses. MicroCT scanning showed the presence of mineralized material in homozygous *Crygc*^{dup} lenses, but not in wild-type lenses. The Alizarin-red stained and the radio-dense mineral had similar regional distributions to the cataracts visualized by darkfield microscopy.

Conclusions: These results demonstrate that expression of a mutant form of a soluble lens protein can alter the levels of two membrane proteins and cause cataracts by disrupting lens fiber cell gap junctional communication leading to formation of calcium precipitates. These data support the hypothesis that cataracts of different etiologies result from pathologic mineralization of the organ.

A new spin on lens biomechanics – the roles of steady state water and intracellular pressure

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Purpose: The ability to focus on near and far objects, known as accommodation, is directly influenced by the biomechanical properties of the lens. It is predicted that age-related change in lens stiffness (biomechanics) is the primary factor in the development of presbyopia. However, how this increase in lens stiffness occurs is still currently unknown. We hypothesise the increase in lens stiffness is driven by an age-dependent decline in the ability of the lens to maintain either the steady state water content or the intracellular pressure gradient. To investigate we collectively utilize published data from MRI (water), an intracellular pressure system (pressure) and (now) a lens spin system (biomechanics) as a new dimension to understand the possible causes.

Methods: A custom implementation of a lens spinning system coupled with a finite element model was used to estimate the stiffness of bovine lenses under physiological modulation without decapsulation. Experimentally, lenses post-dissection were spun (1000 RPM) and subsequently placed in either control isotonic-AAH solution, or modified-AAH media for 4h to reach steady-state. The same experimental process was repeated post-incubation to evaluate changes in shear modulus.

Results: Extracted shear modulus estimates indicate that for hypotonic-AAH lenses, the core modulus decreased and the outer cortex modulus increased. In contrast, the shear modulus of ouabain-AAH incubated lenses resulted in a similar decrease in the core modulus but did not demonstrate a corresponding increase in modulus in the outer cortex.

Conclusion: These observations conclude for the first time, unsurprisingly, that different physiological modulations result in different localised changes to lens stiffness. Furthermore, preliminary data suggest that both the steady state water content and pressure collectively govern the localized stiffness of the lens. The data also suggests that the increase in stiffness in presbyopia is likely due to a combined increase in both total water and pressure.

Ouabain-induced hydrostatic pressure changes influence the shape, optics, and stiffness of the lens during simulated accommodation in a lens stretcher

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Purpose: To determine the effect of ouabain-induced hydrostatic pressure changes on the crystalline lens shape, optics, and stiffness during simulated accommodation.

Methods: Four lenses from two cynomolgus monkeys (Macaca fascicularis, ages: 4.8–5.8 years; post-mortem time: 11.8±12.4 hours) were mounted in a lens stretcher. Stretching experiments were performed on each lens submerged in Dulbecco's Modified Eagle's Medium (DMEM). The media was then swapped for DMEM + 1mM Ouabain and the stretching experiments were repeated every hour for up to 4 hours. The lens diameter, thickness, optical power, and the stretching force were measured at each stretch position for each experiment run. The axial shift of the tissue chamber wall from the OCT images was used to calculate the average refractive index of the lens. (Uhlhorn *et al.*, 2008) The changes in each parameter with stretching in the DMEM (control) and ouabain group were compared.

Results: The thickness of the lens increased and the diameter decreased with the submersion in ouabain over time (corresponding to a “rounding up” effect) for all lenses. There was no statistically significant change in lens power. The force required to stretch the tissue decreased, on average, from 3.04 g to 2.73 g after 4 hours in ouabain. The lens refractive index increased, on average, from 1.407 to 1.415 after 4 hours in ouabain, consistent with a decrease in water content.

Conclusions: Ouabain produces a consistent change in lens shape and group refractive index. The decrease in force required to stretch the tissue after the ouabain treatment suggests that the inhibition of water transport induced by ouabain causes the lens to become less stiff. These results provide insight into how changes in hydrostatic pressure contribute to optical and mechanical changes in the crystalline lens.

Proteomic measurement of aging influx networks in the lens microcirculatory system

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Purpose: To measure differential proteome composition as a function of cell age at fiber cell suture tips and relate expression at the sutures to the equator for microcirculatory influx inference.

Methods: Fixed bovine lenses were axially sectioned prior to laser capture microdissection of tissue, separating anterior and posterior fiber cell tips from the equator in two cell age groups each. Antigen retrieval was performed prior to a proteomic preparation. Data-independent acquisition mass spectrometry proteomics was used to quantify each protein. Complimentary qualitative evaluation of differential expression was performed with immunofluorescence for validation of proteomic results.

Results: Proteomic measurement of fixed tissue resulted in the quantitation of 700 distinct proteins and 7,323 peptides in 6 lens regions. The most significant changes measured were between young and old fiber cells, with adherens junctions and catenin protein networks among those enriched at the young sutures relative to the equator. Additionally, the aged posterior sutures showed particular enrichment in expression of GLUT1 and GLUT3 transporters relative to the equator. Similarly, each glucose transporter was enriched in the posterior suture relative to anterior suggesting enhanced affinity for glucose or dehydroascorbic acid influx through the vitreous humor. CLIC5, TMEM47, and GCLC were among other proteins enriched in the suture relative to equator while GJA8 was decreased in the suture.

Conclusion: This first study of the aging suture proteome supports previous reports that adherens junction protein networks are enriched in the suture relative to the equator. Novel measurement of GLUT1 and GLUT3 enrichment in the inner suture suggests a greater affinity for glucose in the lens nucleus relative to cortical fibers. In addition to providing an anaerobic glycolysis energy source to the lens nucleus, GLUT-mediated influx may be partially responsible for lens osmotic regulation and oxidative stress response.

TUESDAY

Tuesday, December 6th

Session 6: Crystallins

Chairs: Kirsten Lampi and Rachel Martin

Talks are in the format of 12 minutes presentation with 3 minutes discussion.

Time	Speaker Coauthors	Title
9:00 - 9:15 am	Steve Reichow Kirsten Lampi Russell J. McFarland Adam P. Miller	Mechanisms of amorphous and fibrillar aggregation of the α-crystallins visualized by electron microscopy
9:15 - 9:30 am	Dorota Skowronska-Krawczyk Fangyuan Gao Qianlan Xu	The expression of the alpha and beta crystallins upon stress in aged retina
9:30 - 9:54 am	Adam Miller Susan E. O'Neill Kirsten J. Lampi Steve L. Reichow	Client-induced expansion, elongation, and co-aggregation of the lens α-crystallins
9:45 - 10:00 am	Meghan Alma Rocha Collin Sroge Mina Mozafari Jessica Kelz Nicholas Whitman Rachel W. Martin	Probing the dynamics of deamidated human γS-crystallin
10:00 - 10:15 am	Hassane Mchaourab Jinhee Park Samantha MacGavin Laurie Niederbrach	Transcriptional coupling between Nrf2 and αB-crystallin in the lens and heart of zebrafish under proteostatic stress
10:15 - 10:30 am	Rachel Martin Amanda Abiad Megan A. Rocha Domarin Khago	How crystallin structure impacts refractive index in the lens

Mechanisms of amorphous and fibrillar aggregation of the α-crystallins visualized by electron microscopy

Steve L. Reichow[1,2], Kirsten Lampi[3], Russell J. McFarland[1,2], Adam P. Miller[1,2]

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Purpose: Light-scattering opacities responsible for age-related cataract are a result of aggregation and precipitation of the lens crystallins (α, β, and γ-crystallins). The α-crystallins assemble as polydisperse oligomeric complexes and function as molecular chaperones (holdases). Both these properties are thought to guard against aggregation events that would disrupt the delicate proteostasis of the lens. Environmental stress and chemical modifications that accrue over our lifetimes destabilize these proteins and induce complex forms of protein-protein interactions that lead to aggregation (amorphous and potentially fibril) and ultimately cataract formation. However, gaining direct evidence for these mechanisms has been a challenge, due to the lack of effective methods to characterize the inherently polydisperse structure of α-crystallin, the heterogeneity of chaperone-client aggregate formations, and evasiveness of fibril aggregation states under physiological conditions. Using methods in single-particle (cryo-) electron microscopy, our laboratory is beginning to unravel these mechanisms, with near-atomic level detail.

Methods: α-crystallins and client-bound complexes were characterized by a combination of single-particle (cryo-)EM, biophysical methods and functional studies.

Results: We illuminate new insights into an expansion and elongation mechanism that is shared by αA- and αB-crystallin used to sequester unfolding client protein; and describe a potential pathway of co-aggregation under saturating client conditions (such as those found in the aging lens). We further demonstrate that a conserved motif located within the NT-domain of αB-crystallin plays a critical role in chaperone activity and in facilitating the hallmark structural feature of polydispersity that is common among mammalian small heat shock proteins. Mutagenic disruption of this motif is shown to result in the formation of a novel fibrillar state in cells and exists in dynamic equilibrium with a native-like caged assembly.

Conclusion: This work reveals insights into the molecular plasticity of α-crystallins and their mechanisms of amorphous and fibrillar aggregation that might contribute to cataract and/or other crystallinopathies.

The expression of the alpha and beta crystallins upon stress in aged retina

Dorota Skowronska-Krawczyk, Fangyuan Gao, Qianlan Xu

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Purpose: The role of alphaA- and B-crystalins are members of the small heat shock proteins (sHSP) family, and they are an important part of the stress response. They are known to be expressed in the lens where they protect the aggregation of beta and gamma crystallins, the core building blocks of the tissue. AlphaB-crystallin is expressed also outside the lens shoring the chaperone activity on several clients. Beta and gamma crystallins are usually not found to be expressed outside of the lens.

Methods: In our recent work, we have shown that natural aging increases retina susceptibility to stress. Aged animals exposed to mild stress respond stronger than young animals, leading to severe tissue damage and pronounced visual function decline. When we closely examined the transcriptome of the aged retinas, one of the highest upregulated molecules, were alpha and beta crystallins. Data was confirmed on protein level. Interestingly, there was no upregulation of crystallins in young tissue that had undergone the same treatment.

Results: Next, we have exposed young retinas to multiple instances of mild stress and have shown that it led to response resembling the old retina including the vision loss. Unexpectedly, using epigenetic clock based on DNA methylation signatures, we discovered that young tissue which was exposed to several instances of stress significantly aged. When we looked at the expression levels of crystallins in young retinas upon repetitive stress we noted highly upregulated alpha and beta forms.

Conclusion: Taken together, our data show that both types of crystallins are present upon IOP-related stress in the retina and that expression of both crystallins upon stress is dependent on age-related changes in the tissue. Current experiments in the lab are focused on deciphering the role of crystallins expression upon stress in the retina.

Client-induced expansion, elongation, and co-aggregation of the lens α-crystallins

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Purpose: The α-crystallin molecular chaperones (αAc and αBc isoforms) function in preventing protein aggregation events in the eye lens. However, in old age it is thought that saturating conditions of unfolding clientele overwhelm the α-crystallin chaperone capacity, leading to light-scattering chaperone/client co-aggregates associated with cataract formation. However, our mechanistic understanding of the co-aggregation process is still not well understood, due to the difficulty in characterizing such structurally heterogeneous systems.

Methods: Quantitative structural comparison of the αAc and αBc co-aggregation pathways was achieved by visualizing and statistically characterizing the morphological changes in chaperone/client complexes by single-particle electron microscopy (EM) under varying client (lysozyme) concentrations, which were further correlated with solution-state biochemical and biophysical characterizations.

Results: We describe a mechanism by which αAc and αBc chaperone/client co-aggregation proceeds through the formation of an "initiation complex" and progresses through a mechanism involving a remarkable degree of expansion and elongation. Ultimately, under saturating conditions of unfolded client, the chaperone/client complexes appear to collapse (or cluster) to form large light-scattering co-aggregates (up to microns in diameter). αAc and αBc appear to share a similar elongation/expansion mechanism, however αAc is potentiated toward larger co-aggregates and exhibits a significantly wider size distributions of co-aggregation states as compared to αBc.

Conclusion: The high degree of structural plasticity of α-crystallin allows for the formation of a broad range of stable and soluble co-aggregation states, involving pathways of elongation and expansion, preceding formation of catastrophic light-scattering aggregates. Our statistical approach for analyzing single-particle EM data provided a quantitative evaluation of the structurally heterogeneous small heat shock protein co-aggregation pathway. Key variations observed between αAc and αBc could be correlated to isoform-specific differences in chaperone potency toward the model client, lysozyme. This approach may be effectively extended to quantitatively compare the chaperone activity toward other unfolding clients – including β/γ-crystallins.

Probing the dynamics of deamidated human γS-crystallin

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Purpose: Cataract is an opacification of the lens that results from protein aggregation. In order to rationally design therapeutics that will prevent cataract it is critical to elucidate the mechanisms of protein aggregation.

Methods: The majority of the protein content in the human lens is comprised of crystallins, specifically, α-, β-, and γ-crystallin. Exposure to harmful radiation concomitant with a loss of homeostasis equipment in the lens allows for post-translational modifications (PTMs) to occur on crystallins. The most common PTM found in aged and cataractous lenses is deamidation, the conversion of asparagine or glutamine to aspartic acid or glutamic acid, respectively.

To determine if deamidation negatively alters the biophysical properties of crystallins, our lab created four variants of human γS-crystallin that range from minimally to abundantly deamidated. These variants include 3-, 5-, 7-, and 9- site deamidation variants. Previous work in my lab revealed that these variants have increased susceptibility to oxidation and aggregation despite having little structural perturbation as revealed by crystallography.

Results: My hypothesis is that altered dynamics, rather than large structural rearrangement, are the source of altered biophysical properties.

Conclusion: Here I present my progress in investigating the dynamics and aggregation behavior of the variants. I have collected the 1H-15N HSQC and 1H-15N heteronuclear NOE experiments to determine the transverse relaxation rate (R1), the spin-lattice relaxation rate (R2), and NOE values necessary for probing the fast motion of these variants. These results are accompanied by investigations into backbone solvent accessibility and monitoring of dimerization rates.

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

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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.

Methods: 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. 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.

Results: 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.

Conclusion: Multiple molecular pathways, some implicated in cardiomyopathy, are revealed from transcriptome profiling thus identifying novel targets for further investigation. Together our transcriptome/phenotypic analysis establishes an intersection between the oxidative stress and chaperone responses in the lens and the heart.

How crystallin structure impacts refractive index in the lens

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Purpose: The γ-crystallins of the vertebrate lens are remarkably soluble structural and refractive proteins. They are a topic of intense interest from researchers worldwide, who have often focused on their solubility and stability, which must be maintained over many years. Recent work in my group is directed at understanding another important property of γ-crystallins, their refractivity. The prevailing model in the literature holds that the protein refractive index increment is a weighted average of those of the component amino acids and that crystallin refractivity is therefore independent of structure.

Methods: We recombinantly expressed and purified several refractive crystallins from humans and aquatic organisms. The proteins were then characterized using experimental biophysical methods, and the refractive index increment measured.

Results: We find that the refractive index of crystallin proteins greatly exceeds values predicted by the additive model, especially for lens proteins from aquatic organisms, and in contrast to control proteins not selected for refractivity, for which this model does match the measured values. The difference is particularly large for fish crystallins, which would be expected because aquatic organisms do not benefit from an air-water interface at the cornea and the proteins therefore must provide all of the focusing power for these visual predators. I will discuss and interpret these results in light of structural models for the proteins and present partial corrections to the additive model.

Conclusions: We conclude that crystallin structures are selected by evolution for refractivity as well as solubility and stability. In some cases, particularly in the crowded fish lens, these properties may be at odds, as some of the residues that maximize refractivity can also negatively impact solubility and vice versa.

Session 7: Aging Conditions of the Lens

Chairs: Kevin Schey and Santosh Paidi

Talks are in the format of 12 minutes presentation with 3 minutes discussion.

Time	Speaker Coauthors	Title
11:00 - 11:15 am	Kevin Schey Zhen Wang David MG Anderson Jessica Paredes Jarod Fincher Lee S. Cantrell	Molecular Aging in the Human Lens
11:15 - 11:30 am	John Clark Roy A. Quinlan	Establishment and Maintenance of Lens Transparency
11:36 - 11:54 am	Petr Sherin Marina Kuimova	Visualisation of viscosity changes within plasma membranes of eye lens
11:45 - 12:00 pm	Santosh Kumar Paidi Qinrong Zhang Yuhan Yang Chun-Hong Xia Na Ji Xiaohua Gong	Illuminating cellular organization of ocular lenses in vivo using adaptive optical two photon fluorescence microscopy
12:00 - 12:15 pm	Aiden Grosas Jeeeun Shin David C. Thorn Henry W. Orton Mithun C. Mahawaththa Martyna M. Judd Li Lynn Tan Joe A. Kaczmarski Nicholas Cox Thomas Huber Gottfried Otting Colin J. Jackson John A. Carver	Structural, functional, and mechanistic basis for the oligomerisation of the major eye lens protein βB2-crystallin
12:15 - 12:30 pm	Kirsten Lampi Sam Wheeler David Anderson Larry David	Deamidation increases the unusual disulfide crosslink between cysteines 24 and 36 in gammaS-crystallin

Molecular Aging in the Human Lens

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Purpose: To measure the effects of aging on ocular lens biomolecular (proteins & lipids) structure and function.

Methods: Proteomics and imaging mass spectrometry (IMS) were used to identify and localize age-related changes to ocular lens proteins and lipids. For proteomic analyses, human lenses of varying age were manually dissected into cortex, outer nucleus and inner nucleus regions, homogenized and separated into water soluble, urea soluble or urea insoluble fractions. After trypsin digestion, both data-independent acquisition (DIA) and data-dependent acquisition (DDA) LC-MS/MS methods were applied to dive deeply into the proteome and to identify/quantify protein modifications, respectively. For imaging mass spectrometry, 10-micron thick sections were coated with matrix and either proteins or lipids were imaged by MALDI IMS.

Results: Proteomic measurement resulted in the quantitation of 4,788 distinct proteins and 46,681 peptides by DIA analysis. Unsupervised hierarchical clustering revealed a remodeling event that occurred at ~50 years of age. Specific proteins involved in the remodeling include: connexin 50, SLC24A2, and aquaporin-5. DDA proteomics revealed multiple modifications such as dehydroalanine (DHA) and succinimide (Asu), that are intermediates to protein-protein crosslinking events and multiple protein-protein crosslinks were identified. For most crystallins, DHA modified peptides appear enriched in the urea insoluble fraction suggesting a role in crystallin aggregation. IMS analysis shows cataract specific localization of C-terminal g-crystallin fragments and ganglioside degradation. Functional assays are ongoing to assess the effects of age-related protein and lipid modifications on aquaporin function.

Conclusion: A variety of age-related molecular changes have been detected, some that are cataract-specific. Such changes are expected to cause functional alterations of major lens proteins.

Establishment and Maintenance of Lens Transparency

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Purpose: To consider the importance of post-translational modifications to the development and maintenance of short-range order (SRO).

Methods and Results: The contributions of selected, published results are reviewed and referenced. Image formation in the human depends on a high refractive index, “n”, of the symmetric, transparent cornea and lens, the optics of the eye. Transparency results from short-range order (SRO) in the spatial arrangement of collagen fibrils in the cornea and soluble crystallins in the lens, generated by interactions between protein constituents. While corneal collagen is remodeled continuously and replaced, lens crystallins are very long-lived and are not replaced, accumulating post-translational modifications, PTMs, over a lifetime. In lens, the accumulation of PTMs begins before birth and continues during normal development of SRO in the growing eye. The premise is that PTMs have a protective effect through stabilization of protein-protein interactions in the lens cytoplasm during differentiation. The continuous increase in PTMs with growth contributes to a “cataractogenic load” (CL) that is observed in the slit lamp as a subtle increase in light scattering with age. Eventually the increase in protein interactions reaches a “tipping point” when protein aggregates are large with respect to the wavelength, λ, of visible light ($400 < \lambda < 700$ nm) forming an age-related cataract (ARC), the most common protein-condensation disease in humans.

Conclusion: We hypothesize that ARC is preventable by protecting the biochemical and biophysical properties of lens proteins needed to maintain transparency, refraction, and optical function.

Visualisation of viscosity changes within plasma membranes of eye lens

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Purpose: The lens of a mammalian eye has no protein and lipid turnover. With age they accumulate numerous post-translational modifications leading to defects in the whole tissue structure. We assume that the age-related damage to plasma membrane structure may play crucial role in the retarding of the metabolite exchange predisposing the lens nucleus to oxidative stress – a key factor in the formation of cataracts. Here we present a novel method proving the spatially resolved access to dynamic properties of plasma membranes, namely polarity and viscosity, under conditions as close as possible to in vivo.

Methods: Fluorescence Lifetime Imaging Microscopy (FLIM) in combination with fluorescent probes sensitive to polarity and viscosity (so-called molecular rotors), were applied to plasma membranes of ex vivo porcine eye lens [1, 2]. The undeniable advantage of lifetime measurements is the insensitivity to dye concentration and the multiphoton excitation of dyes – the non-invasive access to the tissue. Lenses were cryosliced to thin slices (10-20 um), stained at room temperature with low concentrations of dyes and sealed to prevent the sample drying.

Results: FLIM with molecular rotors and polarity sensors has shown that plasma membranes of eye lens exhibit unprecedentedly high degree of lipid and protein organization, close to so-called 'lipid rafts' [1]. At the same time, these highly ordered structures of high viscosity are susceptible for photooxidation and cumulative age-related changes. We demonstrated that the viscosity within plasma membrane decreases in the case of electron transfer (Type I) or increases in the case of reactions initiated by singlet oxygen (Type II) [2].

Conclusions: 'FLIM – fluorescent probe' approach was successfully applied to the animal eye lens. This opens opportunities for further studies of age-related and pathological changes in dynamic properties within the ex vivo human eye lens.

References: *1. P.S. Sherin et al., Chem. Sci., 8 (2017) 3523-3528. 2. P.S. Sherin et al., J. Photochem. Photobiol. B: Biol., 225 (2021) 112346.*

Illuminating cellular organization of ocular lenses in vivo using adaptive optical two photon fluorescence microscopy

Santosh Kumar Paidi[1], Qinrong Zhang[2,3], Yuhan Yang[2], Chun-Hong Xia[1,6], Na Ji[2,3,4,5,7], Xiaohua Gong[1,6,7]

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Purpose: The ocular lens is an avascular multicellular organ, which grows continuously during the lifespan of the animal. Studies on the cellular organization of postnatal lenses have been carried out ex vivo on sections of enucleated lenses using light and electron microscopy. Here, we have visualized the lens cellular organization of live mice in vivo at different ages by using two photon fluorescence microscopy and adaptive optics.

Methods: We employed direct wavefront sensing to measure and correct aberrations in two photon fluorescence microscopy measurements of cellular organization deep inside the anterior part of the lens in vivo in live mice. We employed transgenic mice that express tdTomato fluorescent proteins in lens cell membranes and carried out longitudinal imaging of lens fiber cellular organization of same mice at different ages.

Results: We obtained significant improvements in signal, contrast, and spatial resolution of lens cell images up to >700 μm depth inside the lens in vivo. We observed novel morphological features such as anterior voids, enlarged vacuoles in fiber cells, and inhomogeneous organization of fiber cells with depth in different regions up to near the lens core that have not been reported in the previous ex vivo studies. We unexpectedly observed large ‘abnormal cavities’ in the deeper regions of these live lenses. The longitudinal studies of lens images revealed the incorporation of layers of new fiber cells as well as the retention of size and shape of the anterior voids at the different ages.

Conclusion: Taken together, non-invasive longitudinal in vivo imaging of lens morphology using two photon fluorescence microscopy and adaptive optics revealed novel cellular information about lens suture formation and alterations of lens fiber cell morphogenesis such as unique voids that might reflect fiber cell subcellular structures in the lenses of live animals at different ages and be related to the inward flow pathways of the lens internal circulation.

Structural, functional, and mechanistic basis for the oligomerisation of the major eye lens protein βB2-crystallin

Aidan B. Grosas, Jeeeun Shin, David C. Thorn, Henry W. Orton, Mithun C. Mahawaththa, Martyna M. Judd, Li Lynn Tan, Joe A. Kaczmarski, Nicholas Cox, Thomas Huber, Gottfried Otting, Colin J. Jackson, John A. Carver

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Purpose: Crystallin proteins in the translationally quiescent lens fiber cells must maintain their structure and soluble properties over a lifetime to preserve normal vision. However, environmental and chemical insults to the crystallins can lead to predominantly amorphous aggregation pathologically characterized as age-related cataract. βB2-crystallin is the principal β-crystallin isotype in the mammalian lens and has several oligomeric forms. Curiously, β-crystallin oligomers shift from dimers to tetramers with age, and homo-tetrameric βB2-crystallin is a major species. Given the relevance to age-related cataract, we investigated the formation of βB2-crystallin tetramer from its stable dimeric form and characterized its structure, conformation and biophysical properties.

Methods: We used size-exclusion chromatography (SEC) to study βB2-crystallin oligomerization. Crystallization of a homogenous tetramer yielded suitable crystals for X-ray diffraction and allowed us to solve βB2-crystallin as a tetramer in a novel space group. We used double electron-electron resonance (DEER) electron paramagnetic resonance (EPR) spectroscopy, ion mobility-mass spectrometry (IM-MS) and SEC coupled small angle X-ray scattering (SEC-SAXS) to study conformational changes that occur to βB2-crystallin upon oligomerization. Nuclear magnetic resonance (NMR) spectroscopy and molecular dynamics (MD) simulations were used to infer flexibility of the N- and C-terminal extensions for βB2-crystallin dimer and tetramer. Various spectroscopic methods were used to study dimer and tetramer stability and aggregation propensity.

Results: Low pH and high salt conditions are required to form βB2-crystallin tetramer from dimer. We identified pivotal interfacial ion-pairs that explain the conditions of tetramer formation. βB2-crystallin undergoes a large conformational rearrangement from a compact ‘face-en-face’ dimeric conformer to a domain-swapped tetramer, utilizing a domain-swapped dimer as a conformational intermediate. As a result of this oligomerization and conformational change, tetrameric βB2-crystallin has reduced terminal extension flexibility and increased thermal stability of ~12 ºC and no aggregation with time at 60 ºC, relative to the dimeric form.

Conclusion: βB2-crystallin oligomerizes from a face-en-face dimer to a domain-swapped tetramer. This occurs via a domain-swapped dimer intermediate using key ion-pairs and charge screening. This increases the stability of the complex thereby acting to stabilize the lens proteome with age to stave off the formation of age-related cataract.

Deamidation increases the unusual disulfide crosslink between cysteines 24 and 36 in gammas-crystallin

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Purpose: During normal aging and cataract formation the predominant proteins in the lens of the eye, crystallins, undergo extensive deamidation and disulfide formation. One of the major crystallins, gammaS-crystallin, has multiple potential deamidation sites and 7 Cysteines with all but 3 buried. GammaS-crystallin is found in the isolated insoluble proteins from aged lenses. We have previously identified the major deamidation sites occurring at Asn14, 76, and 143. In the present series of experiments, we identified the effects of oxidation and deamidation together.

Methods: We subjected recombinantly expressed gammaS-crystallin with deamidations mimicked at the in vivo sites to oxidation by incubating anaerobically for 5 days with excess oxidized glutathione. Samples were then analyzed by mass spectrometry to measure either whole masses or tryptic peptides.

Results: Whole masses in both wildtype and deamidated gammaS-crystallin indicated the presence of 1 and 2 disulfide bonds while tryptic peptide masses identified the disulfide between Cys24 and Cys36, with lesser amounts between Cys24 and Cys82. Deamidation increased oxidation of gammaS-crystallin. The major mass peak in the oxidized, deamidated gammaS-crystallin matched gammaS-crystallin with two disulfide bonds. Given the buried nature of Cys36, molecular modeling was performed. The structural rearrangement to form 24-36 was surprisingly minimal. The second strand in the structure must relax for the bond to form, providing much more flexibility to C22 and to C26.

Conclusions: We conclude that while the unique arrangement of Cys 22, 24, and 26 suggest a possible role in maintaining the redox potential of the protein, deamidation disrupts this potential facilitating an unusual bond between Cys24 and 36 associated with insolubilization.

Tuesday, December 6th

Session 8: Poster Presentations and Flash Talks

Chairs: Michael Wormstone and Viviana Berthoud

Each presenter has the opportunity to give a 2-minute flash talk, followed then by an interactive poster session when presenters should be available to entertain questions and discussion.

Time	Poster Presenter Coauthors	Title
2:00 - 2:02 pm	Russel McFarland Steve Reichow	Disruption of a conserved N-terminal IXI motif of αB-crystallin leads to the formation of dynamic fibrillar aggregates
2:02 - 2:04 pm	Sanjaya Shrestha Sandeep Aryal Archana D. Siddam Francisco G. Hernández Salil A. Lachke	The transcription elongation factor Ell2 is necessary for achieving optimal expression levels of a specific cohort of genes, including several crystallins, in lens development
2:04 - 2:06 pm	Michael Vu Cathy Cheng	Mapping the universe of Eph receptor and ephrin ligand transcripts in epithelial and fiber cells of the eye lens
2:06 - 2:08 pm	Xiaolu Xu Christopher Materna Yan Wang Shuo Wei Melinda K. Duncan	Modeling lens development in Xenopus tropicalis
2:08 - 2:10 pm	Zhaohua Yu Faisal Raeme	In vivo Cytochrome C release in the lens epithelial cells after subthreshold exposure to UVR-300 nm

Poster 1

Disruption of a conserved N-terminal IXI motif of αB-crystallin leads to the formation of dynamic fibrillar aggregates

Russell J. McFarland[1,2], Steve L. Reichow[1,2]

[1] *Department of Chemistry, Portland State University, Portland, OR, USA*
[2] Chemical Physiology and Biochemistry, Oregon Health and Sciences University, Portland, OR, USA

Purpose: The α-crystallins (αA- and αB- isoforms) function as ATP-independent chaperones that bind and sequester destabilized proteins in the lens, delaying the nucleation of light-scattering aggregates that give rise to age-related cataract. Chaperone activity and the functional polydispersity of the α-crystallins are mediated by two intrinsically disordered regions, the N-terminal domain (NTD) and a C-terminal extension (CTE). The CTE contains an oligomerization motif with sequence IXI and is highly conserved among small heat-shock proteins. A similar IXI-motif is present on the NTD of α-crystallins. While comparatively under-studied, the NT-IXI is thought to contribute to client recognition and to compete with the CTE IXI-motif for binding sites within the core α-crystallin domain.

Methods: To investigate the structural and functional role of the NTD IXI-motif of αB-crystallin, conserved isoleucine residues were replaced with alanine by mutagenesis. This NT-AXA construct was produced in E. coli and characterized by a combination of high-resolution CryoEM, biophysical methods and functional studies.

Results: Disruption of the NT-IXI motif in αB-crystallin led to the discovery of an ordered fibrillar state accessible to αB-crystallin. This helical state is morphologically distinct from previously described amyloid-like states and is completely reversible with the native-like caged assemblies. Single particle CryoEM reveals a flexible helical assembly composed of pair-wise stacks of α-crystallin domain dimers stitched together by interweaving CTE domains. Fibrils are filled with an internal density with limited resolvability attributed to the NTD, presumably facilitating fibril assembly. Functional characterization shows the native-like caged form of this mutant retains substantial chaperone activity, albeit decreased in comparison to wild-type αB-crystallin. In contrast, the fibrillar-state of the NT-AXA mutant appears to potentiate chaperone-client co-aggregation.

Conclusion: These studies revealed the NT-IXI motif of αB-crystallin contributes an important role to chaperone activity and in preventing large-scale filamentous aggregates that could potentially contribute to cataractogenesis and/or other crystallinopathies.

Poster 2

The transcription elongation factor Ell2 is necessary for achieving optimal expression levels of a specific cohort of genes, including several crystallins, in lens development

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Purpose: It is established that dynamic control over gene expression is critical in lens development. Indeed, several transcription factors in this process have been identified. Further, it is also understood that abundant levels of specific transcripts (e.g., crystallins) need to be generated for making high levels of their encoded proteins in fiber cells. However, it is unknown whether "general" components that regulate transcription play a specific role for achieving optimal levels of transcripts that are highly expressed in fibers. To address this knowledge-deficit, we examined whether the iSyTE-identified transcription elongation factor Ell2 (elongation factor for RNA polymerase II 2) has a specific function in regulating key genes in lens development.

Methods: Lens Ell2 expression was examined by RT-PCR, in situ hybridization, Western blotting and immunofluorescence. $Ell2^{cKO}$ mice were generated using Pax6GFPCre. RNA-seq was performed on $Ell2^{cKO}$ lenses. EdgeR and DAVID analysis identified differentially expressed genes and altered pathways.

Results: iSyTE predicted Ell2 as a high-priority candidate based on its enriched expression in the mouse lens. ZFIN database described Ell2 expression to be high in fish lens development. Ell2 expression was validated in mouse lens embryonic and postnatal development and found to be robustly present in fiber cells. $Ell2^{cKO}$ mice exhibited reduced lens size at postnatal day 15. RNA-seq showed reduced transcript levels of a specific subset of fiber genes, including several crystallins, Aqp1, Birc7, Dmrta2, Lgsn, Mip, etc. Thus, sub-optimal levels of key fiber genes may likely contribute to the small lens defect in $Ell2^{cKO}$ mice.

Conclusion: $Ell2^{cKO}$ mice exhibit small lens and reduced expression of a cohort of fiber cell genes. These findings strongly suggest that a general component of the transcription machinery, Ell2, has been recruited in the lens developmental program to enhance expression of key genes and achieve their optimal transcript levels in fiber cells. Funding: R01EY021505.

Poster 3

Mapping the universe of Eph receptor and ephrin ligand transcripts in epithelial and fiber cells of the eye lens

Michael P. Vu and Catherine Cheng

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Purpose: EphA2 receptor and ephrin-A5 ligand mutations have been linked to congenital and age-related cataracts. It has also been found that this receptor and ligand are unlikely to be exclusive binding partners in most of the lens. We conducted a wide investigation using 6-week-old wild-type and knockout mouse lenses to identify and map the transcripts of all Eph receptors and ephrin ligands in the lens.

Methods: Lenses from 6-week-old mice were used for RNA isolation, reverse transcription, PCR, and DNA sequencing to determine the presence of transcripts for Eph and Efn (encodes for ephrin ligands) genes. We examined whole lens, epithelial cell, and fiber cell RNA samples.

Results: Most of the transcripts in the Eph receptor family and all the transcripts in the Efn ligand family were present in the lens. Our results revealed the presence of transcripts for 12 out of 14 Eph receptors and 8 out of 8 Efn ligands in various fractions of lens cells. We verified the variants of each gene that is expressed, and we found two epithelial-cell-specific genes. Surprisingly, we also identified one Eph receptor variant that is expressed in KO lens fibers but is absent from control lens fibers. We also identified one low expression ephrin variant that is only expressed in ephrin-A5 control samples.

Conclusion: These results indicate that the lens expresses almost all Ephs and ephrins, and there may be many receptor-ligand pairs that play a role in lens homeostasis. Our data is the first to reveal the wide variety of Eph receptors and ephrin ligands that are present in the lens. Future studies will utilize real-time PCR on Eph/Efn genes and variants identified in this study to determine whether there is any potential up- or downregulation of transcripts in our knockout and/or aging models.

Poster 4

Modeling lens development in *Xenopus tropicalis*

Xiaolu Xu; Christopher Materna; Yan Wang; Shuo Wei; Melinda K. Duncan

Department of Biological Science, University of Delaware, Newark, DE, USA

Purpose: Lens fiber differentiation from lens epithelial cells (LECs) requires the morphological remodeling of LECs into elongated lens fiber cells (LFCs), however, the mechanisms regulating this process are not well described. Previously, we found that Prox1, a transcription factor critical for the expression of crystallins and other LFCs markers, is also essential for the elongation of LFCs. However, the mechanisms by which Prox1 regulates this phenomenon are unclear although many cytoskeletal regulators are aberrantly expressed in Prox1 null lenses. Here we evaluate the feasibility of using Xenopus (X.) tropicalis, to screen genes with potential importance in lens development.

Methods: We generated a time series of tadpole sections to elucidate the stages of lens morphogenesis. In situ hybridization (ISH) was used to visualize the spatial expression patterns of three cytoskeletal regulators whose mRNA levels are downregulated with loss of Prox1: kinesin 1A (Kif1a), microtubule associated end binding protein (Mapre3) and tubulin-β6 (Tubb6). Translation blocking morpholino was used to knockdown each of these genes while the resulting phenotypes were assessed by plastic sections.

Results: The mammalian lens placode arises from the surface ectoderm and invaginates to form the lens vesicle. In X. tropicalis, however, it keeps thickening to form a flattened spherical cell mass without a vesicle structure. The lens mass then reorganizes dramatically to form a single layer of epithelial cells at the surface surrounding the lens core, which composed of many concentric layers of elongated LFCs. ISH suggested that all three genes have concentrated expression in the eye at lens vesicle stage. Loss of Mapre3 and Tubb6 produces microphthalmia associated with lens structural defects, whereas inactivating Kif1a produces a stronger defect with the absence of lens structures.

Conclusion: X. tropicalis is a valuable model to reveal the molecular mechanism regulating lens development, in which cytoskeletal regulators play important roles.

Poster 5

In vivo Cytochrome C release in the lens epithelial cells after subthreshold exposure to UVR-300 nm

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Purpose: The maximum tolerable dose (MTD) of UVR-B at 300 nm in rat was estimated at 3.65 kJ/m^2. Subthreshold doses have additive effects. Daily exposure to 1 kJ/m^2 accumulates over time leading to cataract formation. The subthreshold UVR damage takes longer than 24 h to repair. Thus, this study aimed to investigate the time evolution of Cytochrome C (Cyt C) release in the rat lens epithelial cells after in vivo subthreshold exposure to ultraviolet radiation (UVR).

Methods: Twelve six-week-old female albino Sprague-Dawley rats were unilaterally exposed to UVR of 1 kJ/m^2. At a latency of 1, 8, 16 and 24 hours after exposure, both exposed and contralateral non-exposed eyes were enucleated and processed for immunohistochemistry. The Triton X-100 concentration was first determined by comparing the pixel intensity of Cyt C staining at different Triton concentrations. Three mid-sagittal sections from each lens were stained with the selected Triton concentration. The stained lens epithelial cells were masked for estimation of mean pixel intensity.

Results: The pixel intensity of Cyt C staining increased as Triton concentration increased, and 0.09 % Triton was used in order to dissolve cell membranes but not mitochondria membranes. There was no significant difference of Cyt C release among the post-exposure time groups. There was no significant difference between exposed and contralateral non-exposed eyes. No significant difference was indicated between exposed and contralateral non-exposed eyes at different post-exposure time groups.

Conclusions: UVR-B of 1 kJ/m^2 at 300 nm does not induce cytochrome C release from mitochondria into cytosol in the lens epithelial cells within 24 hours after exposure. It might indicate a delayed onset of Cyt C release.


WEDNESDAY

Wednesday, December 7th

Session 9: Autophagy in Lens Differentiation, Maintenance, and Disease

Chairs: Marc Kantorow and Lisa Brennan

Talks are in the format of 12 minutes presentation with 3 minutes discussion.

Time	Speaker Coauthors	Title
9:00 - 9:15 am	Fielding Hejtmancik Xiaodong Jiao Zhiwei Ma Shahid Y. Khan Muhammad Ali Firoz Kabir Chan Hyun Na Michael Delannoy Yinghong Ma Caihong Qiu M. Joseph Costello S. Amer Riazuddin	The role of FYCO1-dependent autophagy in lens fiber cell differentiation and cataractogenesis
9:15 - 9:30 am	Rifa Gheyas Sue Menko	Role of PI3K Signaling Inhibition in Inducing Autophagy-Dependent Organelle Elimination
9:30 - 9:45 am	Lisa Brennan Josh Disatham Rachel Zabizin Judy Yang Marc Kantorow	Hypoxia-dependent mitophagy regulates formation of the lens OFZ
9:45 - 10:00 am	Om Srivastava Akousa Boateng Roy Joseph	Dual function of βA3/A1-crystallin in the lens: Structural protein and as an Autophagy modulator
10:00 - 10:15 am	Alan Shiels Thomas M. Bennett Thomas W. White Yuefang Zhou	A novel role for charged multivesicular-body protein 4b in the lens
10:15 - 10:30 am	Marc Kantorow Joshua Disatham Lisa Brennan	HIF1 is a master regulator of hypoxia-dependent lens fiber cell gene expression

The role of FYCO1-dependent autophagy in lens fiber cell differentiation and cataractogenesis

J. Fielding Hejtmancik[1], Xiaodong Jiao[1], Zhiwei Ma[1], Shahid Y. Khan[2], Muhammad Ali[a], Firoz Kabir[2], Chan Hyun Na[3], Michael Delannoy[4], Yinghong Ma[5], Caihong Qiu5, M. Joseph Costello[4], and S. Amer Riazuddin[2]

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Purpose: FYVE and coiled-coil domain containing 1 (FYCO1) is an adaptor protein expressed ubiquitously and required for microtubule-dependent plus-end-directed transport of macroautophagic/autophagic vesicles. We investigated the role of FYCO1 mutations in human cataract genetics and pathophysiology and the role of FYCO1 in autophagy and organelle clearance during lens fiber cell differentiation.

Methods: Linkage analysis, WES and Sanger DNA sequencing, Transcriptome, proteome, and metabolome profiling, flow cytometry, confocal microscopy, and transmission electron microscopy

Results: Loss-of-function mutations in FYCO1 cause inherited cataracts without additional ocular or extra-ocular phenotypes. FYCO1 is expressed in the mouse embryonic and adult lens, peaking at P12d. FYCO1 proteins partially colocalize to microtubules and are found adjacent to Golgi, but are primarily colocalize to autophagosomes. Fyco1 homozygous knockout mice recapitulate the cataract phenotype in humans. Transcriptome, proteome, and metabolome profiling identifies many autophagy-associated genes, proteins, and lipids perturbed in Fyco KO mouse lenses. FYCO1 (c.2206C>T) knock-in human lens epithelial cells exhibit decreased autophagic flux and autophagic vesicles secondary to loss of FYCO1. Damaged cellular organelles accumulate in FYCO1 (c.2206C>T) knock-in lens organoids and in Fyco1 KO mouse lenses.

Conclusion: Mutations in FYCO1 are a common cause of autosomal recessive congenital cataracts in humans, acting through a loss of FYCO1 function with secondary diminished autophagic flux and impaired organelle removal.

Role of PI3K Signalling Inhibition in Inducing Autophagy-Dependent Organelle Elimination

Rifah Gheyas, and A. Sue Menko

Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA, USA

Purpose: Previous studies performed in our lab show that the spatiotemporal elimination of mitochondria and ER during development to form the Organelle Free Zone (OFZ) is autophagy-dependent. These studies investigated the role of suppression of PI3K signaling pathways in autophagy linked elimination of mitochondria, ER, and nuclei during lens development.

Methods: At E12, a developmental time prior to formation of the OFZ, chick embryo lenses in organ culture were exposed to the pan-PI3K inhibitors, LY294002 or CH5132799, the PI3K/Akt-signaling axis inhibitor MK-2206, or the vehicle DMSO for 24hrs. Impact on organelle removal was analyzed by immunoblot of differentiation state-specific fractions isolated by microdissection and immunolocalization of cryosections. Antibodies included those to autophagy promoting molecules, PI3K signaling pathway intermediates, and organelle-specific proteins. Primary lens cell cultures that had differentiated to form lentoid structures were immunolabeled for autophagy markers.

Results: Exposure of lenses to pan-PI3K inhibitors or the Akt-specific inhibitor induced autophagy and the premature removal of mitochondria and ER from lens fiber cells. While blocking all PI3K downstream signaling also induced the premature elimination of nuclei and complete formation of the OFZ, blocking just the PI3K/Akt signaling axis alone was alone insufficient to execute nuclear elimination. However, immunostaining of lentoid structures in primary lens cultures showed the presence of autophagolysosomes adjacent to nuclei and containing chromatin fragments.

Conclusion: Elimination of lens fiber cell mitochondria and ER to form the OFZ involves an autophagic-dependent mechanism activated through suppression of the PI3K/Akt signaling axis. While the elimination of nuclei is also PI3K-regulated, its induction involves blocking multiple downstream effectors of PI3K. Autophagy also appears to play a role in disposal of fragments of nuclear material during the final stages of lens fiber cell denucleation.

Hypoxia-dependent mitophagy regulates formation of the lens OFZ

Lisa Brennan, Josh Disatham, Rachel Zabizin, Judy Yang and Marc Kantorow

Department of Biomedical Science, Charles E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, FL USA

Purpose: A long-sought question in lens biology is how organelles are removed to form the lens organelle-free zone. An answer to this question came from early studies from our lab showing that lens deletion of the mitophagy protein BNIP3L resulted in retention of non-nuclear organelles during lens fiber cell maturation. We also showed that non-nuclear organelle retention was dependent on the low oxygen levels in fiber cells resulting from the hypoxic environment of the lens itself. These studies suggested that hypoxia dependent BNIP3L regulation and key autophagy requirements govern the elimination of non-nuclear organelles during lens formation. Here we provide evidence for these mechanisms.

Methods: Cultured embryonic chick lenses and lenses from WT and BNIP3L knockout mice and were visualized by IHC and confocal microscopy. Lenses were also examined for protein levels by western analysis. Embryonic chick lenses were also exposed to hypoxia and analyzed similarly. Mutant forms of BNIP3L deleted for specific functional domains were generated and analyzed for their ability to eliminate non-nuclear organelles upon over expression in cultured embryonic chick lenses.

Results: BNIP3L co-localizes mitochondria (MT), endoplasmic reticulum (ER), and Golgi apparatus (GA) in lens fiber cells. These organelles were retained in BNIP3L KO relative to WT mouse lenses. Exposure of cultured chick lenses to hypoxia resulted in accelerated elimination of MT, ER and GA relative to lenses cultured under normoxic conditions. Hypoxia exposure of the lens resulted in activation of the master regulator of the hypoxic response transcription factor HIF1 and HIF1 was confirmed to bind to the BNIP3L transcriptional regulatory region. Deletion of specific BNIP3L domains will be discussed.

Conclusion: Hypoxia-dependent activation of BNIP3L drives elimination of non-nuclear organelles during lens formation and is transcriptionally regulated by HIF1. The results confirm the importance of autophagy for establishing the structure and function of the mature lens.

Dual function of βA3/A1-crystallin in the lens: Structural protein and as an Autophagy modulator

Om P. Srivastava, Akousa Boateng and Roy Joseph

Department of Optometry and Vision Science, University of Alabama at Birmingham, Birmingham, AL, USA

Purpose: In the lens, βA3/A1-crystallin has been known to function as a structural protein. Studies in retina show that the crystallin modulates autophagy by being a lysosomal-resident protein. The primary purpose of the study was to determine whether βA3/A1-crystallin functions as an autophagy modulator in the lens.

Methods: We have generated two mouse models: (1) βA3/A1-crystallin complete knockout mouse model (named: βA3/A1KO, where βA3/A1 is absent in the lens), and (2) βA3/A1 G-91-deletion knock-in mouse model (named βA3ΔG91, where βA3/A1 is dysfunctional). Relative to wild-type lenses, lenses of both mouse models were examined for the potential role of the crystallin as an autophagy modulator by determining levels of autophagy markers and double-membraned vesicles containing undegraded organelles by immunohistochemical, cytochemical and electron microscopic (EM) analyses.

Results: Relative to wild-type lenses, the lenses of βA3/A1KO and βA3ΔG91 mice exhibited attenuation of organelle degradation, greater cellular apoptosis, reduced lysosomes numbers and developed congenital cataract. On examination of lens frozen sections and lens cultured epithelial cells of βA3/A1KO and βA3ΔG91 mice, greater levels of autophagic markers [ubiquitinated proteins, p62 (a ubiquitin-binding scaffold protein that colocalizes with ubiquitinated protein aggregates), LC3 (a 17-kDa microtubule-associated soluble protein)], were present in the lenses of the two mouse models relative to those from wild-type mice. Additionally, during EM analysis, the lenses of βA3/A1KO and βA3ΔG91 mice showed double-membraned vesicles containing undegraded organelles relative to wild-type lenses. The results suggested that an absence or dysfunctional βA3/A1-crystallin in lenses of βA3/A1KO and βA3ΔG91 mice, respectively, result in blockade of autophagic organelles degradation.

Conclusions: The results suggest that either an absence βA3/A1 (in βA3/A1KO) or a dysfunctional βA3/A1 (in βA3ΔG91) causes autophagic blockade at the lysosomal level to prevent organelles degradation in the lens.

A novel role for charged multivesicular-body protein 4b in the lens

Alan Shiels[1], Thomas M. Bennett[1], Thomas W. White[2], Yuefang Zhou[1]

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[2] *Department of Physiology and Biophysics, Stony Brook University, Stony Brook, NY, USA*

Purpose: Charged multivesicular-body protein 4b (CHMP4B) is a core subunit of the endosomal sorting complex required for transport-III (ESCRT-III) machinery that serves many remodeling and scission processes of biological membranes including, but not limited to, plasma and lysosome membrane repair, (macro)autophagy, and mitophagy. Sequence variations in the human gene for CHMP4B have been associated with inherited and age-related forms of cataracts and CHMP4B is required for lens growth and differentiation in mice. Here, we determine the sub-cellular distribution of CHMP4B in the lens and uncover a novel association with gap-junction alpha-3 protein (GJA3) or connexin-46 (Cx46) and GJA8 or Cx50.

Methods: Mouse eye sections and isolated lens fiber cells (LFCs) were imaged using immunofluorescence confocal microscopy (IFCM) and the in situ proximity ligation assay (isPLA). Lens protein complexes were analyzed by immunoprecipitation and immunoblotting (IP-IB).

Results: IFCM revealed that CHMP4B localized to plasma-membranes of elongated LFCs in the outer cortex of the lens - where gap-junction plaques often associated with ‘ball-and-socket’ double-membrane junctions first form - particularly on the broad faces of these flattened hexagon-like cells in cross-section. Dual IFCM showed that CHMP4B co-localized with gap-junction plaques containing Cx46 and/or Cx50. When combined with the isPLA, IFCM indicated that CHMP4B lay in close physical proximity (< 40-nm apart) to Cx46 and Cx50. In Cx46-knockout (KO) lenses, CHMP4B-membrane distribution was similar to that of wild-type, whereas, in Cx50-KO lenses CHMP4B localization to LFC membranes was lost. IP-IB analysis revealed that CHMP4B formed complexes with Cx46 and Cx50 in vitro.

Conclusions: Our data suggest that CHMP4B forms cell-membrane complexes with Cx46 and Cx50 and may be associated with ball-and-socket junctions during LFC differentiation. Further, they raise the possibility that Cx46 and/or Cx50 are both substrates and regulators of autophagy as previously shown for other connexins.

HIF1 is a master regulator of hypoxia-dependent lens fiber cell gene expression

Marc Kantorow, Joshua Disatham and Lisa Brennan

Charles E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, FL, USA

Purpose: The lens contains a decreasing oxygen gradient from the surface to the core that parallels the elimination of organelles and the expression of key genes required for lens fiber cell formation and function. Consistently our lab has demonstrated that the mitophagy protein BNIP3L that is required for elimination of non-nuclear organelles is induced by exposure of the lens to hypoxia and its transcription is dependent on activation of the master regulator of the hypoxic response, transcription factor HIF1a. Here we sought to identify other genes regulated by HIF1a and the requirement for chromatin remodeling in the expression of these genes.

Methods: We employed a multiomics approach combining CUT&RUN, RNA-seq and ATAC-seq analysis to establish the genomic complement of lens HIF1α binding sites, genes activated or repressed by HIF1α and the chromatin states of HIF1α-regulated genes.

Results: CUT&RUN analysis revealed 8,375 HIF1α-DNA binding complexes in the chick lens genome. 1,190 HIF1α-DNA binding complexes were significantly clustered within chromatin accessible regions (χ^2 test $p < 1x10^{-55}$) identified by ATAC-seq. Formation of the identified HIF1α-DNA complexes paralleled the activation or repression of 526 genes, 116 of which contained HIF1α binding sites within 10kB of the transcription start sites. GO and pathway analysis implicate HIF1α in control of a wide variety of cellular pathways potentially critical for lens fiber cell formation, structure and function including autophagy, mitophagy, glycolysis, cell cycle regulation, chromatin remodeling, Notch and Wnt signaling, differentiation, development, and transparency.

Conclusions: These data identify HIF1α as a key regulator of genes required for lens fiber cell formation, structure and function and they provide a basis for understanding the role in chromatin in hypoxia-dependent lens gene expression.

Wednesday, December 7th

Session 10: Redox Biology of the Lens

Chairs: Xingjun Fan and Eugene Serebryany

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

Time	Speaker Coauthors	Title
11:00 - 11:18 am	Marjorie Lou	The Ying-Yang effect, or the damage and benefit of reactive oxygen species (ROS) to the lens – A review
11:18 - 11:36 am	Hongli Wu Kayla Green Jinmin Zhang Yu Yu Majorie Lou	Glutaredoxin Activators with Multiple Antioxidative Activities Protect the Lens from Oxidative Damage
11:36 - 11:54 am	Elizabeth Whitcomb Sheldon Rowan Eloy Bejarano Rebecca L. Pfeiffer Kristie L. Rose Kevin L. Schey Bryan W. Jones Maria Miranda Allen Taylor	Expression of a mutant ubiquitin in the lens results in cataract and alterations in redox status and amino acid metabolism
11:54 - 12:12 pm	Eugene Serebryany Sourav Chowdhury Christopher N. Woods David C. Thorn Nicki E. Watson Arthur McClelland Rachel E. Klevit Eugene I. Shakhnovich	Myo-inositol, natively abundant in the human eye lens, acts as a chemical chaperone suppressing redox-dependent γ-crystallin misfolding and aggregation
12:12 - 12:30 pm	Xingjun Fan Zongbo Wei Caili Hao Vincent Monnier Mark Haamrick Meghan McGee-Lawrence	Blocking glutathione synthesis enzyme GCLC truncation delays cataract formation

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

Marjorie F. Lou

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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 first two are very unstable and short lived while the last one is freely diffusible and relatively long-lived.

Methods: They can be made internally through various enzyme systems or externally from ultraviolet light or ionizing radiation and other environmental toxins. Although the lens is rich in antioxidants, such as glutathione (GSH), plus other detoxification enzyme systems to protect the lens from oxidative stress and damage, the prolonged exposure of the lens to ROS during the long lifespan of an individual can be overwhelmed by these toxic and harmful molecules. Thus, ROS are generally considered as the leading cause for senile cataract formation. Yet ROS have also been shown as beneficial to the lens, as they are essential elements in mediating growth factor's mitogenic function during cell proliferation.

Results: This review is to describe first the Ying-side or the damage-side of H_2O_2. How it can oxidize lens protein thiols, leading to cascade results of protein-protein disulfide formation, aggregation, and eventual cataract formation. The review will then describe the Yang-side, or the good side of ROS by demonstrating how H_2O_2 at certain levels can 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 confirms the importance of redox regulation and balance for the general health of cells and tissues, and certainly the lens.

Glutaredoxin Activators with Multiple Antioxidative Activities Protect the Lens from Oxidative Damage

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Purpose: To evaluate the function and therapeutic potential of the glutaredoxin (Grx) system, both glutaredoxin 1 (Grx1) and glutaredoxin 2 (Grx2), using Grx1/Grx2 double knockout (DKO) mice and the Grx activators as research models.

Methods: Two-month-old Grx1/Grx2 DKO and age-matched wild-type (WT) mice were exposed to 20.6 kJ/m^2 UV radiation for 15 mins to induce cataracts. Mice were euthanized at 4 days post-exposure. The degree of the cataract and lens morphology were evaluated under a dissecting microscope. To further define the crosstalk between the Grx system and nuclear factor erythroid 2-related factor 2 (Nrf2) antioxidant pathway, Nrf2 and its downstream target proteins were examined by using Western blot analysis. Additionally, we have developed a series of Grx modulators, molecules L1-L4, which are a family of pyridol-containing N-heterocyclic amines. The therapeutic potential of these Grx activators was evaluated using primary lens epithelial cells and ex-vivo lens organ culture system.

Results: We found that UV radiation caused more severe anterior subcapsular cataract in Grx1/Grx2 DKO than that of WT mice. Lenses of Grx1/Grx2 DKO mice contained significantly lower levels of glutathione (GSH) and higher levels of glutathionylated proteins (PSSG), a marker for protein thiol oxidation. Deletion of Grx1 and Grx2 also decreased the expression of the antioxidant enzyme transcription factor regulator, Nrf2, and its downstream antioxidant genes, including catalase, superoxide dismutase (SOD), and thioredoxin (Trx). Our ex vivo study showed that L1-L4 dose-dependently protected the lens from H_2O_2-induced opacification. L4 exerted anti-ferroptotic function by binding iron (II) and (III), upregulating glutathione peroxidase 4, and preventing MDA formation.

Conclusions: Grx1 and Grx2 gene deletion impairs Nrf2-dependent antioxidant response, causing elevation of oxidative stress that may increase the lens susceptibility to UV-induced damage. Grx activators can improve redox homeostasis in the lens and prevent H_2O_2-induced cataract formation.

Expression of a mutant ubiquitin in the lens results in cataract and alterations in redox status and amino acid metabolism

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Purpose: While cataracts can develop through multiple mechanisms, they often occur through common pathways and lead to common endpoints. Protein aggregation, impaired fiber cell differentiation, and absence of fiber cell denucleation are all frequently observed in congenital cataracts. It is now apparent that other metabolic abnormalities associate with cataractogenesis, including alteration in levels of amino acids, glutathione, taurine, and redox homeostasis. We have analyzed the proteome and spatial metabolome of mice expressing a mutant ubiquitin protein (K6W-Ub) in the lens. Furthermore, we have analyzed redox related metabolites to determine the molecular mechanisms underlying formation of the congenital cataract.

Methods: Lenses from C57BL/6J wild-type or cataractous K6W-Ub transgenic mice were dissected at E15.5, P1, or P30 and proteins were analyzed via MS-based tandem-mass-tag (TMT) quantitative proteomics. Spatial metabolomics were quantified using computational molecular phenotyping (CMP). Validation of proteomics findings was performed using Western blot analysis and immunohistochemistry. Glutathione and taurine were quantified using HPLC.

Results: Pathway analyses of the proteomic data revealed processes that were altered during lens differentiation, by expression of K6W-Ub, or both including glutathione metabolism; glycolysis/gluconeogenesis; and glycine, serine, and threonine metabolism. Analysis of the metabolome via CMP revealed statistically significant decreases in taurine and glutathione and smaller decreases in glutamate, glutamine, aspartate, and valine in all ages of K6W-Ub lenses. Lens metabolites were spatially altered in the cataractous K6W-Ub lens. Quantification of glutathione and taurine levels in lenses demonstrated decreases in taurine levels and GSH:GSSG ratios in the cataractous lenses.

Conclusions: The large reductions in levels of taurine and glutathione may be general signatures of cataract development, as human cataracts also have reduced glutathione and taurine. Key roles for amino acid metabolism and glycolysis/gluconeogenesis in cataractogenesis are emerging. Together our data point toward potential common metabolic/proteomic signatures of cataracts.

Myo-inositol, natively abundant in the human eye lens, acts as a chemical chaperone suppressing redox-dependent γ-crystallin misfolding and aggregation

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Purpose: Light-scattering protein aggregates in the lens cause cataract, one of the most prevalent protein aggregation disorders. Lens cytoplasm becomes progressively more oxidizing with age and especially with cataract onset. Proteomic and in vitro studies have converged on the importance of lens γ-crystallin misfolding via formation of non-native disulfide bonds for light scattering aggregation. Delivery of aggregation suppressors (or any drugs) to the lens is notoriously difficult. We wondered whether a previously overlooked aggregation suppressor could be gleaned from the native lens metabolome itself.

Methods: We quantified aggregation suppression by the unusually abundant lens metabolite myo-inositol and several structurally or chemically similar compounds, using our previously well-characterized in vitro aggregation assays of oxidation-mimicking human γD-crystallin variants and investigated myo-inositol's molecular mechanism of action using solution NMR, negative-stain TEM, differential scanning fluorometry, thermal scanning Raman spectroscopy, turbidimetry in redox buffers, and free thiol quantitation.

Results: Myo-inositol strongly suppressed aggregation of multiple γD-crystallin variants, in a concentration dependent manner under physiologically relevant conditions. Unlike many known chemical chaperones, myo-inositol's primary target was not the native, unfolded, or final aggregated states of the protein; rather, we propose that it was the rate-limiting bimolecular step on the aggregation pathway. Notably, myo-inositol suppressed both misfolding and formation of non-native intramolecular disulfides in γD-crystallin in mildly oxidizing glutathione buffers, despite containing no redox-capable chemical moieties.

Conclusion: One of the evolved mechanisms of lens resilience involves myo-inositol suppressing oxidative γ-crystallin misfolding and consequent aggregation. Given recent metabolomic evidence that myo-inositol is severely depleted in human cataractous lenses compared to age-matched controls, maintaining or restoring healthy levels of this compound in the lens may be a simple, safe, and globally accessible strategy to prevent or delay lens opacification due to age-onset cataract.

Blocking glutathione synthesis enzyme GCLC truncation delays cataract formation

Xingjun Fan[1], Zongbo Wei[1], Caili Hao[1], Vincent Monnier[2], Mark Haamrick[1], Meghan McGee-Lawrence[1]

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[2] *Department of Pathology and Biochemistry, Case Western Reserve University*

Purpose: Oxidation has been documented as the major risk factor in age-related cataract formation. Glutathione (GSH), the most abundant lens antioxidant, is continuously decreasing in the aging lens, particularly in the lens nucleus. The critical role of GSH in maintaining lens redox status and transparency is very well recognized. But the underlying mechanisms of impaired GSH biosynthesis and profound reduction of lens GSH levels in the aged lens are still poorly understood. We found that an age-related truncation of the GSH synthesis enzyme GCLC might be responsible for impaired GSH synthesis in aged lenses. We created knock-in mice to block GCLC truncation.

Method: The posttranslational processing of γ-glutamyl cysteine ligase, catalytic subunit (GCLC) in human and mouse lenses at different ages, was measured by immunoblot and mass spectrometry. The cleavage site was predicted by in-silico computational analysis and was confirmed by mass spectrometry and amino acids mutagenesis approach. Aspartate to glutamate mutation (D499E) knock-in mice were created by Crispra-Cas9 gene editing. The degree of cataracts was monitored by the slit-lamp.

Results: Age-related GCLC truncation produces 60KD (G60) and 13KD (G13) fragments. The G60 fragment contains a catalytic site but loses catalytic activity. However, G60 and G13 can form heterodimers intracellularly when both fragments are present. G60-G13 heterodimer gains catalytic activity but is significantly lower than native GCLC. We monitored 150 20-month-old mice and found that D499E KI mice had substantially fewer cataracts than wild-type mice.

Conclusion: Our study reveals that age-related truncation of the GSH synthesis enzyme GCLC is responsible for declined lens GSH during aging and blocking such truncation may delay cataract formation.


THURSDAY

Thursday, December 8th

Session 11: Lens Regeneration and Lentoids

Chairs: Michael Wormstone and Barbara Pierscionek

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

Time	Speaker Coauthors	Title
9:00 - 9:18 am	Antonio Carlos Lottelli	A clinical perspective on pediatric cataract treatment
9:18 - 9:36 am	Katia Del Rio-Tsonis Sophia Carmen Ratvasky Georgios Tsissios Anthony Sallese J. Raul Perez-Estrada Jared A. Tangeman Weihao Chen Byran Smucker Arielle Martinez Hui Wang	Macrophages are essential for newt lens regeneration
9:36 - 9:54 am	Michael O'Connor Rachel Shparberg Stephanie Watson	Investigating in vivo human lens regeneration via lens cell transplantation
9:54 -10:12 am	Sofia Moriam	Using stem cell-derived human lens cells to explore responses to BMPs
10:12 - 10:30 am	Sadia Islam Catherine Cheng Velia M. Fowler	NMIIA:F-actin contractility regulates meridional row cell shape transformation & alignment during lens differentiation

A clinical perspective on pediatric cataract treatment

Antonio Carlos Lottelli[1,2]

[1] *Botucatu Medical School, Universidade Estadual Paulista Júlio de Mesquita Filho, Brazil*
[2] Medical University of South Carolina, USA

Purpose: The aim of this presentation is to show the difficulties encountered in the treatment of pediatric cataract through the currently available means and how new perspectives of treatment using stem cells can overcome these difficulties.

Methods: Clinical evaluation studies and related case studies.

Results: A red reflex exam was implemented in the area of the Botucatu Medical School Clinical Hospital (São Paulo, Brazil). This led to the creation of a reference Center for treatment of childhood cataract and related difficulties. Fixed diopter intraocular lenses without accommodation present significant challenges in a growing eye at risk of amblyopia. Difficulties are encountered in the use of contact lenses for pediatric cataract management, especially in developing countries and families with low socioeconomic status.

Conclusions: The treatment currently available for pediatric cataracts generates ametropia that is difficult to treat, with a risk of amblyopia and impossibility of accommodation recovering. Treatment involving stem cells might overcome these difficulties providing better anatomical and functional results.

Macrophages are essential for newt lens regeneration

Sophia Carmen Ratvasky [1,2,3], Georgios Tsissios [1,2,3], Anthony Sallese [1,2], J. Raul Perez-Estrada [1,2] , Jared A. Tangeman [1,2,3], Weihao Chen [2,3,4], Byran Smucker[2, 5], Arielle Martinez [1,2], Hui Wang [2,4], Katia Del Rio-Tsonis [1,2,3]

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Purpose: For many decades, scientists have been trying to better understand the fascinating scar-free injury response known as regeneration. Many studies have shown that macrophages are required for successful regeneration across a wide variety of animals. We aimed to investigate the role of macrophages during newt lens regeneration.

Methods: To assess the role of macrophages during newt lens regeneration, we systematically depleted macrophages using clodronate liposomes. To test this, control or clodronate liposomes were added immediately after lentectomy, and at 2 and 4 days post-lentectomy (dpl) in the optic cup. In addition, we introduced an additional injury to the iris 60 dpl and assessed for regeneration. Eyes were in vivo imaged via optimal coherence tomography and/or collected and processed for histology, iris cell proliferation, gene expression, alpha-smooth actin and extracellular matrix presence.

Results: All macrophage depleted eyes failed to regenerate a new lens. In addition, early macrophage depletion during the lens regeneration process resulted in a significant decrease in iris cell proliferation, an unresolved cell accumulation, and prolonged inflammation. Moreover, a fibrotic-like response and abnormalities in extracellular matrix remodeling were observed. Remarkably, a secondary injury to the iris was sufficient to re-start the regeneration process and cleared the fibrotic response.

Conclusion: We determined that macrophages are necessary for newt lens regeneration as they modulate the inflammatory response during injury, preventing fibrosis and contributing to extracellular matrix clearance and proliferative signals. Most impressive was that the introduction of a new injury was sufficient to re-set the fibrotic path to a regenerative path. Further investigating the cellular and molecular mechanisms that regulate macrophage-iris interactions within this regeneration paradigm will provide further insights on how of these immune cells could be modulating repair processes in other species including mammals.

Investigating in vivo human lens regeneration via lens cell transplantation

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Purpose: Cataracts are a leading cause of low vision and blindness in children. Implantation of an IOL is typically avoided in young children as their eye growth changes the required IOL characteristics. Standard replacement of lens function via contact lenses or aphakic glasses is traumatic for the children and caregivers, and vision outcomes are often suboptimal. Unsurprisingly, childhood cataract patients report quality of life as low as child cancer patients. These factors lead to impaired motor, cognitive, language and social development. with lifelong social, educational and employment disadvantage. Lens regeneration, from cataract mutation-free lens epithelial cells (LECs), might avoid these problems by providing a replacement biological lens capable of growing with the patient.

Methods: We performed a pilot lens cell transplantation study in New Zealand white rabbits to assess the impact of the rabbit eye environment. Purified human LECs were produced from pluripotent stem cells via established methods (Murphy et al., 2018). Cells were seeded onto a biodegradable attachment substrate for transplantation (Taylor et al., 2015). One eye of one rabbit received the substrate only. One eye of a second rabbit received the stem cell-derived LECs on the substrate. Three months after transplantation, treated and untreated eyes were analyzed via a characterization pipeline consisting of MRI, histology and mass spectrometry.

Results: More lens material was present in the eye that received the stem cell-derived LECs compared to the eye that received only the attachment substrate. The MRI and histology showed the cell attachment substrate was not degraded and induced an immune response. Mass spectrometry showed human lens fiber cell proteins, including crystallins, only in the LEC-transplanted eye.

Conclusions: These data indicate the rabbit eye can stimulate human LECs to differentiate into lens fiber cells, supporting continued investigation of LEC transplantation as a potential approach to lens regeneration for childhood cataract.

Using stem cell-derived human lens cells to explore responses to BMPs

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Purpose: Crystallins are the predominant proteins in the lens, with different crystallins expressed in different lens locations. Bone morphogenetic proteins (BMPs) are a diverse class of growth factors that have been reported to regulate lens development and crystallin expression. This study used human lens epithelial cells (LECs) derived from pluripotent stem cells to investigate the role of BMPs in crystallin expression.

Methods: Human pluripotent stem cells were used to generate ROR1-expressing (ROR1e) LECs, and subsequently light-focusing micro-lenses (mi-ls), using established methods (Dewi et al., 2021). The LECs and mi-ls were treated with BMP inhibitors or BMPs, with the effect of these treatments compared to control treatments. Effects were quantified for crystallin mRNA (via qPCR) and protein (via mass spectrometry), as well as various mi-l parameters including light focusing (via light microscopy and image analyses).

Results: Consistent with our published data (Murphy et al., 2018), ROR1e LECs treated with BMP inhibitors showed a significant decrease in mRNA and protein expression for some crystallins. Similarly, mi-ls treated with BMP inhibitors (without exogenous BMPs) showed impaired development in vitro, decreased expression of a range of crystallin proteins, and decreased light-focusing ability. Conversely, treating mi-ls with exogenous BMPs caused increased light focusing and increased expression of some crystallins.

Conclusions: These data suggest the expression of various crystallin proteins in stem cell-derived human lens cells is promoted by endogenous BMPs. This expression can be altered using exogenous BMPs and BMP inhibitors. Whether this BMP-mediated regulation of crystallin expression is direct or indirect is yet to be determined.

NMIIA:F-actin contractility regulates meridional row cell shape transformation & alignment during lens differentiation

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Purpose: Lifelong lens transparency and focusing ability depend on the precise alignment of equatorial epithelial cells that differentiate into secondary fiber cells during lens development and morphogenesis. During this process, the equatorial epithelial cells transform from randomly packed cells into precisely aligned, hexagonally shaped meridional row cells that elongate to form the hexagonally packed, long fiber cells. We have shown that non-muscle myosin IIA (NMIIA) is required for epithelial cell alignment and fiber cell hexagonal packing, based on misaligned meridional rows and disordered fiber cells in mouse lenses with mutations in NMIIA. To determine how actomyosin networks control epithelial cell alignment, we investigated actomyosin network organization prior to and during these shape transformations in wild-type lenses.

Method: We used reporter knock-in mice with enhanced GFP (eGFP) fused to the head domain of the NMIIA heavy chain. Lens whole mounts were stained with fluorescent phalloidins (F-actin) and Hoechst (nuclei) and imaged by confocal microscopy to investigate actomyosin organization.

Result: We observed distinct GFP-NMIIA:F-actin networks in lens anterior epithelial and equatorial meridional row cells. GFP-NMIIA associates with F-actin at the basal surface and apical polygonal arrays in anterior epithelial cells and is present in the cytoplasm surrounding the nucleus. However, GFP-NMIIA does not colocalize with sequestered F-actin bundles and cortical F-actin near the lateral membranes of anterior epithelial cells. In contrast, cortical membrane-adjacent GFP-NMIIA:F-actin and cytoplasmic stress fibers crossing the cells are observed at the basal surface of meridional row cells. In the middle and apical regions of these elongating cells, NMIIA-F-actin stress fibers are no longer present, and NMIIA:F-actin is highly concentrated at anterior-posteriorly oriented membranes, and depleted from equatorially-oriented membranes.

Conclusion: Actomyosin remodeling accompanies the lens epithelial cell shape transformations and alignment that take place during differentiation to form precisely aligned, hexagonally packed fiber cells.

Thursday, December 8th

Session 12: Large Scale Data in Lens Research

Chairs: Salil Lachke and Ales Cvekl

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

Time	Speaker Coauthors	Title
11:00 - 11:18 am	Ales Cvekl William K. Chang Danielle Rayee Michael Camerino Yilin Zhao Qing Xie Masako Suzuki Chi Zhang Carolina Eliscovich Deyou Zheng	Lens development and lens fiber cell differentiation: New insights on gene control mechanisms from multi-omics and imaging
11:18 - 11:36 am	Hélène Choquet Chen Jiang Jie Yin Thomas J. Hoffmann Pirro G. Hysi M. Maria Glymour Eric Jorgenson Ronald B. Melles Salil A. Lachke	Genome-wide association study of cataract: identification of risk loci and shared genetics with other common vision disorders
11:36 - 11:54 am	Salil Lachke Sandeep Aryal Sarah Coomson Sanjaya Shrestha Deepti Anand	Applying iSyTE to uncover regulatory networks in lens development and its associated defects
11:54 - 12:12 pm	Matthieu Duot Carole Gautier-Courteille Yann Audic Agnès Mereau Archana D. Siddam Deepti Anand David Reboutier Justine Viet Catherine Le-Goff-Gaillard Salil A. Lachke Luc Paillard	Application of a multi-omics approach using iCLIP-seq and RNA-seq to the lens to identify downstream RNA targets of the cataract-linked RNA-binding protein CELF1
12:12 - 12:30 pm	Adrienne Giannone Caterina Sellitto Barbara Rosati David McKinnon Thomas W. White	Single Cell RNA Sequencing of the Lens Epithelium in Wild Type Postnatal Mice

Lens development and differentiation: New insights on gene control mechanisms from multi-omics and imaging

Ales Cvekl, William K. Chang, Danielle Rayee, Michael Camerino, Yilin Zhao, Qing Xie, Masako Suzuki, Chi Zhang, Carolina Eliscovich, and Deyou Zheng

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Purpose: A systematic use of multi -omics methods applied to the earliest stages of lens development and differentiation generates novel insights into the molecular and cellular mechanisms of lens morphogenesis, transcriptional mechanisms including gene regulatory networks (GRNs), and posttranscriptional mechanisms in differentiating lens fibers. Recent progress in imaging of RNA and proteins with single molecule resolution offers unprecedent insights into the most fundamental mechanisms of gene control.

Methods: Mouse lenses from embryonic (E14.5) and newborn (P0.5) mice were micro dissected into epithelium and fibers. “Open” chromatin domains were visualized using ATAC-seq and DNA methylation by whole genome bisulfite sequencing. For comparison, methylation data on ES cells and neural progenitor cells (NPCs) were included. H3.3 histone variant and CTCF were analyzed in P0.5 lens chromatin by ChIP-seq, our earlier RNA-seq and ChIP-seq datasets were also included. Hi-C analyses included ES cells. Single cell RNA-seq analyses (10xGenomics) were conducted within the earliest lens development window, from E8.5 to E10.5. Visualization of crystallin mRNAs employed multiplexed error-robust FISH (MERFISH).

Results: We identified dynamics of open chromatin changes by defining differentially accessible regions, low methylated regions, and unmethylated regions during mouse lens fiber cell (Path1: epiE14.5→fibE14.5→fibP0.5) and epithelium (Path2: epiE14.5→epiP0.5) differentiation. Lens, NPCs and ES cells show dramatically different DNA methylation patterns of pluripotency loci such as Nanog and Oct4 and lineage specific Pax6. DNA methylation does not limit Pax6 binding to DNA. Mouse E9.5 micro dissected embryos were used for scRNA-seq and over 10,000 ells were analyzed by the UMAP algorithm to identify a distinct cluster of lens progenitor cells.

Conclusions: Reduced DNA methylation correlates with expression of important genes involved in lens morphogenesis and lens fiber cell differentiation. Together, the data open the field for mechanistic studies of lens-specific enhancers, GRNs that govern lens morphogenesis, and identification of cataract-causing mutation in non-coding sequences.

Genome-wide association study of cataract: identification of risk loci and shared genetics with other common vision disorders

Hélène Choquet[1], Chen Jiang[1], Jie Yin[1], Thomas J. Hoffmann[2,3], Pirro G. Hysi[4,5,6], M. Maria Glymour[3], Eric Jorgenson[7], Ronald B. Melles[8], and Salil A. Lachke[3,10]

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Purpose: Cataract is the leading cause of blindness among the elderly worldwide. Although observational studies have identified environmental and clinical factors, including ophthalmic conditions, that influence cataract risk, twin and family studies support an important role for genetic factors with heritability estimates up to 58%. However, it is not clear if these observational associations represent causal risk factors, and the genetic etiology of cataract formation remains to be discovered.

Methods: We conducted a multiethnic GWA meta-analysis, including 585,243 individuals (67,844 cataract cases and 517,399 controls) from two cohorts: the Genetic Epidemiology Research on Adult Health and Aging (GERA) and the UK Biobank, with replication in 3,234,455 participants (347,209 cataract cases and 2,887,246 controls) from the 23andMe research cohort. We then undertook genome-wide genetic correlations using LD score regressions to estimate shared genetics between cataract and more than 700 diseases/traits, including vision disorders. Finally, potential causal effects were assessed between primary open-angle glaucoma or myopic refractive error and cataract risk using 2-sample Mendelian randomization analyses.

Results: We reported 55 genome-wide significant loci, 38 of which were not previously reported. We found significant genetic correlations between cataract and other vision disorders, including glaucoma (r_g=0.30, P=4.57x10^{-6}) and myopia (r_g=0.25, P=1.10x10^{-5}). Finally, we reported that genetically determined primary open-angle glaucoma was significantly associated with cataract risk (inverse-variance weighted model: OR=1.04; se= =1.02; P=0.018) and that a more negative mean spherical equivalent refractive error was associated with an increased risk of cataract (OR per diopter=0.92; se=1.01; P=6.51x10^{-13}).

Conclusions: Our work provides further insight into the complex genetic architecture of cataract risk and demonstrates a shared genetic basis and an association between primary open-angle glaucoma or myopic refractive error and cataract risk. This may support population cataract risk stratification and screening strategies, based on primary open-angle glaucoma and refractive error information.

Applying iSyTE to uncover regulatory networks in lens development and its associated defects

Salil A. Lachke[1,2], Sandeep Aryal[1], Sarah Coomson[1], Sanjaya Shrestha[1], Deepti Anand[1]

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Purpose: The development and maintenance of lens transparency involves modulation of gene expression control in epithelial and fiber cells. Many genes, for example crystallins, are transcribed at high levels in fiber cells. However, it is unknown whether transcription in fiber cells involves lens-specialized function of components of the "general" transcription machinery to achieve the extremely high levels of such mRNAs. Further, stability and/or translation of lens-expressed mRNAs need to be controlled by post-transcriptional mechanisms to achieve optimal transcriptome and proteome in epithelial and fiber cells. Here, we apply the bioinformatics tool iSyTE to identify the transcription elongation factor Ell2 and the RNA-binding protein (RBP) Elavl1 that are involved in orchestrating these events in the lens.

Methods: Lens-specific compound conditional deletion mice for Elavl1 and Ell2 were generated using Pax6GFPCre and termed Elavl1^{cKO} and Ell2^{cKO}, respectively. Lens tissue from cKO mice was characterized by immunostaining, RNA-sequencing (RNA-seq) and RT-qPCR.

Results: Elavl1^{cKO} and Ell2^{cKO} lenses exhibit morphological defects that are detected in embryonic and early postnatal stages, respectively. RNA-seq identifies mis-expression of many genes in both cKO lenses. El12^{cKO} lenses show significantly reduced transcript levels of a cohort of fiber-expressed genes such as crystallins, while Elavl1^{cKO} lenses show abnormal mRNA levels of several novel factors in the lens, as well those with known function in the lens.

Conclusion: These data demonstrate that the new iSyTE-predicted genes, the RBP Elavl1, and transcription elongation factor Ell2, mediate regulation of key genes in lens development, and provide new insights into the molecular pathology of cataract and lens defects in animals deficient in these genes. Importantly, Ell2 is found to be mis-expressed in mouse cKO lenses for the RBPs Elavl1 and Celf1, suggesting a crosstalk between transcriptional and post-transcriptional regulatory networks that governs optimal transcriptome and proteome in the lens.

Application of a multi-omics approach using iCLIP-seq and RNA-seq to the lens to identify downstream RNA targets of the cataract-linked RNA-binding protein CELF1

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Purpose: Celf1 encodes a conserved RNA-binding protein (RBP) that post-transcriptionally regulates gene expression by distinct mechanisms, including control over pre-RNA alternative splicing, mRNA stability or translation into protein. Lens-specific conditional knockout (cKO) of Celf1 in mice results in early-onset cataract. Further, knockdown of Celf1 in zebrafish and Xenopus also lead to lens defects, suggesting its conserved function in vertebrate lens development. However, a global-level understanding of downstream targets of CELF1 in the lens is unaddressed. Therefore, we applied a multi-omics approach to identify the RNA targets of CELF1 protein in the lens, whose mis-regulation, especially abnormal splicing, potentially contributes to lens defects.

Methods: To identify Celf1-regulated candidates in the lens, we performed an integrative analysis using two distinct omics approaches. We applied individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP) followed by high-throughput RNA-sequencing (iCLIP-seq) to identify the RNA-binding sites of CELF1 protein in adult wild-type (WT) mouse lens. We next did a integrative analysis of iCLIP-seq data to RNA-seq data on Celf1cKO and control mouse lenses to identify RNA that are directly bound by CELF1 protein and have abnormal alternative splicing events upon Celf1-deficiency.

Results: These analyses identified 22 mRNAs whose specific splicing pattern in the lens is potentially directly regulated by CELF1. Of these candidates, 10 encode proteins linked to cytoskeleton (e.g., ABLIM1, ANK2, CLTA, CTNNA2, SPTBN1, SEPTIN8, YWHAE, etc.). Thus, abnormal splicing of these cytoskeletal protein encoding RNAs in Celf1cKO may contribute to the abnormal lens fiber cell morphology observed in these animals.

Conclusions: Multi-omics analysis using CELF1 protein iCLIP-seq in the lens and RNA-seq on Celf1cKO lens led to prioritization of 10 CELF1 potential direct mRNA targets that are linked to the cytoskeletal biology. The altered splicing of these mRNAs upon Celf1 deficiency may contribute to the lens pathology.

Single Cell RNA Sequencing of the Lens Epithelium in Wild Type Postnatal Mice

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Purpose: The epithelial monolayer of the ocular lens is a complex network that maintains the overall health of the organ. To date, single cell RNA sequencing (scRNA-seq) of the epithelium has not been analyzed in depth. We used scRNA-seq technology to assess potential transcriptional and functional heterogeneity between cells in the postnatal epithelium and better understand lens growth, differentiation and homeostasis.

Methods: scRNA-seq on P2 mouse lenses was performed using the 10X Genomics Chromium Single Cell 3' Reagent Kit (v3.1) and short-read Illumina sequencing. Pre-processing of data and sequence alignment was conducted using the 10X Genomics Cell Ranger software. Quality control was performed in RStudio using the Seurat and DoubletFinder packages. Data was processed using the Seurat standard integration pipeline with 20 principal components, 2000 variable features and a UMAP resolution of 0.3. Cell populations were identified with the FindAllMarkers function, and lens epithelial cells subsetted. Epithelial subclusters were contrasted using the FindMarkers function, alongside comparative PROGENy and GO analyses. A pseudotime trajectory was then plotted using Monocle v3 to simulate cell differentiation across subclusters.

Results: Lens epithelial cells were divided into distinct subclusters, each classified by gene markers. Most notably, the expression of crystallins, ion channels, and transporters was not uniform, and indicated differential expression in cycling versus differentiating cell clusters. Signaling pathways, such as NOTCH and PI3K, were also differentially expressed in subclusters. Comparative GO analysis between clusters showed enrichment in ribosomal, mitochondrial, and lens development pathways upon differentiation.

Conclusion: scRNA-seq corroborated known markers of epithelial differentiation and proliferation, while providing further insights into the pathways and genes directing these processes. Interestingly, we demonstrate that the epithelium can be divided into distinct subpopulations. These clusters reflect the transcriptional diversity of proliferation, signaling, and maintenance in the postnatal lens epithelium.

Session 13: Lens Maintenance and Anti-Cataract Strategies

Chairs: Juliet Moncaster and Vincent Monnier

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

Time	Speaker Coauthors	Title
2:00 - 2:18 pm	Vincent Monnier Zhenxiang Gao Maria Gorenflo David C Kaelber Rong Xu)	Novel pharmacological approaches for the delay of cataract surgery based on drug repositioning and artificial intelligence
2:18 - 2:36 pm	Krishna Sharma Goutham Shankar Puttur Santhoshkumar	Specific sequences in the N-terminal domain of αB-crystallin control oligomerization and chaperone activity
2:36 - 2:54 pm	Caili Hao Zongbo Wei Xingjun Fan	Lens Gpx4 deficient mice induce lipid peroxidation and cataract
2:54 - 3:12 pm	Marta Soltesova Prnova Milan Stefek Lucia Kovacikova Magdalena Majekova Abel Santamaria Cimen Karasu	Cemtirestat - novel indole-based bifunctional aldose reductase inhibitor/antioxidant as a promising drug for treatment of diabetic complications
3:12 - 3:30 pm	Juliet A. Moncaster Douglas Parsons Olga Minaeva Lee E. Goldstein	Phenotyping of P301S tau mutant Alzheimer's Disease mice lenses

Novel pharmacological approaches for the delay of cataract surgery based on drug repositioning and artificial intelligence

Vincent M Monnier[1], Zhenxiang Gao[2], Maria Gorenflo[3], David C Kaelber[4], and Rong Xu[2]

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Purpose: Age-related cataract is the leading cause of blindness worldwide and associated with impaired quality of life. Cataract surgery with intraocular lens implantation is the only cure; however, surgery is associated with complications and not readily available in developing countries. Thus, pharmacological prevention of cataract is an unmet medical need. Currently, no medications are approved or known to delay cataract progression in humans.

Methods: A drug repurposing strategy combining an artificial intelligence (AI)-based drug discovery system and clinical corroboration using electronic health records (EHRs) with twelve diabetes mellitus cataract-associated genes was used to create a “knowledge graph” for ranking of drug candidates. Retrospective cohort studies using TriNetX to access 90 million EHR data were then performed to evaluate the top 10 repurposed candidate drugs for cataract outcomes at 3, 5, 10 and 20 years for patients with cataract and diabetes of which 172,886 patients had cataract extraction.

Results: Among top-10 AI predicted repurposed candidate drugs four common drugs (M, I, AP, A) were associated with a reduced 20-year cataract extraction risk in diabetic patients (adjusted hazard ratio (HR): 0.83 [0.80-0.87], 0.78 [0.68-0.88], 0.83 [0.77-0.88], 0.78 [0.74-0.81], respectively). Overall suppression of cataract prevalence at 20 years was 40% by Drug M, 15% for Drug I, 7% for Drug AP, and 0-7% for A, respectively. Drug effects were not additive implying perhaps a common mechanism of action.

Conclusions: While surgery is the only cure for cataract, four repositioned drugs have the potential to delay cataract progression in diabetes. Of these, Drug M appears to have the most robust and sustained potential in both genders. Promises and limitations of these data will be discussed.

Specific sequences in the N-terminal domain of αB-crystallin control oligomerization and chaperone activity

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Purpose: Studies show that the N-terminal domain in αB-crystallin plays a critical role in the oligomerization. This study was undertaken to determine the role of specific sequences in the N-terminal domain.

Methods: Sequences, 21-28 and 54-61, that were shown to have an independent effect on the αB-crystallin oligomerization were deleted by site-directed mutagenesis to create αBΔ21-28,Δ54-61. In addition, another mutant was engineered to get Tag-αBΔ21-28,Δ54-61 where Tag was MASMTGGQQMGRGSEF served an N-terminal extension. The recombinant proteins were expressed and purified. The critical features such as molecular size, hydrophobicity, chaperone activity and stability of the proteins were compared with that of αB-WT protein. Chaperone-like activities of the αB-WT and the deletion mutants (αBΔ54-61 and αBΔ21-28Δ54-61) were evaluated using luciferase and alcohol dehydrogenase as substrates. The ability of the proteins to inhibit Aβ1-42 oligomerization and fibril formation in-vitro was tested using TEM. Cell integrity, ROS detection assays were performed to access the cytoprotective ability of the crystallins in sodium selenite-challenged ARPE-19 cells.

Results: The molar mass of αBΔ21-28,Δ54-61 ranged from 60 to 160 kDa whereas Tag-αBΔ21-28,Δ54-61 oligomers were in 80 to 200 kDa range, significantly lower (520 to 790 kDa) than that for αB-WT. The hydrodynamic radii (Rh) of the mutants also showed a similar reduction. Both αBΔ21-28Δ54-61 and Tag-αBΔ21-28,Δ54-61 mutants exhibited up to 25-fold increase in chaperone activity when compared to αB-WT with the substrates we used. The αBΔ21-28Δ54-61 double mutant also suppressed the Aβ1-42 fibril formation in-vitro and Aβ1-42-induced cytotoxicity in ARPE-19 cells to a greater extent compared to αB-WT. Cytotoxicity and ROS detection studies showed that the double mutant protein has higher anti-apoptotic and anti-oxidative activity than the αB-WT in oxidatively stressed cells.

Conclusion: Our study shows that the residues 21-28 and 54-61 in αB-crystallin contribute to the oligomerization and regulate the chaperone activity.

Lens Gpx4 deficient mice induce lipid peroxidation and cataract

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Purpose: Both lens crystallins and lipids are long-lived and have little or no turnover, especially in the core region. Long-lived proteins and lipids are more prone to the accumulation of damage during lens aging, which is considered the central pathogenesis of age-related cataractogenesis. An age-related increase in lipid peroxidation has been found in both human and mouse lenses. However, the role of lipid peroxidation in cataractogenesis remains inclusive. Our previous study (Free Rad Bio Med, 2021, 167:94-108) demonstrated that aging lens epithelium is susceptible to ferroptosis. Here, we investigated the role of lipid peroxidation in cataract formation by targeting the key detoxification enzyme glutathione peroxidase 4 (GPX4).

Methods: Mouse lens cataracts were examined by slit lamp and darkfield microscopy. Lens epithelium cell number was counted with serial HE sections. Both apoptosis and ferroptosis markers were determined by Western blotting and immunostaining. Rescued experiments were performed by intraperitoneal injection of Liproxstatin-1 in Gpx4 KO mice. Gpx4 was knockout by CRISPR-Cas9 technology in Human lens epithelial cells (FHL124). Cell viability was investigated by CCK8 assay. Lipid peroxidation was determined by C11-Bodipy 581/591.

Results: Cataracts were observed in the Gpx4 KO lens at postnatal day zero. Microphthalmia was developed in Gpx4 KO adult mice. Lens epithelium cell number was dramatically reduced, suggesting the death of lens epithelial cells in the Gpx4 KO lens. Further study showed that makers of ferroptosis but not apoptosis significantly elevated in the Gpx4 KO lens. In vitro studies indicated that Gpx4 KO FHL124 cells cannot survive without a ferroptosis inhibitor, Liproxstatin-1. Furthermore, cataracts were alleviated by treating Gpx4 KO mice with ferroptosis inhibitor, Liproxstatin-1.

Conclusion: 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.

Cemtirestat - novel indole-based bifunctional aldose reductase inhibitor/antioxidant as a promising drug for treatment of diabetic complications

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Purpose: Cemtirestat, 2-(3-thioxo-2H-[1,2,4]triazino[5,6-b]indol-5(3H)-yl)acetic acid, was recently designed and patented as a highly selective and efficient aldose reductase inhibitor endowed with antioxidant activity ((WO2015057175; J Med Chem 58:2649, 2015). High resolution X-ray crystallographic assay of the human aldose reductase AKR1B1 crystallized with cemtirestat revealed a peculiar mode of cemtirestat binding, leaving the selectivity pocket closed, in contrast to binding of structurally related lidorestat. Presently the drug is under complex preclinical investigation. Here we studied effects of cemtirestat in in vivo and ex vivo animal models of diabetes on progression of the eye lens opacification and peripheral neuropathy.

Methods: Streptozotocin (STZ) induced rats and genetically modified ZDF rats were used as models of type 1 and type 2 diabetes, respectively. The in vivo experiments were complemented with ex vivo investigations in the isolated rat eye lenses and the rat brain cortical slices. Stages of the eye lens opacification and the indices of peripheral neuropathy were determined.

Results: Long term medication of STZ treated animals with cemtirestat led to delay in development of the advanced stages of cataract. At the end of the experiment, the visual cataract score was significantly decreased in the diabetic groups treated with cemtirestat. Cemtirestat inhibited accumulation of sorbitol in the isolated rat eye lenses exposed to high glucose and in the lenses of the fatty ZDF rats. In both in vivo models, cemtirestat attenuated symptoms of peripheral neuropathy with high significance. Moreover, antihypertriglyceride activity of cemtirestat was recorded in diabetic rats after STZ treatment. In the isolated rat brain cortical slices exposed to the neurotoxic quinolinic acid, cemtirestat restored thiol-disulfide homeostasis most likely by releasing free GSH from the pool of endogenously bound disulfides.

Conclusions: Considering the obtained results, cemtirestat represents a practical example of a therapeutic strategy against chronic complications in diabetes based on multiple pharmacological activities.

Phenotyping of P301S tau mutant Alzheimer's Disease mice lenses

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Purpose: We previously discovered that Aβ accumulates in the cortical/supranuclear region of the lens in the eyes of 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 Tau. Tau is a microtubule associated protein that is expressed in the brain and becomes hyperphosphorylated in AD eventually forming neurofibrillary tangles. Tau has previously been reported to be expressed in the lens (Bai et al., 2007; Zhao et al., 2013). Here we investigated whether cataracts may develop in the P301S tau mutant AD mouse model.

Method: P301S mice were bred and maintained at Boston University School of Medicine. 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 3-13 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.

Result: There was no overt difference in lens phenotype between the P301S transgenic and non-transgenic mice. No cataracts were observed in these lenses at 12-13 months of age.

Conclusion: There was no overt difference in lens phenotype between the P301S transgenic and non-transgenic mice. 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.

Session 14: TGFb and Lens Fibrosis

Chairs: Frank Lovicu and Judy West-Mays

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

Time	Speaker (Coauthors)	Title
4:00 - 4:18 pm	Melinda Duncan Jiawen Xiang Anthony Pompetti Adam Faranda Yan Wang Samuel Novo David W. Li	Atf4 Regulates Genes Controlling Nutrient Metabolism in the Avascular Lens
4:18 - 4:36 pm	Aftab Taiyab Vanessa Wong Yasmine Belahlou Judith West-Mays	Understanding the role of biomechanical signaling during lens fibrosis
4:36 - 4:54 pm	Mary Flokis Frank Lovicu	FGF differentially regulates lens epithelial cell behavior during TGF-β-induced EMT
4:54 - 5:12 pm	Michael Wormstone Lucy J Dawes Lixin Wang Julie A. Eldred	Is EMT a pre-requisite for matrix contraction
5:12 - 5:30 pm	Zongbo Wei Caili Hao Jian-kang Chen Lin Gan Xingjun Fan	A Tamoxifen-inducible Cre Knock-in Mouse for Lens-specific Gene Targeting

Atf4 Regulates Genes Controlling Nutrient Metabolism in the Avascular Lens

Melinda K. Duncan[1], Jiawen Xiang[1,2], Anthony Pompetti1, Adam Faranda[1], Yan Wang[1], Samuel Novo[1], and David W. Li[2]

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Purpose: Atf4, a transcriptional regulator of the unfolded protein (UPR) and integrated stress responses (ISR), is required for lens development, however the underlying mechanisms mediating its function have been a mystery for over 20 years. This investigation fills this knowledge gap.

Methods: The phenotype of Atf4 null mice was re-evaluated using standard methods, and lenses isolated from embryonic day (E) 16.5 mice were subjected to RNAseq. Differentially expressed genes (DEGs) were identified bioinformatically and confirmed using semi-quantitative confocal immunofluorescence. Free amino acid levels were evaluated by LC-MS, and glutathione levels were evaluated using a luminescent assay.

Results: As previously reported, adult Atf4 null mice lack lenses due to decreases in cell proliferation and increases in apoptosis beginning at E16.5. Next gen RNA sequencing revealed that the E16.5 Atf4 null lens transcriptome was profoundly abnormal, with numerous lens preferred genes downregulated including Foxe3, Dnase2b and Crybb2. Further, many genes regulating nutrient transport were down regulated including membrane solute carriers (Slc), as well as amino acid biosynthesis and sugar metabolic enzymes. This resulted in a reduction in free amino acid and total protein levels demonstrating that Atf4 null lenses are starved for nutrients. Further genes regulating glutathione synthesis and transport were downregulated in Atf4 null lenses leading to significant decreases in glutathione levels and increases in reactive oxygen species. This appears to result in chronic activation of pathological autophagy as the expression of the autophagy-related gene Sqstm1/p62 was elevated in Atf4 null lens.

Conclusion: Atf4 null lenses have reduced levels of numerous nutrient transporters leading to elevations in autophagy and reactive oxygen species resulting in lens disintegration by birth. These data demonstrate that Atf4 is a key regulator of the metabolic specializations that allow the lens to survive and grow in an avascular environment that is low in nutrients and oxygen.

Understanding the role of biomechanical signaling during lens fibrosis

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Purpose: Fibrotic cataracts, posterior capsular opacification and anterior subcapsular cataract are attributed to transforming growth factor-β (TGFβ) induced epithelial-to-mesenchymal transition (EMT) of lens epithelial cells (LECs). Recent investigations from our laboratory have shown the novel role of biomechanical signaling, specifically yes associated protein (YAP)-mediated signaling, during TGFβ-induced EMT of LECs. Here, we have investigated the effect of YAP inhibition on TGF-β-induced EMT.

Methods: Immunohistochemistry was performed for YAP and alpha smooth muscle actin (α-SMA) using lenses from wild-type (WT) and TGFβ-overexpressing transgenic (TGFβtg). For validation and molecular analyses, LEC explants from either 30-days old mouse or 17-19 days old Wistar rat pups were used. Explants were either treated with dimethyl sulfoxide (DMSO), 6ng TGFβ (6ng/mL) with or without Verteporfin (100nM), a specific inhibitor of YAP signaling, for 48 hrs. Immunofluorescence and Western Blot analyses were performed using appropriate antibodies and imaged to detect and compare the expression levels.

Results: The immunohistochemistry (IHC) analyses on ocular sections from TGFβtg show increased expression of YAP and α-SMA in the fibrotic plaques when compared to wild-type littermate lenses (N = 3). The incubation of rat lens explants with verteporfin prevented the TGFβ-induced fiber-like phenotype (N=12). Furthermore, co-treatment of rat lens explants with verteporfin prevented TGF-β-induced α-SMA expression ($p<0.001$, N=4), as well as delocalization and degradation of E-cadherin (N=6). In addition, verteporfin prevented TGF-β-induced nuclear translocation of YAP in LECs (N=6).

Conclusion: The increased expression of YAP in TGFβtg lenses indicate specific role of YAP-mediated signaling during lens fibrosis. The absence of α-SMA expression and presence of peripheral E-cadherin along with a decrease in nuclear YAP in TGFβ-treated lens explants co-incubated with verteporfin shows the role of YAP in TGFβ-induced EMT of LECs.

FGF differentially regulates lens epithelial cell behaviour during TGF-β-induced EMT

Mary Flokis[1] and Frank J. Lovicu[1, 2]

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Purpose: Fibroblast growth factor (FGF) and transforming growth factor-beta (TGF-β) can regulate and/or dysregulate lens epithelial cell (LEC) behavior, including proliferation, fiber differentiation, and epithelial-mesenchymal transition (EMT). Earlier studies have investigated the crosstalk between FGF/TGF-β in dictating lens cell fates, that appears to be dose dependent. Here we tested the hypothesis that a fiber differentiating dose of FGF differentially regulates lens epithelial cells undergoing TGF-β-induced EMT.

Methods: Postnatal 21-day-old rat lens epithelial cell explants were treated with a fiber differentiating dose of FGF-2 (200ng/ml) and/or TGF-β2 (50pg/ml), over a 5-day culture period. We compared central LECs (CLEC) and peripheral LECs (PLEC), using immunolabelling for changes in markers for EMT (α-SMA), fiber differentiation (β-crystallin), and cytoskeleton (Tpm1.6-1.9), as well as Smad2/3- and MAPK/ERK1/2-signalling.

Results: LEC explants co-treated with FGF-2 and TGF-β2 exhibited a differential response, with CLECs undergoing EMT while PLECs favoring a fiber differentiation response, compared to only TGF-β-treated explants where all cells underwent an EMT. The CLECs cotreated with FGF and TGF-β immunolabelled for α-SMA, with minimal β-crystallin, whereas PLECs demonstrated strong β-crystallin reactivity, and little α-SMA. Interestingly, compared to TGF-β-only treated explants, α-SMA was significantly decreased in CLECs when co-treated with FGF/TGF-β. Smad-dependent and independent signaling was increased in FGF-2/TGF-β2-treated CLECs with heightened nuclear localization of t-Smad2/3, while PLECs favored ERK1/2-signalling over Smad2/3 activation.

Conclusion: The current study confirmed that FGF-2 is influential in differentially regulating LECs during TGF-β-induced EMT, leading to a heterogenous cell population, typical of that observed in PCO development. This highlights the cooperative relationship between FGF and TGF-β contributing to lens pathology, providing a different perspective when considering the implications of preventing PCO.

Is EMT a pre-requisite for matrix contraction?

Michael Wormstone, Lucy J Dawes, Lixin Wang, Julie A. Eldred

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Purpose: Fibrosis has defining features such as hyperproliferation, matrix deposition and matrix contraction and plays an important role in lens wound healing events following cataract surgery. Transforming growth factor β (TGFβ) is strongly implicated in this process. The response to surgical injury can lead to disruption of visual quality and is known as posterior capsule opacification (PCO). It is a widely held view that transdifferentiation from an epithelial cell to a myofibroblast is required to give rise to matrix contraction, but rarely is this relationship investigated. Therefore, we will provide an overview of our studies (published and unpublished), to provide a broader perspective on the relationship between myofibroblast expression and matrix contraction.

Methods: The human lens epithelial cell line, FHL124, was used. TGFβ2-induced contraction was assessed with a patch contraction assay. The myofibroblast marker alpha smooth muscle actin (αSMA) was determined at the gene and protein levels using QRT-PCR and western blots respectively. The effects of additional growth factors/hormones (basic fibroblast growth factor (bFGF), 17β-estradiol) or pathway inhibitors/disruptors (Y27632 Rho kinase inhibitor, RGDS peptide, αSMA siRNA) were assessed in the presence and absence of TGFβ stimulation.

Results: TGFβ induced significant matrix contraction and upregulation of αSMA. αSMA expression was suppressed by bFGF, 17β-estradiol and Rho kinase inhibitor. However, while Rho kinase inhibitor suppressed matrix contraction, 17β-estradiol had no effect and basic FGF accelerated the process. Suppression of αSMA and disruption of fibronectin/integrin binding using RGDS peptide also accelerated TGFβ induced matrix contraction.

Conclusion: Expression of the myofibroblast marker αSMA is not an essential pre-requisite for lens epithelial cell mediated matrix contraction in response to TGFβ.

A Tamoxifen-inducible Cre Knock-in Mouse for Lens-specific Gene Targeting

Zongbo Wei,[1] Caili Hao,[1] Jian-kang Chen,[1] Lin Gan,[2] Xingjun Fan[1]

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Purpose: Mouse models are valuable tools in studying lens biology and biochemistry, and the Cre-loxP system is the most used technology for gene targeting in the lens. However, numerous genes are either indispensable in lens development, and the conventional knockout method either prevents lens formation or causes simultaneous cataract formation, hindering the studies of their roles in lens structure, growth, metabolism, and cataractogenesis during lens aging. An inducible Cre-loxP mouse line is an excellent way to achieve such a purpose. In the present study, we aim to establish an inducible knock-in mouse model for lens-specific gene targeting (LeCreERT2) in a spatial-temporal manner.

Methods: LeCreERT2 mice were created by in-frame infusion of a P2A-CreERT2 at the C-terminus of the last coding exon of the gene alpha A crystallin (Cryaa). Mouse lens morphology and opacity were examined by slit lamp, darkfield microscopy, and HE staining. Gene deletion was evaluated by Western blotting and immunostaining.

Results: LeCreER^{T2} mice express tamoxifen-inducible Cre recombinase uniquely in the lens. Through ROSA$^{mT/mG}$ and two endogenous genes (Gclc and Rbpj) targeting, we found no Cre recombinase leakage in the lens epithelium, but 50-80% leakage was observed in the lens cortex and nucleus. Administration of tamoxifen almost completely abolished target gene expression in both lens capsule and cortex but only mildly enhanced gene deletion in the lens nucleus. Notably, no overt leakage of Cre activity was detected in developing LeCreER^{T2} lens when bred with mice carrying loxP floxed genes that are essential for lens development.

Conclusion: This newly generated LeCreER^{T2} line will be a powerful tool to target genes in the lens for gene functions study in lens aging, posterior capsule opacification (PCO), and other areas requiring precision gene targeting.


FRIDAY

Friday, December 9th

Session 15: Lens Matrix Biology

Chairs: Melinda Duncan and Ram Nagaraj

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

Time	Speaker Coauthors	Title
9:00 - 9:18 am	Ram Nagaraj Mi-Hyun Nam Cibin T. Rghavan Marcus A. Glomb Michael Wormstone	Role of the lens capsule in lens epithelial cell to mesenchymal transition
9:18 -9:36 am	Matt Reilly Wade Rich Bharat Kumar Heather L. Chandler	What the Cells See: A Mechanobiological Model of Lens Stretching
9:36 - 9:54 am	Leah O'Neill Samuel G. Novo Adam P. Faranda Mahbubul H. Shihan Yan Wang Melinda K. Duncan	Mechanisms Regulating Inflammatory and Fibrotic Responses of LECs to Lens Injury
9:54 - 10:12 am	Alyssa L. Lie Xingzheng Pan Ehsan Vaghefi Thomas W. White Paul J. Donaldson	The mechanics of water circulation in the in vivo accommodating human lens
10:12 - 10:30 am	Sepideh Cheheltani Sadia Islam Megan Coffin Velia M. Fowler	Deletion of Cap2 causes altered F-actin distribution in lens fiber cells, affecting the lens stiffness

Role of the lens capsule in lens epithelial cell to mesenchymal transition

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Purpose: Lens epithelial cell mesenchymal transition (EMT) is central to posterior capsule opacification (PCO) after cataract surgery. The lens capsule proteins accumulate post-translational modifications during aging. One of the major modifications occurs through glycation. Glycation is the reaction of aldehydes and ketones with lysine and arginine residues in proteins; it produces advanced glycation end products (AGEs). We have investigated the role of lens capsule AGEs in PCO.

Methods: Human lens capsule AGEs were measured by LC-MS/MS by the standard addition method. Effects of lens capsule AGEs on TGFβ2-mediated EMT were studied using human lens epithelial cells cultured on human lens capsules of varying ages and extracellular matrix modified by AGEs. The role of RAGE, a receptor of AGEs in AGE-mediated promotion of EMT LECs was studied in mouse lens epithelial cells using specific inhibitors of RAGE.

Results: AGEs progressively accumulate in aging lens capsules and the levels in cataract lens capsules are significantly higher than in non-cataractous lens capsules. We found that capsule-AGEs promote EMT of lens epithelial cells. Nascent young lens capsules and mouse extracellular matrix modified by AGEs also promoted the effects of TGFβ2 on lens epithelial cells. Aged lens capsules (that contain high levels of AGEs relative to young lens capsules) promoted the TGFβ2-mediated EMT of lens epithelial cells more than young lens capsules. Capsule AGEs bind to RAGE, a receptor for AGEs in lens epithelial cells and generate reactive oxygen species during the promotion of lens epithelial cell EMT.

Conclusions: AGEs present in lens capsules promote the TGFβ2-mediated EMT of lens epithelial cells and such promotional activity is directly related to the levels of AGEs. Capsule AGEs, through binding to RAGE in lens epithelial cells, are likely to contribute to PCO.

What the Cells See: A Mechanobiological Model of Lens Stretching

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Purpose: We recently discovered that lens epithelial cells (LECs) proliferate in response to zonular tension. The mechanism through which LECs transduce this signal remains unknown, except that it involves the YAP pathway [1]. In this study, both computational and experimental methods were used to determine how, in a biomechanical sense, LEC proliferation is stimulated in response to zonular tension.

Methods: Encapsulated porcine lenses were attached to custom lens stretchers via the sclera and connective tissues as previously described [1]. After 24 hours, capsules were lightly fixed, flat mounted, labeled using Ki67 antibodies, and visualized using fluorescence microscopy. Automated image processing was used to quantify local cellular proliferation. A computational model of porcine lens stretching was also developed based on previous measurements of porcine lens shape [2] and biomechanical properties [3], as well as properties of the capsule [4]. The resulting strains in the lens capsule were calculated and used in a mechanobiological model of cell behavior. The proliferation rate of the LECs was tied to a volumetric growth of the whole lens to mimic the production of lens fiber cells.

Results: Static stretching resulted in significant proliferation near the point of zonular insertion. Strain energy density in the epithelium was highly predictive of where proliferation occurred during lens stretching experiments. This coincides with the so-called proliferative zone observed in murine lenses. The behavior of this zone depended significantly on the aspect ratio of the lens.

Conclusions: As in many other tissues, LECs likely proliferate to minimize their potential energy consumption. This behavior allows the lens to survive and function with a minimal metabolism. The size and shape of the young lens may drive growth patterns in the adult lens and even control the accommodative ability of species.

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Mechanisms Regulating Inflammatory and Fibrotic Responses of LECs to Lens Injury

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Purpose: The success of cataract surgery (CS) is acutely compromised by post-surgical inflammation and chronically compromised by the development of posterior capsular opacification (PCO). PCO is a wound healing response where residual lens epithelial cells (LECs) undergo multiple processes of proliferation and migration – interrupting clear vision. This study seeks to understand the molecular mechanisms by which CS acutely induces inflammation and PCO.

Methods: WT mice were subjected to lens fiber cell removal to model CS; LECs were collected at 0 and 6 hours for RNA seq profiling. Bioinformatic analysis revealed differentially expressed genes (DEGs) and enriched pathways. The injury response of mice lacking 2 DEGs: fosb and egr1 from lens cells were obtained and studied using RNA seq and immunostaining.

Results: RNA seq analysis revealed that mouse LECs upregulate expression of numerous genes by 6h post-cataract surgery (PCS), including several cytokines, while the expression of pro-fibrotic and lens preferred genes are unchanged. Notably, the upregulated DEGs were highly enriched in immediate early transcription factors (IETFs) including fosb and egr1. We found that IETF upregulation also occurs rapidly in LECs explanted in serum free media, indicating cell autonomous regulation. Pathway analysis and immunostaining revealed that ERK/MAPK signaling is upregulated in LECs within 1h PCS suggesting a mechanism for IETF upregulation. LECs lacking Egr1 or FosB both attenuated a portion of the injury response although they appear to regulate different genes.

Conclusion: We show that LECs rapidly remodel their transcriptome by 6h PCS and identified various IETFs that might regulate later LECs responses to lens injury. These rapid responses likely prime LECs for later cell proliferation, epithelial-mesenchymal transition, and lens cell differentiation in response to CS.

The mechanics of water circulation in the in vivo accommodating human lens

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Purpose: Development of modern solutions for the age-old problem of presbyopia has been largely impeded by a poor understanding of its origins. An accurate description of the lens deformation process in accommodation would provide insights to the mechanisms underlying presbyopia. Therefore, we have employed in vivo magnetic resonance imaging (MRI) techniques to investigate whether a redistribution of free water within the lens is associated with external shape changes in accommodation.

Methods: Geometric dimensions and free water distribution maps were obtained from lenses of eleven young adults (mean ± SD: 21 ± 2 years) using previously established protocols on a 3T MRI scanner (MAGNETOM Skyra; Siemens, Germany). Measurements were acquired without and with accommodation (3 D stimulus) for each subject. The cross-sectional area (CSA), surface area (SA), and volume (VOL) of lenses were calculated from MR images using custom-written software in MATLAB ((The MathWorks, Natick, MA). Free water distribution was independently described for the anterior and posterior axial lens thickness by a power function. Changes with accommodation were tested statistically with paired t-tests.

Results: Only lens SA decreased with accommodation (P=0.015); CSA and VOL showed no changes (both P>0.05). Irrespective of accommodative status, free water always varied parabolically across the lens. However, the rate of free water variation across the anterior lens was more gradual with accommodation (P=0.037). Posteriorly, free water variation showed no changes with accommodation, nor did the free water content at the central and peripheral lens (all P>0.05).

Conclusion: The preservation of lens VOL and CSA despite a decrease in SA implies accommodative deformation is not due to lens compression or fluid exchange with its surroundings. The accompanying smoother lens free water distribution observed anteriorly further supports this and suggests protein syneretic processes of water binding-unbinding may instead be involved in accommodative deformation of the lens.

Deletion of Cap2 causes altered F-actin distribution in lens fiber cells, affecting the lens stiffness

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Purpose: Lens fiber cells are composed of a variety of actin filament (F-actin) networks whose stability is regulated by actin-binding proteins that function to maintain normal lens stiffness and resilience. Cyclase Associated Proteins (CAPs) are actin-binding proteins with 2 subfamily members, Cap1 and Cap2. Cap2 enhances F-actin disassembly in the actin cytoskeletal system by promoting the dissociation of ADF/cofilin-bound G-actin subunits from the pointed ends of filaments. To determine a function for Cap2 in lens actin cytoskeleton organization and biomechanics, we studied the Cap2-/- lens phenotype at the cellular and tissue levels.

Methods: The lens-specific Cap2-/- mouse was generated by crossing cap2loxP mice to Mlr10-Cre mice. Western blotting, immunostaining, confocal microscopy and mechanical testing were used to study Cap2-deficient mouse lenses.

Results: Cap2-deficient lenses had normal shape and size with no signs of cataracts. Immunostaining of equatorial cross sections of wild-type lenses shows that Cap2 is expressed in both lens epithelial and fiber cells. In fiber cells, Cap2 staining appears to be enriched with F-actin on the broad sides and at the vertices, as well as diffuse in the cytoplasm. Further, Cap2 appears to be localized with F-actin near the membrane in both differentiating and maturing fiber cells, but more abundant in the cytoplasm of inner cortical fiber cells. Although fiber cell morphogenesis and hexagonal packing remain unchanged in Cap2-/- lenses, these lenses show an increase in the F-actin signal intensity in lens fiber cells, as well as a significant increase in stiffness under compression, compared to the wild-type lenses.

Conclusion: Deletion of Cap2 in the lens leads to a significant increase in the lens stiffness. This is likely due to an increase in the proportion of F-actin vs G-actin in fiber cells caused by a net reduction of actin disassembly in the absence of Cap2.

Friday, December 9th

Session 16: PCO Mechanisms and Management

Chairs: Michael Wormstone and Grace Cooksley

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

Time	Speaker Coauthors	Title
11:00 - 11:18 am	Aram Saeed Michael Wormstone	3D printing of foldable, transparent, implantable intraocular lenses
11:18 - 11:36 am	Grace Cooksley Joseph Lacey Marcus Dymond Yury Gogotsi Susan Sandeman	Positive resolution of the wound healing response contributing to posterior capsule opacification development byTi3C2Tx (MXene)
11:36 - 11:54 am	Hiro Matsushima	Is it possible to maintain visual function permanently after cataract surgery?
11:54 - 12:12 pm	Liping Tang Joyita Roy Arjun Jaitli Le Hoang Samira I. Izuagbe Amjad Chatila	An In Vitro System to Investigate IOL: lens capsule interaction
12:12 - 12:30 pm	Closing Remarks from Kona 2022 Organizers	

3D printing of foldable, transparent, implantable intraocular lenses (Oral Presentation)

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Purpose: A cataract is a region of cloudiness in the crystalline lens. It is the biggest cause of blindness in the world and the second leading cause of severe vision impairment. During cataract surgery, the clouded lens is removed and replaced with an artificial intraocular lens (IOL), which restores visual strength. Rapid prototyping and fabrication processes using 3D printing (namely additive manufacturing) have been proposed to supplement and/or replace traditional moulding and lathing techniques to shorten lens development cycles, enable unlimited complex design iteration, and improve access to premium lenses and clinical outcomes.

Method: To create foldable, transparent, and biocompatible 3D printed IOLs, stereolithography and digital light processing have been developed with new photocurable resins. The photocurable resin composition has been optimized to produce various IOL designs with acceptable transparency, mechanical properties, and surface optical quality. The accuracy, reproducibility, and transparency of the lenses were evaluated using SEM and optical transmittance measurements. The new lenses' ability to fold and unfold in cell culture conditions was investigated using digital light imaging, compression, and tensile testing. FHL124 cell survival was assessed using the LDH test to determine OL biocompatibility.

Results: New materials and methods for rapid 3D printing of foldable, transparent, and high-quality lenses have been developed. The LDH analyses demonstrated that the FHL124 cells survived and tolerated the new IOL exudate and contact and the mechanical properties found to be comparable to existing commercial lenses.

Conclusion: Rapid prototyping of intraocular lenses is possible using stereolithography and digital light processes to reduce fabrication time, provide access to unlimited design iterations, and accelerate the development of new and premium lenses. However, this task necessitates access to carefully developed, highly advanced materials and know-how.

Positive resolution of the wound healing response contributing to posterior capsule opacification development by*Ti3C2Tx* (MXene)

Grace Cooksley,[1,2] Joseph Lacey,[2] Marcus Dymond,[1] Yury Gogotsi,[3] Susan Sandeman[1]

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Purpose: To investigate the impact of Ti3C2Tx (MXene) on the wound-healing response of lens epithelial cells (LEC) contributing to posterior capsule opacification (PCO).

Methods: Human LECs were incubated with Ti3C2Tx and positive inflammatory control, interleukin 1 beta (IL-1β). Enzyme immunoassay (ELISA) and real-time polymerase chain reaction (RT-PCR) was used to measure pro-inflammatory cytokines IL-6 and IL-1β. RNA sequencing was performed to elicit any gene pathway suppression by Ti3C2Tx on cytokine-induced inflammation. Lipid profile of LECs was investigated to determine the interaction of Ti3C2Tx with the cell membrane.

Results: Ti3C2Tx did not upregulate IL-6 and IL-1β expression. Cells treated with Ti3C2Tx and IL-1β showed a significant reduction in IL-1β expression. RNA sequencing found Ti3C2Tx downregulated hallmark inflammation genes which coordinated with upregulation in cells treated with IL-1β. A similar profile was found in cells treated with Ti3C2Tx and IL-1β although several subfamilies of the cytokine-cytokine receptor interaction pathway were more-weakly induced. Lipidomic profile showed no significant changes in phospholipid composition after 24-hour treatment with Ti3C2Tx. However, certain signaling lipids involved in inflammation showed similar upregulation in IL-1β-treated cells.

Conclusion: Genomic profile validates the use of IL-1β as an in vitro positive control for trauma-induced inflammation post-cataract surgery. Ti3C2Tx does not promote pro-inflammatory cytokine expression in HLEC, suggesting its presence within the capsule bag may not promote the wound healing response of residual LEC leading to PCO development.

Is it possible to maintain visual function permanently after cataract surgery?

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Purpose: It well known that the advances in cataract surgery equipment and intraocular lenses (IOLs) have increased visual function in the early postoperative period for cataract patients. However, postoperative complications such as anterior capsular constriction and secondary posterior capsular opacification are associated with the wound healing response, and it is difficult to suppress tissue changes surrounding the IOL.

Methods/Results: We have tried to overcome postoperative complications by using various methods such as IOL shape, materials, and surface modification, and have achieved some results, but they inevitably progress when the observation period is long. Recently, many relatively new complications such as opacification of the IOL itself and deterioration of the IOL surrounding tissue, such as IOL displacement and dislocation, have been reported and are often discussed at clinical conferences.

Conclusion: In this presentation, what kind of problems are there in maintaining the visual function for long time after cataract surgery, and how far have we been solved? I would like to consider the future possibilities as well.

An *in vitro* System to Investigate IOL: lens capsule interaction

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Purpose: The interactions between IOL and Posterior Lens Capsule (PLC) may influence the extent of Posterior Capsule Opacification (PCO) formation. It is hypothesized that a strong binding of IOL to PLC may impede lens epithelial cell (LEC) migration and proliferation and thus reduce PCO formation. Unfortunately, this hypothesis cannot be tested via the existing in vitro and in vivo models. Thus, we need a new system to study IOL-PLC interaction and predict PCO.

Methods: An in vitro model with simulated PLC system and a custom-designed micro-force tester is established to predict PCO potential of IOL. For that, 3D simulated LCs were developed by imprinting 3D-printed human lens onto molten gelatin. A custom-designed micro-force tester was created to measure the IOL: PLC adhesion force. The LECs-seeded PLC were generated to assess the influence of IOL: PLC interaction on LEC responses.

Results: Simulated PLC was optimized to possess mechanical properties resembling “real” PLC (elastic modulus of 0.023 N/mm^2, ultimate stress of 0.021 N/mm^2). Using the system, we observed binding forces on different commercially available IOLs in the following sequence: Acrysof (7.80 ± 0.87 mN) > Silicone (1.50 ± 0.12 mN) > PMMA (1.14 ± 0.33 mN) which are in a perfect agreement with the clinical observations about PCO performance of the tested IOL in the following order. Our preliminary results also show that Acrysof IOLs allow substantially less (>4X) LEC infiltration and proliferation than PMMA IOLs.

Conclusion: Our results thus far support the overall hypothesis. We believe that this system can provide valuable insight into the IOL:LC interplay and their relationship to the clinical PCO outcome.

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