NATIONAL FOUNDATION FOR EYE RESEARCH

TABLE OF CONTENTS

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

ORGANIZING COMMITTEE

Scientific

Paul Donaldson, Ph.D.
Auckland, New Zealand

Julie Lim Ph.D.
Auckland, New Zealand

Gus Grey, Ph.D.
Auckland, New Zealand

Ehsan Vaghefi
Auckland, New Zealand

Administrative

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

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

NATIONAL FOUNDATION FOR EYE RESEARCH AWARDS

Kinoshita Lectureship

K. Krishna Sharma, Ph.D.
University of Missouri
Columbia, Missouri

Henry Fukui Travel Award

Eric C. Beyer, M.D., Ph.D.
University of Chicago
Chicago, Illinois

Frederick Bettelheim Travel Award

Ali Mohammed, Ph.D.
University of East Anglia
Norwich, England

W. Gerald Robison Travel Award

Nathalie Houssin, Ph.D.
The Ohio State University
Columbus, Ohio

Alvira Reddy Travel Award

Rijo Hayashi, M.D.
Dokkyo Medical University
Saitama, Japan

Donald Champagne Travel Award

Elizabeth A. Whitcomb
Tufts University
Boston, Massachusetts

National Foundation for Eye Research
Young Investigator Travel Awards

Yadi Chen, Ph.D.
University of Auckland
Auckland, New Zealand

Shannon J. Das, Ph.D.
University of Sydney
Sydney, NSW, Australia

Thao Huynh, Ph.D.
University of East Anglia
Norwich, UK

Sidra Islam, Ph.D.
Case Western Reserve University
Cleveland, OH

Oscar Jara, Ph.D.
University of Chicago
Chicago, IL

Shruthi Karnam, Ph.D.
Duke University School of Medicine
Durham, NC

Yuxing Li, Ph.D
University of California Berkeley
Berkeley, CA

Alyssa Lesley Lie, Ph.D.
University of Auckland
Auckland, New Zealand

National Foundation for Eye Research
Young Investigator Travel Awards

Douglas Parsons, Ph.D.
Boston University School of Medicine
Boston, MA

David Thorn, Ph.D.
The Australian National University
Canberra, Australia

Kehao Wang, Ph.D.
Nottingham Trent University
Nottingham, UK

Tayler F. L. Wishart, B.S.
The University of Sydney
Sydney, NSW, Australia

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

Deepti Anand, Ph.D.
University of Delaware
Newark, DE

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

Yan Ma, Ph.D.
National Institutes of Health
Bethesda, MD

Qian Nie, Ph.D.
Sun Yat-Sen University
Guangzhou, China

National Foundation for Eye Research
Young Investigator Travel Awards

Samuel Novo, B.S.
University of Delaware
Newark, DE

Shaili Patel, B.S.
University of Delaware,
Newark, DE

Mahbubul H. Shihan, M.S.
University of Delaware
Newark, Delaware

Venkat Pulla Rao Vendra, Ph.D.
National Institutes of Health
Bethesda, MD

Yu Zhang
First Affiliated Hospital of Chongqing Medical University
Chongqing, China

ACKNOWLEDGEMENTS

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for the meeting received
from the following:

National Foundation for Eye
Research

The Japanese Society for
Crystalline Lens Research

Senju Pharmaceutical Company

Presentation Guidelines

Instructions for platform presenters

- Unless indicated by the Session Chair all presentations are limited to 18 minutes in total duration, of which 3 minutes should be left for questions/discussion.

- Presentations should be prepared in PowerPoint.

Instructions for poster presenters

- Poster boards are 36 inches high x 48 inches wide. Posters should be put up either Sunday evening or Monday morning and taken down immediately at the end of the meeting at noon Friday.

- Presenters who self-identify as young investigators (PhD student or within 5 years post PhD) will be evaluated for the best poster prize.

ICL 2019 OVERVIEW (DEC. 8-10)

Time	Dec. 8 (Sun)	Dec. 9 (Mon)	Dec. 10 (Tue)
8:00			
		8:30 – 10:00 Session 1: Development of lens structure and function *(Barbara Pierscionek & Kehao Wang)* S1-1 Salil Lachke S1-2 Kehao Wang S1-3 Ehsan Vaghefi S1-4 Irene Vorontsova S1-5 Melinda Duncan	**8:30 – 1000 Session 5: Lens in the middle ages – new insights into presbyopia** *(Paul Donaldson & Bianca Maceo Heilman)* S5-1 Bianca Maceo Heilman S5-2 Steve Bassnett S5-3 Rick Mathias S5-4 Paul Donaldson
9:00			
		10:00 - 10:30 Break	**10:00 – 10:30 Break**
10:00		**10:30 – 12:00 Session 2: Lens development** *(Frank Lovicu & Yuki Sugiyama)* S2-1 Kristen Koenig S2-2 Nathalie Houssin S2-3 Shali Patel S2-4 David Li S2-5 Tayler Wishart	**10:30 – 12:00 Session 6: Cellular biomechanics** *(Velia Fowler & Cathy Cheng)* S6-1 Velia Fowler S6-2 Justin Parreno S6-3 Yuki Sugiyama S6-4 Tim Plageman Jr. S6-5 Cathy Cheng
11:00			
12:00		**12:00 – 1:00 Lunch**	**12:00 – 1:00 Lunch**
1:00		**1:00 - 2:30 Session 3: Cell signaling in the lens** *(Xin Zhang & Kristen Koenig)* S3-1 Xin Zhang S3-2 Yuki Takeuchi S3-3 Eri Kubo S3-4 Elizabeth Whitcomb S3-5 Frank Lovicu	**1:00 – 2:30 Session 7: Lens structure and function - Compaction** *(Joe Costello & Steve Bassnett)* S7-1 Steve Reichow S7-2 M. Joseph Costello S7-3 Shruthi Karnam S7-4 Rosica Petrova S7-5 Barbara Pierscionek
2:00		**2:30 – 4:30 Session 4: Poster session with refreshments**	2:30 – 3:00 Break
			3:00 – 4:30 Session 8: Ion channels and transporters *(Tom White & Nick Delamere)* S8-1 Woo-Kuen Lo S8-2 Eric C. Beyer S8-3 Xiaohua Gong S8-4 Nick Delamere S8-5 Tom White S8-6 Yadi Chen
3:00			
4:00	**4:00 – 6:30 Registration**		
	5:00 Welcome Reception		
5:00			
		6:00 – 7:00 Luau Dinner	
6:00	**7:00 – 7:30 Opening Remarks Travel Award Presented**	**7:00 Luau**	
7:00	**7:30 – 8:30 Kinoshita Lecture By Dr. Krishna Sharma**		

ICL 2019 OVERVIEW (DEC. 11-13)

Time	Dec. 11 (Wed)	Dec. 12(Thu)	Dec. 13 (Fri)
8:00			
	8:30 – 10:00 Session 9: The old lens–age related changes to lens structure and function *(Kevin Schey & Roger Truscott)* S9-1 Roger Truscott S9-2 Roy Quinlan S9-3 Kevin Schey S9-4 John Carver S9-5 Juliet Moncaster	**8:30 – 10:00 Session 11: Loss of transparency and treatment options** *(John Clark & Hiroshi Sasaki)* S11-1 Hiroshi Sasaki S11-2 John Clark S11-3 Peter Kador S11-4 Vincent Monnier	
9:00			**9:00 – 10:30 Session 15: Cataract surgery and posterior capsule opacification** *(Michael Wormstone & Sulin Chen)* S15-1 Mahbubul Shihan S15-2 Michael Wormstone S15-3 Thao Huynh S15-4 Xingjun Fan S15-5 Ali Mohammed
10:00	**10:00 – 10:30 Break**	**10:00 – 10:30 Break**	
	10:30 – 12:00 Session 10: Crystallin structure and function *(Kirsten Lampi & Eugene Serebryany)* S10-1 Kirsten J. Lampi S10-2 Hassane Mchaourab S10-3 Jayanti Pande S10-4 David Thorn S10-5 Eugene Serebryany	**10:30 – 12:00 Session 12: Redox regulation of lens cell differentiation, homeostasis and defese** *(Marc Kantorow & Julie Lim)* S12-1 Julie Lim S12-2 Oscar Jara S12-3 Hongli Wu S12-4 Shannon Das S12-5 Marc Kantorow	10:30 – 11:00 Break
11:00			**11:00 – 12:30 Session 16: Genetics and big data in the lens** *(Ales Cvekl & Salil Lachke)* S16-1 Ales Cvekl S16-2 Anne Slavotinek S16-3 Deepti Anand S16-4 Alan Shiels S16-6 Suraj Bhat
12:00	**12:00 Box Lunch**	**12:00 – 1:00 Lunch**	
	Afternoon free	**1:00 – 2:30 Session 13: Protein modifications in cataract** *(Gus Grey & Takumi Takata)* S13-1 Viviana Berthoud S13-2 Takumi Takata S13-3 Venkata Pulla Rao Vendra S13-4 Lee Goldstein S13-5 Gus Grey	**Final Remarks**
1:00			
2:00		**2:30 3:00 Break**	
3:00		**3:00 – 4:15 Session 14: New ideas and issues in the lens - an eye on the future** *(Melinda Duncan)* **Panel discussion**	
4:00		**4:15 - 4:45 Remembering Venkat Reddy** *(Frank Giblin & Peter Kador)*	
5:00		**5:35 Spreading Venkat Reddy's ashes**	
6:00		**6:00 – 7:00 Cocktails**	
7:00		**7:00 – 9:00 Banquet**	
8:00			

SCIENTIFIC PROGRAM

***(All talks are 18 minutes (15 minutes plus 3 minutes for questions) unless announced by Session Chairs).**

Sunday 8th of December

4:00pm-6:30pm	**Registration**
5:00pm-7:00pm	**Welcome Reception**
7:00-7:30	Opening remarks and presentation of travel awards
7:30-8:30	Kinoshita Lecture by Dr. K. Krishna Sharma, University of Missouri

Monday 9th of December

8:30am-10:00am Session 1: Development of lens structure and function -

Moderators*: *Barbara Pierscionek & Kehao Wang

Salil Lachke	RNA-binding protein mediated post-transcriptional control in lens development
Kehao Wang	Early development of gradient index profile in zebrafish eye lens
Ehsan Vaghefi	Hand-in-hand modifications of physiological optics of the lens with age
Irene Vorontsova	Aquaporin 0a is required for water homeostasis in the zebrafish lens in vivo
Melinda Duncan	Clues into the pathogenesis of juvenile-onset aniridic cataract; lens development into adulthood

10:00am-10:30am COFFEE BREAK / MORNING TEA

10:30am-12:00pm Session 2: Lens development

Moderators: Frank Lovicu & Yuki Sugiyama

Kristen Koenig	Lens development and evolution in the squid, doryteuthis pealeii
Nathalie Houssin	Characterization of epithelial cell arrangements during lens placode invagination
Shaili Patel	The conserved RNA-binding protein Rbm24 post-transcriptionally controls key transcription factors in early eye and lens development
David Li	The cAMP Responsible element binding protein (CREB) regulates lens differentiation through control of Pax6 and other target genes
Tayler Wishart	Differential expression of HSPGs in lens development: implications for the regulation of lens cell activity and architecture

12:00pm-1:00pm LUNCH

1:00-2:30pm Session 3: Cell signaling in the lens

Moderators: Xin Zhang & Kristen Koenig

Xin Zhang	Abelson kinases regulate FGF signaling in lens vesicle closure

Yuki Takeuchi	Decoy receptor, Fgfrl1, suppresses FGF signaling to ensure equator-specific onset of lens fiber differentiation
Eri Kubo	Effect of tropomyosin 1 knockout in lens development and fiber differentiation
Elizabeth Whitcomb	Control of lens fiber cell denucleation by CDK1 and its regulators
Frank Lovicu	Spreds in lens development: regulators of ERK-signaling leading to epithelial cell proliferation and fiber differentiation

2:30pm-4:30pm **Session 4: Posters with Refreshments**

See list at end of program

6:00pm-7:00pm **Luau Dinner**

7:00pm **Luau Show**

Tuesday 10th of December

8:30am-10:00am **Session 5: Lens in middle age - new insights into presbyopia**

Moderators*: *Paul Donaldson & Bianca Maceo Heilman

Bianca Maceo Heilman	The optics of accommodation and presbyopia and therapeutic strategies to treat presbyopia
Steve Bassnett	The lens in middle age: structural aspects
Rick Mathias	Effects of age on the mouse lens circulation and homeostasis
Paul Donaldson	Studying the physiological optics of the lens: new insights into the development of presbyopia

10:00am-10:30am **COFFEE BREAK / MORNING TEA**

10:30am-12:00pm **Session 6: Cellular biomechanics**

Moderators: Velia Fowler & Cathy Cheng

Velia Fowler	Determinants of lens biomechanics
Justin Parreno	Contribution of the lens capsule to whole lens biomechanical properties
Yuki Sugiyama	Cell rearrangement and shape change cooperatively drive lens cell movement to form a spherical tissue
Tim Plageman Jr.	The cadherin-associated protein, Arvcf, is an important regulator of lens fiber cell morphology and lens function
Cathy Cheng	Age-related changes in eye lens biomechanics, morphology, refractive index and transparency

12:00pm-1:00pm **LUNCH**

1:00pm-2:30pm **Session 7: Lens structure and function - compaction**

Moderators: Joe Costello & Steve Bassnett

Steve Reichow	Connexin-46/50 intercellular communication channels in a dual lipid bilayer environment resolved at near-atomic resolution by CryoEM

M. Joseph Costello	Galago (bush baby) monkey lenses employ nuclear excisosomes that are unique in origin and structure
Shruthi Karnam	Dystrophin (Dp71) is required for mechanical stiffness and membrane organization of the ocular lens
Rosica Petrova	The subcellular localisation of AQP5: Is it regulated by zonular tension and/or the activation of TRPV1 and 4 ion channels?
Barbara Pierscionek	Development of refractive index in the embryonic chick lens

2:30pm-3:00pm COFFEE BREAK / AFTERNOON TEA

3:00pm-4:50pm Session 8: Ion channels and transporters

Moderators: Tom White & Nick Delamere

Woo-Kuen Lo	AQP1 upregulation in lens epithelium is associated with cataractogenesis in AQP0-deficient lens fibers in mice
Eric C. Beyer	Connexin mutants impair the lens circulation leading to calcium accumulation/precipitation and cataracts
Xiaohua Gong	The roles of Cx46 and periaxin in fiber cell morphogenesis and cataratogenesis
Nick Delamere	Studies on TRPV1, NKCC1 and hydrostatic pressure responses in mouse lens.
Tom White	The ciliary muscle and zonules of zinn modulate lens intracellular hydrostatic pressure through transient receptor potential vanilloid channels
Yadi Chen	Pharmacological regulation of the lens water transport and its effects on physiological optics of the bovine lens

Wednesday 11th of December

8:30am-10:00am Session 9: The old lens - age related changes to lens structure and function

Moderators: Kevin Schey & Roger Truscott

Roger Truscott:	What the lens can tell us about aging and human age-related diseases
Roy Quinlan	Ageing, cataractogenic load and repair in the lens – an old dog still learning old tricks
Kevin Schey	The molecular impact of aging in the lens and beyond
John Carver	Crystallin proteins: post-translational modifications and amyloid fibrils
Juliet Moncaster	Age-related opacity changes in lenses of healthy centenarian humans and old wild-type mice

10:00am-10:30am COFFEE BREAK / MORNING TEA

10:30am-12:00pm Session 10: Crystallin structure and function -

Moderators: Kirsten Lampi & Eugene Serebryany

Kirsten J. Lampi	Increased aggregation and light scattering of human γS-crystallin due to cataract-associated deamidations

Hassane Mchaourab	a-crystallins, oxidative stress, and zebrafish lens development: recent confounding observations
Jayanti Pande	Protein condensation and cataract: an overview
David Thorn	The crystal structure of the disulfide-linked γS-crystallin dimer provides insight into an aggregation-prone oxidation product associated with cataractous lenses
Eugene Serebryany	Catalysis of aggregation by interface opening and disulfide exchange in cataract-associated variants of human γD crystallin

FREE AFTERNOON

Thursday 12th of December

8:30am-10:00am Session 11: Loss of transparency and treatment options

Moderators*: *John Clark & Hiroshi Sasaki

Hiroshi Sasaski	Prevalence of and visual impairment by waterclefts and retrodots
John I Clark	Loss of transparency and opacification of the lens
Peter Kador	The process of developing an FDA approved anti-cataract drug
Vincent Monnier	Approaches to controlling chemical protein aging in the loss of transparency in lens

10:00am-10:30am COFFEE BREAK / MORNING TEA

10:30am-12:00pm Session 12: Redox regulation of lens cell differentiation, homeostasis and defense

Moderators: Marc Kantorow & Julie Lim

Julie Lim	Redox imbalance results in the early onset of age related cataracts in cystine/glutamate antiporter knockout mice
Oscar Jara	Connexin50D47A alters the redox status and causes oxidation in the lens
Hongli Wu	Emerging functional crosstalk between the glutaredoxin system and Nrf2 antioxidant pathway: evidence from ultraviolet radiation-induced cataract formation
Shannon Das	Nox4 is required but not essential for TGFβ-induced cataract
Marc Kantorow	Hypoxia regulation of lens fiber cell differentiation

12:00pm-1:00pm LUNCH

1:00pm-2:30pm Session 13: Protein modifications in cataract

Moderators: Gus Grey & Takumi Takata

Viviana Berthoud	A tale of two serines
Takumi Takata	Creation and analysis of isomers-containing lens αA-crystallin
Venkata Pulla Rao Vendra	Human g-crystallin mutations affecting aromatic Greek key pairs and N-terminal tyrosine corner impairs tight packing and causes congenital nuclear cataract
Lee Goldstein	Detection of early Alzheimer's Disease-linked molecular changes in the lens by quasi-elastic light scattering ophthalmoscopy in young subjects with Down Syndrome

Gus Grey	Imaging mass spectrometry-based approaches to support lens biomolecular mapping and anti-cataract therapy development

2:30pm-3:00pm COFFEE BREAK / AFTERNOON TEA

3:00pm-4:15 pm Session 14: New ideas and issues in the lens - an eye on the future

Moderator: Melinda Duncan

4:15pm – 4:45pm Remembering Venkat Reddy's leadership in cataract research and the National Foundation for Eye Research.

Speakers: Frank Giblin and Peter Kador

5:35pm Spreading Venkat Reddy's ashes. Location TBA.

6:00pm-7:00pm Cocktails

7:00pm-9:00pm Banquet

Friday 13th of December

9:00am-10:30am Session 15: Cataract surgery and posterior capsule opacification

Moderators: Michael Wormstone & Sulin Chen

Mahbubul Shihan	Feasibility of bringing anti PCO therapeutics to clinical practice; physician acceptance and identification of a drug target
Michael Wormstone	Assessing the therapeutic potential of resveratrol to inhibit posterior capsule opacification
Thao Huynh	Identifying the mechanisms regulating sulforaphane induced lens cell death in PCO prevention
Xingjun Fan	Lens epithelial cells adaptation to oxidative stress and its relevance to posterior capsule opacification
Ali Mohammed	Fabrication of implantable IOLs using 3D printing technology

10:30am-11:00am COFFEE BREAK / MORNING TEA

11:00am-12:30pm Session 16: Genetics and big data in the lens

Moderators: Ales Cvekl & Salil Lachke

Ales Cvekl	Genetic coding of lens differentiation, -omics and emerging regulatory models
Anne Slavotinek	Foxe3 and downstream genes in lens development
Deepti Anand	iSyTE: a systems resource for gene discovery in lens development and cataract
Alan Shiels	The TRPM3 locus in lens development and cataract
Suraj Bhat	Transcriptional profiling of single cells – insights into lens fiber cell morphogenesis

FINAL REMARKS – MEETING CLOSURE

Poster Presentations
2:30 to 4:30 Monday 9th of December

Poster 1	**Chenjun Guo:** Expression of Grx2 in the capsule tissue of age-related cataract patients
Poster 2	**Douglas S. Parsons:** In vivo quasi-elastic light scattering eye scanner detects molecular aging in humans
Poster 3	**Emily K. Hamlin:** Species-dependent changes in lens crystallin protein solubility during aging
Poster 4	**Hideaki Morishita:** A cytosolic phospholipase-mediated organellar degradation in the lens in zebrafish and mice
Poster 5	**Hidetoshi Ishida:** Cataract-inhibitory effects of water chestnut and lutein in Shumiya cataract rat
Poster 6	**Hirotaka Hashimoto:** The effect of aging on the antioxidative activity of astaxanthin in human aqueous humor
Poster 7	**Hiroyuki Matsushima:** Experimental safety evaluation of intraocular lenses using rabbit cataract model
Poster 8	**Karera Mukunoki:** Effect of capsular tension ring to prevent posterior synechia after cataract surgery using rabbit models
Poster 9	**Kazuhiro Takao:** Hydrogen prevents corneal endothelial damage in cataract surgery
Poster 10	**Kohei Miyata:** Comparison of visual outcomes after implantation of Symfony® and FINE VISION®
Poster 11	**Mayumi Nagata:** Clinical evaluation of surface modified intraocular lenses
Poster 12	**Xiaobin Liu:** More than just a reactive oxygen species scavenger: grapes prevents UV radiation-induced cataract by upregulating anti-apoptotic protein XIAP
Poster 13	**Xiaona Ning:** Effects of Grx2 on the oxidative damage under hyperoxia in human lens epithelial cells
Poster 14	**Yoko Murakami:** Inhibitory effects of topical administration of a ROCK inhibitor on in vivo secondary cataract formation
Poster 15	**Ajay Pande:** Similar structural perturbation in two distinct cataract-associated mutants, Y66N and V41M, of human gammaS–crystallin
Poster 16	**Alyssa Lie**: Does water content in the lens nucleus change during accommodation?
Poster 17	**Samuel G. Novo:** Cataract surgery triggers remnant lens epithelial to initiate the immediate early response
Poster 18	**Chun-hong Xia:** Lens epithelial cell heterogeneity in lens growth and cataract formation
Poster 19	**Jia-Ling Fu:** Pax6 directly regulates Birc7 to control apoptosis of lens epithelial cells
Poster 20	**Qian Nie:** The SUMOylation E3 ligase PIAS1 regulates apoptosis and inflammatory response in the ocular lens
Poster 21	**Rijo Hayashi:** Aquaporin 8 in the lens
Poster 22	**Yazdankhah M:** βA3/A1-crystallin effects on the pathogenesis of persistent fetal vasculature (PFV) disease by regulating EGFR/MTORC1/autophagy signaling pathway
Poster 23	**Yoshiki Miyata:** Analysis of tissue interaction involved in lens glutathione homeostasis in chicken embryo lens ex vivo models

Poster 24	**Yuxing Li:** Lens filament proteins CP49 and filensin are required for cold cataract formation in young mouse lenses
Poster 25	**Mark Petrash:** An unexpected role for aldose reductase in regulation of lens regeneration
Poster 26	**Zhaohua Yu:** In vivo density change of lens epithelial cells induced by UVR exposure
Poster 27	**Yan Ma: m**odeling congenital cataract in vitro using mouse-specific induced pluripotent stem cells in a reproducible, controlled manner
Poster 28	**Natsuko Hatsusaka:** Epidemiological study of nuclear emergency workers at Tokyo Electric Power Company Fukushima Nuclear Power Plant: findings from cataract study 3-6 years after the nuclear accident
Poster 29	**Robert C. Augusteyn:** Lens, zonules and ciliary processes
Poster 30	**Yosuke Nakazawa:** Pharmacological modulation and changes in osmolarity alter the subcellular distribution of TRPV1 and TRPV4 channels in the mouse lens
Poster 31	**Qian Wang:** Etv transcription factors functionally diverge from their upstream FGF signaling in lens development
Poster 32	**Sidra Islam**: Inhibition of gamma crystallins aggregation as a paradigm for the development of anti-cataract drugs
Poster 33	**Yanzhong Hu**: Heat shock factor 4 regulates lysosome activity by modulating the αB-crystallin-ATP6V1A-mTOR Complex in ocular lens
Poster 34:	**Koichiro Mukai:** Morphological changes of lens tissues due to different IOL materials on early cataract
Poster 35	**Thomasz Dzialoszynski:** Effects of North American ginseng extracts on lens health and plasma in streptozotocin diabetic rats using early and late treatment
Poster 36	**Michael D. O'Connor:** Validating novel, lens signal pathway-mediated gene expression using human pluripotent stem cell-derived lens cells
Poster 37	**Yu Zhang:** The safety and cytokine levels in the aqueous humor after delayed sequential bilateral cataract surgery

ABSTRACTS

Session 1-1 **Salil Lachke:** RNA-binding protein mediated post-transcriptional control in lens development

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Session 12-3 **Hongli Wu:** Emerging functional crosstalk between the glutaredoxin system and Nrf2 antioxidant pathway: evidence from ultraviolet radiation-induced cataract formation
Session 12-4 **Shannon Das:** Nox4 is required but not essential for TGFβ-induced cataract
Session 12-5 **Marc Kantorow:** Hypoxia regulation of lens fiber cell differentiation
Session 13-1 **Viviana Berthoud:** A tale of two serines
Session 13-2 **Takumi Takata:** Creation and analysis of isomers-containing lens αA-crystallin
Session 13-3 **Venkata Pulla Rao Vendra:** Human g-crystallin mutations affecting aromatic Greek key pairs and N-terminal tyrosine corner impairs tight packing and causes congenital nuclear cataract
Session 13-4 **Sidra Islam:** Inhibition of gamma crystallins aggregation as a paradigm for the development of anti-cataract drugs
Session 13-5 **Gus Grey:** Imaging mass spectrometry-based approaches to support lens biomolecular mapping and anti-cataract therapy development
Session 15-1 **Mahbubul Shihan:** Feasibility of bringing anti PCO therapeutics to clinical practice; physician acceptance and identification of a drug target
Session 15-2 **Michael Wormstone:** Assessing the therapeutic potential of resveratrol to inhibit posterior capsule opacification
Session 15-3 **Thao Huynh:** Identifying the mechanisms regulating sulforaphane induced lens cell death in PCO prevention
Session 15-4 **Xingjun Fan:** Lens epithelial cells adaptation to oxidative stress and its relevance to posterior capsule opacification
Session 15-5 **Ali Mohammed:** Fabrication of implantable IOLs using 3D printing technology
Session 16-1 **Ales Cvekl:** Genetic coding of lens differentiation, -omics and emerging regulatory models
Session 16-2 **Anne Slavotinek:** Foxe3 and downstream genes in lens development
Session 16-3 **Deepti Anand:** iSyTE: a systems resource for gene discovery in lens development and cataract
Session 16-4 **Alan Shiels:** The TRPM3 locus in lens development and cataract
Session 16-5 **Suraj Bhat:** Transcriptional profiling of single cells – insights into lens fiber cell morphogenesis

Session 1-1

RNA-binding protein mediated post-transcriptional control in lens development

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Purpose: Lens development/homeostasis involves the precise control of gene expression as cells of the epithelium differentiate into fiber cells. Many lens fiber-enriched proteins are up-regulated at the onset of fiber cell differentiation but their mRNAs are found to be already present in lens epithelial cells. These findings suggest that post-transcriptional regulation, which refers to the control of distinct events between the synthesis of an mRNA and its translation into protein, is a key aspect of gene expression orchestration that is necessary for the generation of specific lens cell proteomes. The human genome encodes >1500 RNA-binding proteins (RBPs), involved in distinct aspects of mRNA post-transcriptional control, but current understanding of their function in lens development and cataract is limited. The motivation of the current study lies in the quest to bridge this knowledge-gap.

Methods: Lens-specific compound conditional deletion mice for the conserved RBP gene, *Celf1*, were generated crossing *Pax6GFPCre* and *Celf1* flox and germline knock-in alleles (termed *Celf1*^{cKO}). Lens tissue from *Celf1*^{cKO} mice was characterized by histology, scanning electron microscopy, immunostaining and RNA-sequencing. RNA-Immunoprecipitation (RIP) or cross-linked-IP (CLIP) was performed with Celf1 antibody followed by qPCR/sequencing.

Results: *Celf1*^{cKO} lenses exhibit abnormal elevation and mis-expression of Prox1 protein in the lens epithelium. *Celf1*^{cKO} lenses also show abnormal up-regulation of Pax6 protein in fiber cells. These changes in protein levels occur without analogous changes in mRNA levels. To gain insight into the mechanism of Celf1-based control over these key lens transcription factors, we performed RIP and CLIP assays which show that Celf1 directly binds to *Pax6* and *Prox1* mRNAs. Reporter assays show that Celf1 translationally controls these transcription factors via their 3'UTRs.

Conclusion: These data demonstrate that Celf1 post-transcriptionally controls the key transcription factors, Pax6 and Prox1, in lens development and provide new insights into the molecular pathology of cataract in Celf1-deficient animals.

Session 1-2

Early development of gradient index profile in zebrafish eye lens

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Purpose: To investigate the development of gradient refractive index in the zebrafish eye lens.

Methods: Measurements were conducted on eye lenses from wild-type zebrafish aged from 15 to 880 post fertilization (dpf). Zebrafish were dissected at University of California Irvine and shipped to SPring-8 synchrotron in Japan. Five to six samples at each measured age were scanned using the X-ray synchrotron radiation based Talbot interferometry located at SPring-8. Scans were performed at beamline BL20B2 where X-ray beams were fine-tuned to 25keV to protect biological samples. Lenses were set in agarose gel and measured in groups of six. Each scan took 50 mins.

Results: Zebrafish eye lenses demonstrate clear gradient refractive index profiles at 15 days. The peak index value of this profile rises from 1.42 at 15 dpf to the maximum of 1.58 at around 120 dpf with a concomitant steepening of the cortical gradient. After reaching the maximum refractive index, the peak region broadens and the cortical gradients remain relatively unchanged. The gradient index profile at all developmental stages is without discontinuities and little individual variation is seen at each age.

Conclusions: Zebrafish eye lens develop gradient index profiles at very early age and this profile remains smooth throughout the age group investigated. Growth in optical parameters correlated well with lens size which is linked to eyeball growth most likely for maintenance of requisite optical function in the visual system.

Session 1-3

Hand-in-hand modifications of physiological optics of the lens with age

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Purpose: To further investigate the existence and measure the degree of the "Lens Paradox" phenomenon *in-vivo*.

Methods: A cohort of healthy participants from young (18-40 years), middle-aged (41-60 years) and older (>60 years) age group were recruited, with 22, 20 and 17 participants in each category respectively. Participants went through an ophthalmic exam which included subjective refraction and ocular biometry with LENSTAR. Participants were then scanned using a 3T clinical SKYRA MRI to generate T2 and anatomical ocular maps. T2 images were then post-processed to extract lenticular geometry and gradient of refractive index (GRIN), using the Jones et al (ref) equation. Using ZEMAX optical modelling software and combining LENSTAR and MRI measurements, accurate individual models of whole eye optics were constructed. The dioptric power of each individual lens was then calculated from ZEMAX.

Results: We observed a general hyperopic shift of the whole eye, which would indicate a reduction of lens power in our aging cohort. However, our ZEMAX results finds an increasing trend of lens power with age, unlike the prediction of the 'Lens Paradox' phenomenon. We then investigated potential sources of error in our data acquisition and optical modelling. It appeared that in order to match the clinically observed age-related hyperopic shift, the T2 to GRIN map formula should be modified to take into account an age factor due to age-related protein deformations and truncations.

Conclusions: Our methods of MRI + LENSTAR + ZEMAX optical modelling appear to be able to replicate clinical vision assessment outcome with reasonable precision. This model will be improved by adjusting the two-decade old T2 to GRIN conversion formula (Jones and Pope) to include an 'age' parameter.

Session 1-4

Aquaporin 0a is required for water homeostasis in the zebrafish lens *in vivo*

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Purpose: Aquaporin 0 (AQP0) is essential for lens development and transparency. Mammalian AQP0 has multiple cellular functions including water transport and adhesion, which have been impossible to study individually. An ancestral teleost genome duplication gave rise to two AQP0 orthologs in zebrafish, *aqp0a* and *aqp0b*, which apparently have functionally diverged. *In vitro*, both Aqp0s permeate water. We have shown that CRISPR-Cas9 mediated null *aqp0a*$^{-/-}$ mutants form cataract, while *aqp0b*$^{-/-}$ lenses look like wild-type, consistent with distinct functions. In this study, we test adhesive properties, and the requirements of Aqp0a and Aqp0b for water homeostasis in zebrafish lenses *in vivo*.

Methods: Heterologous expression in adhesion-deficient mouse fibroblast L-cells was used to test adhesive properties of Aqp0s. To study lens water homeostasis *in vivo*, we have employed a permeable, non-toxic solvatochromic fluorescence probe, ACDAN (6-acetyl-2-dimethylaminonaphthalene). Spectral phasor analysis of the ACDAN signal allows mapping of dipolar relaxation (DR) onto lens images, which we then analyzed using a custom-developed algorithm.

Results: An adhesive role was confirmed for Aqp0b, but not Aqp0a. As the lens matures, overall DR increased, and the maximum DR signal moved from the lens nucleus to the cortex. This DR shift coincided with compaction of lens fiber cells and packing of crystallins in the lens nucleus required for lens optics and emmetropia. In *aqp0a*$^{-/-}$, but not *aqp0b*$^{-/-}$ lenses, there was lower DR in all parts of the lens cortex at 4 days postfertilization, revealing a disruption of water homeostasis.

Conclusion: The use of these novel imaging and analysis techniques suggest an essential role for Aqp0a in lens water homeostasis. But loss of Aqp0b, and thus presumably its adhesive properties are dispensable, implying the presence of compensatory adhesive mechanisms. Future studies will address requirements for specific residues of Aqp0a for *in vivo* lens water homeostasis. (Supported by NIH P41-GM103540 and R01-EY05661).

Session 1-5

Clues into the pathogenesis of juvenile-onset aniridic cataract; lens development into adulthood

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Purpose: Aniridia is a rare genetic condition caused by heterozygous mutations in the gene encoding the transcription factor, Pax6. Aniridia patients experience compromised vision at birth due to iris and retinal defects, but later develop potentially blinding sequelae including cataract. Humans and mice carrying Pax6 mutations have similar ocular phenotypes, however, the pathophysiology of aniridic cataract has not been explored.

Methods: The lens phenotype of aniridia patients was assessed using ultrasound biometry and anterior chamber OCT. Lenses from adult mice heterozygous for Pax6 mutations ($Pax6^{tm1/Pgr}$; Sey) were investigated by gross microscopic examination, micro computed tomography, RNA sequencing, and immunofluorescence.

Results: Human aniridic lenses are typically of normal size although they exhibit increased risk of decentration and juvenile onset cataract. Adult Sey mouse lenses are 60% the size of normal and develop cataracts sporadically as adults. Adult mouse lens epithelial cells (LECs) heterozygous for a Pax6 mutation differentially express 1306 genes (DEGs) compared to littermate controls, while 140 DEGs were identified in Pax6 mutant lens fibers. Bioinformatic analysis showed that the mRNAs upregulated in mutant lens fibers are enriched in molecular markers of oxidative stress, a known cataractogenic mechanism. In contrast, the DEGs upregulated in Sey LECs are highly enriched in markers of fibrosis, which may be driven indirectly by the upregulation of known regulators of WNT pathway signaling, and directly by transcriptional regulators of fibrotic gene expression.

Conclusions: Humans and mice heterozygous for Pax6 mutations are discordant for lens size defects, but both develop lens abnormalities with age. These data suggest that the Pax6 haploinsufficiency sensitizes the lens to fibrotic transformation indicating the correct dosage is necessary for adult LECs to maintain their normal epithelial phenotype. As humans with aniridia are susceptible to an aggressive ocular fibrosis denoted as “aniridia fibrosis syndrome”, this may provide mechanistic insight into this condition.

Session 2-1

Lens development and evolution in the squid, *doryteuthis pealeii*

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Purpose: The cephalopod eye (squid, cuttlefish and octopus) and the vertebrate eye is a remarkable case of evolutionary convergence. Both visual systems are highly acute and have independently evolved a single-chambered morphology with a single lens in the anterior. The vertebrate lens development has been extensively studied but little is known about cephalopod lens formation. In this study we show the first functional molecular characterization of lens development in the squid *Doryteuthis pealeii.*

Methods: *In situ* hybridization expression studies of genes enriched during eye and optic lobe development were performed on five embryonic stages spanning eye development. Genes expressed in the anterior segment in conjunction with morphological analysis were used to define cell populations contributing to lens formation. Wnt agonist experiments were performed using LiCl at stage 21 and 23 for 24 hours and fixed immediately or recovered for 24 hours. Anterior segment phenotypes were assessed in whole mount, section and using *in situ* hybridization for target genes.

Results: Cephalopods have a taxon specific duplication of *Sp6-9* with one paralog found using *in situ* hybridization in the developing lens. Gene expression surveys showed enrichment of *Dlx*, *Exd*, and *Meis* in the anterior segment suggesting a co-option of the of the limb outgrowth gene regulatory network in the development of the cephalopod lens. Wnt signaling canonically regulates this network in the limb and *Wnt*, *Frz* and other pathway members are expressed in the developing eye and anterior segment. Ectopically activating the Wnt signaling leads to down regulation of limb cassette and the loss of the lens.

Conclusion: The Sp6-9/Dlx limb outgrowth network has been redeployed during lens formation and down regulation of Wnt signaling is necessary for proper lens formation. This work suggests that gene duplication is important for the cooption of gene regulatory networks and the evolution of novel phenotypes.

Session 2-2

Characterization of epithelial cell rearrangements during lens placode invagination

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Purpose: Development of the vertebrate eye serves as a valuable model for epithelial morphogenesis. At the onset of lens formation, the surface ectoderm thickens to form the lens placode (LP), an epithelium that undergoes a series of cell rearrangements and shape changes that drive invagination of the tissue. Currently, the cellular mechanisms driving epithelial invagination remain incompletely characterized, therefore we aim at elucidating them using live fluorescent microscopy.

Methods: Chicken and mouse embryos were incubated with a SiR-Actin dye to visualize membrane cells in a climate controlled environment. Specimens were live-imaged using either a Zeiss Axio observer inverted microscope or a Nikon A1R confocal microscope. Additionally, fixed whole embryos were immunolabeled using the appropriate antibodies to visualize the distribution of proteins involved in junctional contraction.

Results: It was observed that LP cells behave differently from their surrounding neighbors. Central LP cells reduce their apical area isotropically and maintain their junctions with neighboring cells whereas peripheral LP cells become anisotropic and dynamically rearrange themselves resulting in a central converging motion. Junctional contraction, was found to occur coordinately in adjacent junctions aligned in arcs and it is Rho-kinase and non-muscle myosin II dependent. Moreover, it is coincident with additional markers of actomyosin contraction. Experimentally induced epithelial lacerations further demonstrated that the generation and alignment of the junctional arcs are due to their mechanical environment facilitated by the centralward movement of epithelial cells and the junctional reduction of the Par-complex protein Par3. Besides, Par3-deficient mouse embryos analysis demonstrated that the anisotropic increase in junctional localization of actomyosin proteins is dependent on Par3.

Conclusions: Altogether, these results highlight the role of mechanical cues that, in association with the coordinated relocation of contractile proteins along the apical junctions, contribute to epithelial invagination, the first step of lens morphogenesis.

Session 2-3

The conserved RNA-binding protein Rbm24 post-transcriptionally controls key transcription factors in early eye and lens development

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Purpose: Lens development in mammals initiates when the optic vesicle induces the presumptive lens ectoderm to form the lens placode. We sought to understand if RNA-binding protein (RBP)-based post-transcriptional control played a role in these developmental processes. Therefore, we used iSyTE (integrated Systems Tool for Eye gene discovery) to identify the RBP Rbm24 (RNA-binding motif protein 24) as a high-priority candidate based on lens-enriched expression in mouse development. Here we examined whether Rbm24 is necessary for eye and lens development.

Methods: Rbm24 germline and conditional knockout (KO) mice were generated and phenotypically and molecularly characterized. RNA-immunoprecipitation (RIP) assays were performed on eye and lens tissue lysate using anti-Rbm24 antibody followed by RT-qPCR to identify pulldown mRNAs that are direct targets of Rbm24 protein. EMSA (electrophoretic mobility shift assay) was performed by incubating purified Rbm24 protein with biotinylated and unbiotinylated *Sox2* 3'UTR probes and visualized using chemiluminescence.

Results: Eye development was defective from early stages in both *Rbm24* germline and conditional KO mice, which exhibit microphthalmia and/or anophthalmia. *Rbm24* KO mice exhibited down-regulation of multiple eye and/or lens markers such as Sox2, Pax6, Lhx2, Jag1, E-cadherin and gamma crystallins. RIP-assays indicated that Rbm24 protein directly interacts with *Sox2* and *Lhx2* mRNAs. EMSA demonstrated that conserved AU-rich-element (ARE) sites in the Sox2 3'UTR are necessary for Rbm24 protein binding. Finally, mRNA decay assay showed that Rbm24 protein-based binding of Sox2 mRNA is necessary for maintaining its optimal levels.

Conclusion: The conserved RBP Rbm24 is necessary for proper development of the mouse eye and lens. Several key transcription factors as well as other eye/lens proteins are mis-expressed in the developing eye tissue of *Rbm24* KO mice. Rbm24 protein directly binds to *Sox2* mRNA via conserved ARE sites and controls its stability, suggesting a molecular mechanism for Rbm24 in post-transcriptionally controlling eye gene expression.

Session 2-4

The cAMP responsible element binding protein (CREB) regulates lens differentiation through control of Pax6 and other target genes

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Purpose: The cAMP response element binding protein (CREB) is a general transcription factor whose functions are activated through phosphorylation at S133 by protein kinase A (PKA) and inactivated by PP-1β and PP2Aα. In the present study using mouse model and cell line, we characterized the developmental expression patterns of the CREB in the developing mouse lenses and analyzed its functional mechanisms in promoting lens differentiation.

Methods: RNA-seq, qRT-PCR, Western blot analysis and immunocytochemistry were used to analyze expression patterns of CREB and its target genes. Co-IP and immunofluorescence co-localization were used to determine protein-protein interactions.

Results: CREB was highly expressed in the ocular lens from the very beginning (ED 10.5) to adult mouse eye lens. CREB activity was consistently detected in the differentiating lens fiber cells. Stable overexpression of wild type CREB but not its S133A mutant in lens epithelial cells promotes cell differentiation in the absence of growth factor and withdrawal of serum. RNAseq analysis revealed that cells with CREB overexpression displayed alteration of expression patterns in more than 1700 genes Among which over 100 genes have been shown to be closely related to differentiation and currently we found that CREB can directly regulate expression of Pax-6 and other genes to promote lens cell differentiation.

Conclusion: CREB is an important transcription factor mediating normal lens development. (Supported by grants from National Natural Science Foundation of China, 81570824, 81770910, 81970787, and 81900842 as well as the Fundamental Funds from the State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University).

Session 2-5

Differential expression of HSPGs in lens development: implications for the regulation of lens cell activity and architecture

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Purpose: Heparan sulfate proteoglycans (HSPGs) are composed of a core protein decorated with sulfated HS glycosaminoglycan (GAG) chains. HSPGs localise to the cell surface and pericellular matrix where they bind, via their HS chains, to diverse components of the cell microenvironment, including growth factors. Through their interactions with growth factors HSPGs form receptor-signalling complexes required for proliferation, migration and differentiation; and sequester growth factors to form local gradients. While HS-sulfation has been identified as critical for normal lens development, the expression of different HSPG core proteins in the lens has yet to be investigated. Here we present the full spectrum of HSPGs expressed during lens morphogenesis.

Methods: The expression of different HSPG family members was compared using semi-quantitative RT-PCR in postnatal rat lenses, and immunolabelling of murine lenses at embryonic days 9.5 to 16.5, and postnatal days 0 and 10. Rat lens epithelial explants were used to investigate the effect of inhibition of HSPG/GAG sulfation on growth factor-induced lens epithelial cell proliferation (BrdU-incorporation) and downstream signalling (eg. Western blotting of phospho-ERK1/2).

Results: Most HSPGs are expressed in lens, with cellular (syndecans and glypicans) and secreted HSPGs (eg. perlecan) differentially expressed in the lens epithelium, primary/secondary fibres and lens capsule at key stages of lens development. Functionally, HS activity but not other sulfated GAGS (eg. chondroitin sulfate) is required for growth factor-induced lens epithelial cell proliferation.

Conclusion: The differential spatial and temporal expression of HSPG core proteins suggests that lens cells acquire a distinctive repertoire of HSPGs as they differentiate, resulting in selective patterns of HS-protein interactions and activity. This implies differential roles for HSPGs and HS sulfation enzymes at key stages of lens induction and morphogenesis. Taken together with the current literature, our findings support a requirement for HS sulfation in growth factor-induced cellular processes in the lens.

Session 3-1

Abelson kinases regulate FGF signaling in lens vesicle closure

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Purpose: Abelson kinase (Abl) was first discovered as an oncoprotein constitutively active in human leukemia, where it promotes many aspects of cellular transformation, including cell proliferation, survival and invasion. In mice, knockouts of *Abl* die around birth and the null mutants for its close homologue *Arg* are viable, but combined deletion of *Abl* and *Arg* results in early embryonic lethality at E8, demonstrating the critical requirement of *Abl* genes for embryonic development. Here, we investigated the functional requirement of Abl and Arg in lens development.

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

Results: In the lens specific knockout of *Abl* and *Arg*, the lens vesicle remained attached to the surface ectoderm at E12.5, which is reminiscent of Peter's anomaly in humans. This persisted to E14.5 as a gap in the center of the surface ectoderm and lens epithelium, allowing the lens fiber cells to leak out of the eye. These phenotypes were reproduced in the lens by overexpression of FGF or constitutive activation of Ras signaling. Remarkably, although genetic ablation of *Fgfr1* and *Fgfr2* abolished lens development, further deletion of *Abl* and *Arg* in *Fgfr1/2* mutants resulted in a well formed lens. The rescue of lens development in the *Fgfr*/*Abl* mutants strongly suggests that Abl kinases negatively regulate the FGF signaling pathway.

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

Session 3-2

Decoy receptor, Fgfrl1, suppresses FGF signaling to ensure equator-specific onset of lens fiber differentiation

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Purpose: During lens development, lens epithelial cells proliferate, migrate toward the lens equator, and start to differentiate into lens fiber cells after passing through the equator. FGF promotes lens epithelial cell proliferation and lens fiber differentiation in a dose-dependent manner. However, it is not fully understood how FGF signaling is modulated in developing lens. FGF receptor-like 1 (Fgfrl1) has three extracellular immunoglobulin domains, which bind to FGF ligands, but lacks an intracellular kinase domain. It was reported that proteolytic cleavage of Fgfrl1 releases its ectodomain, which subsequently binds FGF ligands, suggesting that Fgfrl1 functions as a decoy receptor. Here we focus on zebrafish Fgfrl1b, which is exclusively expressed in lens epithelium, and elucidate its role in lens fiber differentiation.

Methods: To inhibit the function of Fgfrl1b, we injected its morpholino antisense into wild-type embryos and examined lens fiber cell differentiation. Next, to confirm the decoy receptor function of Fgfrl1b, we overexpressed Fgfrl1b tagged with GFP and mCherry at N- and C-terminus, respectively, and traced subcellular localization of extracellular and intracellular domains.

Results: In Fgfrl1b morphant, expression of an FGF target, pea3, was elevated, suggesting that Fgfrl1b suppresses FGF signaling. Furthermore, expression of a lens fiber differentiation marker, Prox1, was also enhanced and ectopic Prox1 expression occurred in lens epithelium of Fgfrl1b morphant. In lens epithelial cells expressing fluorescent proteins-tagged Fgfrl1b, N-terminus-tagged GFP was located in plasma membrane or extracellular space, whereas C-terminus-tagged mCherry was observed in the intracellular region. Thus, it is likely that Fgfrl1b ectodomain is cleaved and released into extracellular space for capturing FGF ligands.

Conclusion: Fgfrl1b acts as a decoy receptor and suppresses FGF signaling in the zebrafish lens epithelium. This FGF suppression in the lens epithelium may be important for tuning FGF activation at the equator to ensure the equator-specific onset of lens fiber differentiation.

Session 3-3

Effect of tropomyosin 1 knockout in lens development and fiber differentiation

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Purpose: Tropomyosin (Tpm) 1 and 2 play an important role in the epithelial mesenchymal transition of the lens epithelial cells. In this study we analyzed the developmental and age-related change of the crystalline lens in conditional knock-out mice of *Tpm 1* (*Tpm1*-CKO).

Methods: Floxed alleles of the *Tpm1* gene were conditionally deleted in the lens by using *Pax6-cre* transgenic mice (*Tpm1*-CKO). Adult and timed-pregnant female *Tpm 1 CKO* (gestational day:GD 14) were obtained. Lenses of GD 14, postnatal day (PD) 7, adult 11 and 48 week-old *Tpm1* CKO and wild type mice (WT) as controls were dissected and photographed. Extracted lenses were fixed with paraffin and stained with hematoxylin-eosin. Immunological localization of Tpm1/2, α smooth muscle actin (αSMA) and F-actin was examined. Expressions of *Tpm1 and 2* and*αSMA* mRNAs was examined by RT-qPCR.

Results: In GD 14, lens fiber damage and disorganization of lens epithelial nucleus were observed. Between 1 and 11 weeks of age, the posterior capsule was ruptured, and lens nucleus was dropped into the vitreous in *Tpm1-CKO*. After the posterior rupture, lens sizes were reduced, and the rupture was closed. Lens opacity and significant reduction of lens sizes were observed at 1 week of age. Disorder of lens fiber cells, and cortical and peri-nuclear liquefaction were observed in *Tpm1-CKO*. There was little difference in the phenotype of lenses in heterozygous and homozygous *Tpm1-CKO*. Expression of F-actin in the *Tpm1-CKO* was observed in damaged fiber cells and at all ages. Expressions of Tpm-1/Tpm-2 and αSMA mRNA were decreased in the lenses of *Tpm1-CKO*.

Conclusions: Our results highlight the significance of Tpm1 function in lens fiber differentiation with development and aging. Dysregulation of Tpm1 may induce lens opacity.

Session 3-4

Control of lens fiber cell denucleation by CDK1 and its regulators

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Purpose: The lens of the eye undergoes a regulated differentiation process whereby organelle removal in the fiber cells is necessary for lens clarity. The mechanisms by which this occurs are unclear. Previous work in our lab demonstrated that impaired lens fiber cell denucleation (LFCD) was associated with increased levels of the CDK inhibitor p27. Additionally, we showed that deletion of CDK1 in the lens impaired LFCD. These data indicate that CDK activity is crucial for denucleation. In this study, we examine the role of CDK activators and inhibitors in the denucleation process.

Methods: We used three different mouse models to ask whether increased p27 levels affects lens differentiation. The first model expresses a drug inducible p27 transgene. We induced expression of the transgene during pregnancy to increase p27 during a crucial phase of lens differentiation. The second model is a *Skp2*-/-. Skp2 is part of a ubiquitin ligase complex that is known to ubiquitinate p27 and target it for proteasomal degradation; these mice constitutively express high levels of p27. The third model expresses a degradation resistant mutant p27. To determine how CDK1 regulators are involved in LFCD, we used a chick lens culture model and treated lenses with small molecule drugs that affect the activities of Cdc25, Wee1, CDK1 and PP2A.

Results: Expression of increased levels of p27 led to impaired LFCD in all three mouse models. Inhibition of the CDK1 activator Cdc25 inhibits LFCD, whereas inhibition of the CDK1 inhibitor Wee1 potentiates LFCD in chick lenses. PP2A inhibits CDK1 activity: activation of PP2A inhibits LFCD while inhibition of PP2A increases LFCD.

Conclusions: Inhibition of CDK1 activity by increased p27 protein levels, activation of PP2A or inhibition of Cdc25 inhibits LFCD. Inhibiting CDK1 inhibitors Wee1 and PP2A, led to increased LFCD. Thus, CDK1 activity is a crucial regulator of LFCD.

Session 3-5

Spreds in lens development: regulators of ERK-signaling leading to epithelial cell proliferation and fiber differentiation

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Purpose: The functional properties of the lens are dependent on its precise cellular architecture, established early in embryogenesis and maintained through coordinated epithelial cell proliferation and subsequent differentiation into fibers. These cellular processes require MAPK/ERK1/2-signaling, that in turn is negatively regulated by members of the Spred family of proteins. As Spreds are expressed throughout lens morphogenesis in distinct spatial patterns, we hypothesized that they play an important role in regulating lens epithelial cell proliferation and fiber differentiation.

Methods: Lens development was examined in murine embryos overexpressing Spred1 or Spred 2, and also in embryos deficient for both Spred1 and Spred2 (Spred1/2^{null}). The lens phenotype was characterized with particular attention to cell number, organization and rates of proliferation (using BrdU-incorporation) and fiber cell differentiation.

Results: Mice with elevated levels of Spred in lens showed reduced ERK-signaling leading to a smaller lens, and microphthalmia, with reduced lens epithelial cell proliferation and fiber elongation. Mice deficient for Spred1/2 in the lens also exhibited microphthalmia; however, with increased epithelial cell proliferation and density disrupting the epithelium, along with increased phosphorylated ERK1/2. Despite changes in the spatial arrangement and fate of BrdU-labeled cells, we observed an eventual recovery in the growth rate of Spred1/2 mutant lenses, implicating intrinsic compensatory mechanisms by other related signaling antagonists.

Conclusions: While Spreds are not essential for lens induction, the lens requires Spreds for its early growth phase, specifically for the maintenance of its cellular architecture. This study provides a greater understanding of some of the key molecules that regulate lens developmental processes, and provides insights into developing strategies to preserve and regenerate normal lens cell structure.

Session 5-1

The optics of accommodation and presbyopia and therapeutic strategies to treat presbyopia

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The human crystalline lens is a complex structure that continuously grows throughout life. The lens has a non-uniform distribution of protein concentrations which produces a refractive index gradient within the lens. This gradient index is a unique property that significantly contributes to its optical power and aberrations. One of the fundamental research objectives at the Ophthalmic Biophysics Center is to understand the relationship between the crystalline lens shape, refractive index gradient, and optics, and how it changes with accommodation and age. This presentation will discuss the use of a combined laser ray tracing and optical coherence tomography (OCT) system to measure the shape and wavefront aberrations of human crystalline lens *in vitro* during simulated accommodation, as well as a custom extended-depth OCT system to study the lens *in vivo* during physiological accommodation. The current status of therapeutic strategies to treat presbyopia will also be discussed.

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Session 5-2

The lens in middle age: structural aspects

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Purpose: To present an overview of the structure and cellular organization of the lens in middle age with an emphasis on those aspects that may presage the development of presbyopia.

Methods: Images obtained using a variety of modalities will be presented, including newly developed techniques for visualizing the three-dimensional organization of the ciliary zonule.

Results: The lens is an unusual cellular system in that it increases steadily in size throughout life. The continual addition of new cells to the lens surface is partially offset by the compaction of pre-existing cells in the lens core, with implications for the shape of the refractive surfaces, the internal refractive index gradient, and tension in the ciliary zonule.

Conclusions: To better understand presbyopia, it will be necessary to integrate cell-level structural information with biochemical data on post-translational modifications of extant lens protein. The ultimate goal of such an integrative approach is to generate a realistic optomechanical model of the aging lens founded in the biology of the system.

Session 5-3

Effects of age on the mouse lens circulation and homeostasis

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Purpose: Formation of age related central cataracts involves loss of intracellular homeostasis. We have investigated effects of age on membrane transport, and how changes in membrane transport affect the intracellular milieu.

Methods: 2 and 14 month old lenses are compared. Intracellular microelectrodes were used to study center to surface variations of physiological parameters in freshly isolated intact lenses. Impedance studies determined gap junction coupling and membrane conductances. Intracellular voltage and hydrostatic pressure were mapped. Intracellular ion concentrations were measured by injection of ion sensitive dyes: sodium (SBFI), potassium (PBFI), calcium (FURA2), hydrogen (BCECF).

Results: Data on center to surface intracellular gradients were fit with a model of the circulation that described interactions between voltage, pressure and ion concentrations. The table summarizes average values based on the best fit. [A]i represents everything intracellular except [K]i, [Na]i and [Cl]i. This would include protein, amino acids, and organic acids, bases and phosphates. At 14 relative to 2 months, gap junction coupling was reduced by a factor of 2, causing 2-fold reductions in the effective intracellular diffusion coefficient and hydraulic conductivity, and sodium flux was reduced by a factor of 2.6.

	2 mos		14 mos	
Data	Ctr	Surf	Ctr	Surf
V_i mV	-43	-60	-9	-33
p_i mmHg	336	0	450	0
$[K]_i$ mM	69	154	17	50
$[Na]_i$ mM	19	5	35	8
$[Cl]_i$ mM	33	20	72	49
$[A]_i$ mM	202	120	183	187

Conclusions: Age causes dramatic changes to the intracellular milieu. Reductions in Na/K pump activity and gap junction coupling may in part be consequences of cumulative oxidative damage. Other changes may be programmed. Low cation, depolarization and high chloride lead to a nearly uniform [A]i. If [A]i is parsed into proteins that are gap junction impermeant, and other solutes that are gap junction permeant, the protein component is predicted to have a center to surface gradient of 68 to 24 mM at 2 months, and 74 to 25 mM at 14 months. Stable protein and refractive index gradients may be the purpose of an aging program.

Session 5-4

Studying the physiological optics of the lens: new insights into the development of presbyopia

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Purpose: It appears that the cellular physiology of the lens acts to maintain the refractive and transparent properties of the lens. Based on data obtained from mouse, bovine and human lenses a summary of how the physiological optics of the lens is regulated will be presented with the view to present new insights into how age-dependent changes to lens physiology may explain the development of presbyopia.

Methods: Ex vivo mouse and bovine lenses were isolated from the eye by either cutting the zonules or by removing the lens still attached to the ciliary body via the zonules of Zinn were studied by a variety of techniques including: Western blotting to detect protein modification, immunohistochemistry to monitor membrane protein trafficking, Off-angle light microscopy to visualize changes in cell volume, a microelectrode-manometer system to monitor lens pressure, and MRI and laser ray tracing to monitor lens optics. To mimic lens aging bovine lenses were organ cultured in hyperbaric oxygen. Human lenses were imaged in vivo with MRI to monitor changes in water content.

Results: Taken together our data in animal lenses shows that water transport in the lens is dynamically regulated by a dual feedback pathway that works to maintain the optical properties of the lens constant. This pathway is in turn altered by the tension applied to the lens via the zonules of Zinn. Changes to the water content observed in the human lens *in vivo* suggest that a similar system is working in the human lens.

Conclusions: These new insights into the regulation of lens water content provide new avenues of investigation that can be pursued to understand the mechanisms responsible for the onset of presbyopia in middle age.

Session 6-1

Determinants of lens biomechanics

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Purpose: Eye lens function requires biomechanical integrity, flexibility and resilience. We evaluated the contributions of lens size, nucleus and cortex, and cytoskeletal structures to these properties by comparison across different species and between mouse mutant lenses.

Methods: Biomechanical testing was performed on lenses from mice, rats, and guinea pigs. Cell and cytoskeleton structures were evaluated by confocal fluorescence microscopy.

Results: Biomechanical testing and morphometrics revealed that lens stiffness is complex and does not always scale with lens or nuclear size across species. While rat lenses are larger and stiffer than mouse lenses, guinea pig lenses are even larger than rat lenses but are softer than the rat lens, indicating that lens size is not a sole determinant of lens stiffness. We observed that the rat lens nucleus occupies a greater volume fraction of the lens than the mouse lens nucleus. However, the guinea pig lens soft nucleus and the rat lens hard nucleus occupy a similar volume fraction of the lens, suggesting a role for nuclear stiffness in determining whole lens stiffness. Additionally, in mouse lenses with reduced levels of tropomyosin 3.5 (Tpm3.5), the nucleus is larger while whole lenses are softer than wild-type lenses, indicating that, in mouse lenses, nucleus size does not affect overall lens stiffness. Confocal microscopy of cortical fiber cells in wild-type and Tpm3.5-depleted lenses revealed perturbations in F-actin networks, with transformation from stiffer Tpm3.5-fimbrin-F-actin networks towards a predominance of softer a2b2-spectrin-F-actin and a-actinin-F-actin networks.

Conclusions: These data indicate that whole lens and lens nucleus size do not solely determine whole lens stiffness. Comparisons between guinea pig and rat lenses indicate that nuclear stiffness may contribute to whole lens stiffness, while comparisons of mouse mutant and wild-type lenses indicate that lens cortical stiffness and fiber cell actin cytoskeleton composition also contribute to whole lens stiffness.

Session 6-2

Contribution of the lens capsule to whole lens biomechanical properties

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Purpose: The lens capsule is a thin collagen-rich basement membrane that surrounds the lens. Although it has long been suggested that the capsule is essential for lens shape change during accommodation [Fincham, 1937], there is little empirical evidence to demonstrate the relationship between the capsule and gross lens mechanical properties. In this study, we tested the hypothesis that the lens capsule contributes to gross lens mechanical properties.

Methods: Lenses were isolated from 8 to 10-week old C57BL/6 mice. To examine lens capsule function, we either completely removed the capsule by manual decapsulation or briefly digested the capsule matrix using 0.2% collagenase. To determine collagen content in lenses following these manipulations, we performed a hydroxyproline content assay. Next, to measure cell viability following collagenase treatment, we performed whole mount imaging of Calcein AM (live) and TO-PRO3 (dead) stained lenses. To investigate whole lens biomechanics, we compressed lenses by the sequential application of load (glass coverslips) onto the lenses; axial strain on the lenses was calculated following the application of each increment of load. Finally, lens resiliency was assessed by examining the shape and size of the lenses following removal of the terminal (10 coverslips) load.

Results: Decapsulation or capsule matrix digestion decreases collagen content in lenses. Collagenase treatment did not affect cell viability, epithelial cell morphology, or capsule thickness. Manual decapsulation increases axial strain at all loads (1 to 10 coverslips) tested while collagenase digestion of capsules increases axial strain at high loads (9 to 10 coverslips). Lens resiliency was reduced by decapsulation as well as collagenase digestion.

Conclusions: The lens capsule contributes significantly to the biomechanical properties and resiliency of whole mouse lenses.

Session 6-3

Cell rearrangement and shape change cooperatively drive lens cell movement to form a spherical tissue

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Purpose: The formation of the spherical shape of the eye lens largely relies on cellular rearrangement and shape change of the lens fibre cells, the postmitotic cells that constitute the bulk of the lens. Although the fibre cell movement and shape change involves 3D or 4D dynamics, most studies so far have been based on observation of 2D lens sections. To understand how each lens fibre cell is spatially arranged and integrated to form a spherical lens, we examined the spatial and temporal profiles of the fibre apical tips within entire lenses.

Methods: Zebrafish whole lenses were stained with phalloidin to visualise the filamentous actin that demarcates the fibre cell margins. Transgenic zebrafish lines that express EGFP-tagged non-muscle myosin (Myo2) under the control of the □A-crystallin promoter were generated and used for live-image analyses of actomyosin contraction at the fibre apical domain.

Results: In zebrafish, the morphology of fibre apical tips differed at each latitude. At the equator, apical tips had a hexagonal shape, whereas within the mid-latitude region, these hexagonal tips converted into a pentagon and then diamond shape. This shape change is reminiscent of the junctional shrinkage commonly observed during cell intercalation, whereby junctional shrinkage creates a tetrad junction, and then this energetically unstable state quickly resolves by forming new junctions perpendicularly. In lens fibres, however, the tetrad junctions were not resolved but instead persisted temporarily then shifted into an irregular arrangement. The fibre tips continued to migrate, gradually reducing the apical area until they reached the anterior pole. In living lenses, Myo2 accumulated to the anterior hexagonal edges that were undergoing junctional shrinkage.

Conclusions: These observations suggest that junctional shrinkage drives fibre tip migration from the equator to mid-latitude, and then apical constriction drives further migration to the anterior pole.

Session 6-4

The cadherin-associated protein, Arvcf, is an important regulator of lens fiber cell morphology and lens function

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Purpose: The numerous cellular protrusions that emanate along the length of lens fiber cells are thought to support the transparency, structure, and ionic homeostasis of the lens. However, the molecular components that drive their formation and maintain their shape is not well understood. Previous studies have indicated that the cadherin-binding protein, Arvcf, is expressed in high levels in the lens and the absence of this human gene is linked to several ocular defects including cortical cataracts.

Methods: The role for Arvcf in the lens was tested by analyzing the lenses of knock-out mice missing both copies of the gene. Lens function was assessed by analyzing its transparency and ability to focus light using a helium-neon laser. Biomechanical measurements of lenses were also assessed using a compression-based device capable of applying known forces to anterior/posterior axis. Additionally, high-resolution microscopy methods including super-resolution confocal and scanning electron microscopy were utilized to assess changes to protrusion morphology and protein localization.

Results: Arvcf knock-out mice develop age-related, cortical cataracts by 7 months of age and have altered biomechanical, and light focusing properties. Surprisingly, lens fiber organization is disturbed as early as 4 weeks of age and cells exhibit elongated and irregularly shaped interlocking protrusions. Close examination of lens fiber cell protrusions reveals a preferential localization of Arvcf protein within these structures that often correspond with the tips of these structures.

Conclusions: The Arvcf protein is crucial to the structure of lens fiber cell protrusions and lens function. Given its localization pattern and ability to bind cadherins it is likely an important component of adherens junctions that join the membranes of these protrusions to neighboring cells and support the mechanical integrity of the lens.

Session 6-5

Age-related changes in eye lens biomechanics, morphology, refractive index and transparency

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Purpose: Life-long eye lens function requires high refractive index, biomechanical integrity and transparency. We conducted an extensive study of wild-type murine lenses 1-30 months of age to define common age-related changes.

Methods: Mouse lenses between 1-30 months of age were examined using tissue mechanical testing, interferometric measurements of refractive index and various modes of microscopy.

Results: Biomechanical testing and morphometrics revealed an increase in lens volume and stiffness with age. We routinely observed cataracts in lenses from mice older than 12 months. Anterior cataracts were due to incomplete suture closure, while the cortical ring cataract appeared to be due to a zone of compaction in cortical lens fiber cells as revealed by scanning electron microscopy of older lenses. Peripheral fiber cells from young lenses were hexagonal in cross section and well organized while fiber cells in old lenses varied in shape. Lens capsular thickness and peripheral fiber cell widths increased between 2 to 4 months of age with no additional changes with age. Refractive index measurements showed a rapid growth in peak refractive index between 1 to 8 months of age.

Conclusions: These data provide a comprehensive overview of age-related changes in murine lenses, including lens size, stiffness, nuclear fraction, refractive index, transparency, capsule thickness and cell size. Our results suggest similarities between murine and primate lenses and provide a baseline for future lens aging studies.

Session 7-1

Connexin-46/50 intercellular communication channels in a dual lipid bilayer environment resolved at near-atomic resolution by CryoEM

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Purpose: The lens gap junctions, connexin-46/50 (Cx46/50), form cell-to-cell pathways that effectively couple neighboring fiber cells to facilitate the circulation of water, ions, nutrients and waste products within this avascular organ. To develop an atomic level understanding of cell-to-cell coupling in the lens, we have determined the structure of heteromeric Cx46/50 intercellular channels using single particle CryoEM (resolved at 3.4 Å resolution; Myers et al. 2018). To extend these structural studies and investigate the influence of the local lipid environment, we have reconstituted native lens Cx46/50 intercellular channels into a dual lipid membrane system using recently developed nanodisc technology – effectively mimicking native cell-to-cell environment.

Methods: High-resolution single particle imaging by electron cryo-microscopy (CryoEM) was applied to elucidate the structure of heteromeric Cx46/50 gap junction channels isolated from the mammalian eye lens within a near-native lipid environment.

Results: The near-native lipid environment had a remarkable stabilizing effect on Cx46/50 intercellular channels, which enabled our ability to resolve the structure at a resolution of 2.1 Å – providing an unprecedented level of detail for this family of proteins. The structure reveals a bouquet of ordered lipids, stabilized by a conserved binding pocket formed at the connexin subunit interface. In addition, over 150 water molecules are resolved throughout the channel. These ordered waters contribute to the interaction network that defines the intercellular permeation pathway, and are integrated into the core of the trans-membrane domain of the protein.

Conclusions: Lipid and water binding sites identified in Cx46/50 are remarkably conserved across the connexin family, suggesting these stabilizing features are key contributors to the structure and function of gap junction intercellular communication. The functional relevance of lipid-binding sites in Cx46/50 will be discussed in the context of the native environment of the eye lens.

Session 7-2

Galago (bush baby) monkey lenses employ nuclear excisosomes that are unique in origin and structure

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Purpose: The Nuclear Excisosome (NE) was first described in developing fibre cells of chick embryo lenses (Costello MJ et al., *PLoS One* 2016;11:e0160785) as a unique structure derived from projections of adjacent cells that contacted and degraded the nuclear envelope during the formation of the organelle-free zone (OFZ). Entirely different unique structures were found in the lenses of prosimian Galago monkeys.

Methods: Lenses from four monkeys aged 2-5 years were fixed in formalin, followed by paraformaldehyde, then processed for Airyscan confocal microscopy or transmission electron microscopy (TEM). Nuclei were stained with DAPI, actin with Alexa-phalloidin, lectins with WGA and membranes with DiI.

Results: Fiber cells contained linear structures with a distinctive cross-section of four membranes and lengths up to 30 µm. The outer membranes of these linear structures were observed attaching to the outer nuclear envelope membrane to initiate degradation near the OFZ. The origin of these unique structures was found to be mitochondria in the equatorial epithelium. The initial change in mitochondria appeared to be the collapse of the cristae and release of matrix components that self-associated and bound to an outer mitochondrial membrane forming the core. The collapsed cristae assembled around the core and became covered by another outer mitochondrial membrane creating the four-membrane structures. Several mitochondria were involved in the formation and maturation of these unique complexes that apparently migrated around the fulcrum into the cytoplasm of nascent fiber cells where they were stabilized until the nuclear degradation was initiated.

Conclusions: Unlike the chick embryo model, the Galago lenses degraded nuclear envelopes with a NE derived from multiple mitochondria in the epithelium to form prominent linear assemblies in developing fiber cells. It appears that each species may have a unique pathway for the removal of nuclei during OFZ formation.

Session 7-3

Dystrophin (Dp71) is required for mechanical stiffness and membrane organization of the ocular lens

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Purpose: To determine the role of the dystrophin-glycoprotein complex (DGC) in lens cytoarchitecture, mechanics and function using an ENU (N-ethyl-N-nitrosourea)-induced dystrophin-deficient mouse model (mdx3cv).

Methods: Expression and distribution profiles of Dp71 in embryonic and adult mouse (C57BL/6) lenses, and fiber cell morphology and the distribution of lens membrane cytoskeletal proteins from wild-type (WT) and dystrophin-deficient mice (mdx3cv) were determined by immunoblot, immunofluorescence, and confocal imaging. Changes in lens stiffness of mdx3cv and WT mice (maintained on C57BL/6 genetic background) were determined using a microstrain analyzer. To identify the extracellular ligands of the DGC complex in lens fibers, the fiber mass derived ECM-enriched fraction from adult mice was analyzed by mass spectrometry.

Results: Dp71, the predominant dystrophin isoform of the lens was found to be induced robustly during fiber cell differentiation in mouse lenses. In equatorial lens sections, Dp71 is distributed as a large cluster at the center of the long arm with some localization to the vertices of the hexagonal fiber cells, colocalizing with β-dystroglycan and connexin-50 at the large clusters. Mdx3cv mouse lenses deficient in Dp71 (by >90%) showed no obvious changes in growth and transparency for up to 12 months. However, the levels of DGC proteins, NrCAM, connexin-50, and AQP0 were significantly decreased in the mdx3cv mouse lens membrane fraction relative to WT lenses. Additionally, compressive stress/strain analyses of intact lenses (2 and 4 month-old) revealed a significant decrease in Young's modulus in mdx3cv mice compared to WT lenses. Perlecan (HSPG2) was found to be a major ECM protein in the mature lens fibers, a known ligand of α-dystroglycan. Utrophin, the functional homolog of dystrophin, was found to have a compensatory response (increased by ~1.5-fold) in mdx3cv mouse lenses.

Conclusions: Taken together, this study reveals that Dp71 is required for maintenance of biomechanics and membrane organization of the lens.

Session 7-4

The subcellular localisation of AQP5: is it regulated by zonular tension and/or the activation of TRPV1 and 4 ion channels?

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Purpose: The subcellular localisation of AQP5, and therefore the water permeability (P_{H2O}) of lens fibre cells, has been shown to alter in the rodent lens by organ culturing (Petrova *et al*. 2017). Here we investigate whether the subcellular localisation of AQP5 is altered by changes to the tension applied to the lens via the zonules, or through the activation of the mechanosensitive channels TRPV1 and TRPV4 that dynamically regulate lens water transport.

Method: Eyes from Wistar rats were extracted and the lenses were either left in the globe with their zonules attached, or removed from the eye by cutting the zonules. In both conditions lenses were organ cultured in Artificial Aqueous Humor (AAH) either in the absence or presence of tropicamide or pilocarpine to change ciliary muscle contraction and alter the tension applied to the lens by the zonules. Lenses with their zonules attached were incubated in the presence of activators (TRPV1: Capsaicin and TRPV4: GSK1016790A) and inhibitors (TRPV1: A889425 and TRPV4:HC067047). Lenses were fixed and cut in an axial orientation and double labelled with the membrane marker WGA and an AQP5 antibody and imaged using a confocal microscope.

Result: In lenses with intact zonules the subcellular localisation of AQP5 in peripheral fibre cells was membranous at both the equatorial plane and at the lens sutures. However, cutting the zonules or reducing zonular tension using pilocarpine resulted in a shift in AQP5 labelling from the membrane to the cytoplasm. Pharmacological modulation of TRPV1 or TRPV4 activity did not show any substantial change in the membrane location of AQP5 in lenses with intact zonules.

Conclusion: Our finding that the subcellular location of AQP5 can be dynamically regulated in the lens periphery suggest that changes in zonular tension can alter the P_{H2O} of fibre cells to modulate water influx and efflux at the poles and equator of the lens respectively.

Session 7-4

Development of refractive index in the embryonic chick lens

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Purpose: To investigate the development of the refractive index gradient in the embryonic chicken lens.

Methods: Measurements were conducted on twenty-nine embryonic chick eyes that had been incubated for 10,12,14,16 and 18 days. Six samples were measured for each age apart from 16 days for which five samples were measured. Eyes were fixed and transported from Cardiff University to the SPring-8 facility in Japan. Samples were set in a physiologically balanced media and refractive index measurement made using the X-ray Talbot interferometer on beamline BL20B2 using X-rays of 25 keV. Measurements took 50 minutes per scan; samples were measured in pairs.

Results: A gradient of refractive index was evident from the earliest age and was steep with slight fluctuations along the optic axis but shows distinct troughs between the nuclear and cortical regions in the equatorial plane. This general trend is maintained with development with a widening of the profile. The refractive index contours appear only in the central lens and change rapidly from prolate to increasingly more circular over 10-18 days. The maximum refractive index increases linearly with age from 1.39 (10 days) to 1.43 (18 days).

Conclusions: The gradient refractive index evident in the embryonic chick lens has a distinct central region and the contours within this region change rapidly over a few days incubation. The oblate form in the very youngest samples has not been seen in mammalian lenses and may be more common in their avian counterparts.

Session 8-1

AQP1 upregulation in lens epithelium is associated with cataractogenesis in AQP0-deficient lens fibers in mice

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Purpose: The lens water transport is conducted by AQP1 for the epithelium and AQP0 for fiber cells. Here, we report that the epithelial AQP1 responds directly to the deficiency of AQP0 in underlying fiber cells.

Methods: Lenses from WT and AQP0-/- and AQP0+/- (P3-24wks old) were used for immunolabeling of AQP1 on frozen sections and epithelial wholemounts. Lens morphology was studied.

Results: Two expression patterns of AQP1 were observed in the lens epithelium in WT. At P3-9, AQP1 was labeled only in the central epithelium. After P9, AQP1 labeling was gradually increased and intensified in the equatorial zone (250μm long) with age, but a small area of the labeling was retained in the central epithelium. In sharp contrast, in the AQP0-/- lenses at P3-9, the area and intensity of AQP1 labeling was significantly increased in the central epithelium, while AQP1 was not regularly upregulated in the equatorial zone. After P9, AQP1 was labeled in two discrete central and equatorial zones. The confluent labeling of AQP1 in the entire epithelium was seen at 6 weeks of age. Also, AQP1 was upregulated in the AQP0+/- lenses. Steady AQP1 labeling was observed in the entire epithelium with age, suggesting that upregulation of AQP1 in the epithelium can be induced by a partial deficiency of AQP0 in fibers. Morphological analyses revealed that the swollen anterior sutures were regularly associated with cataractous fibers in AQP0-deficient lenses.

Conclusions: AQP1 is expressed in two distinct epithelial regions during lens development and growth. The unique expression suggests that the equatorial region is the major active route for inflow and outflow of water between the epithelium and fiber cells. In AQP0-deficient lenses, AQP1 was significantly upregulated in the entire epithelium. The large increase in water influx from the epithelium into underlying fibers would cause further damage to fiber cells.

Session 8-2

Connexin mutants impair the lens circulation leading to calcium accumulation/precipitation and cataracts

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Purpose: Mutants of the lens fiber cell gap junction proteins, Cx46 and Cx50, are linked to congenital cataracts. We have been studying mice with connexin mutations (Cx46fs380 and Cx50D47A) that mimic these human defects to elucidate the mechanism by which they lead to cataracts.

Methods: Connexin levels were determined by immunoblotting. Gap junctional coupling conductance was calculated from intracellular impedance studies of intact lenses. Intracellular free calcium ion concentrations ($[Ca^{2+}]_i$) were measured using Fura-2 and fluorescence imaging. Lenses were examined by dark-field microscopy, Alizarin red staining and micro-CT scanning.

Results: Levels of both Cx46 and Cx50 were significantly decreased in heterozygous and homozygous Cx46fs380 and Cx50D47A lenses. In differentiating and mature fibers, gap junctional coupling was markedly reduced in heterozygotes and homozygotes. The gradient of intracellular calcium ion concentrations was significantly increased in Cx46fs380 and Cx50D47A lenses, reaching concentrations of >2000 nM at the center of both homozygous lenses. This suggested that the accumulated Ca^{2+} might form precipitates. Alizarin red, a dye used to detect insoluble Ca^{2+}, stained Cx46fs380 and Cx50D47A lenses. Micro-CT scanning demonstrated the presence of X-ray dense material consistent with mineralization. The distributions of Alizarin red staining and micro-CT-detected mineralization were similar to the cataracts.

Conclusions: These results demonstrate that expression of Cx46fs380 or Cx50D47A disrupts the lens internal circulation leading to accumulation of Ca^{2+} to levels so high that it forms precipitates. The similar distributions of calcium precipitates and opacities suggest that the crystals correspond to the cataracts. We hypothesize that disruption of the lens circulation, Ca^{2+} accumulation and crystallization is a general mechanism of cataract formation.

Session 8-3

The roles of Cx46 and periaxin in fiber cell morphogenesis and cataratogenesis

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Purpose: Genetic variances between C57BL/6J and 129SvJae mouse strains modulate the severity of the nuclear cataracts in Gja3 (Cx46) gene knockout (Cx46KO) mice. The goal is to investigate the genetic interactions between Cx46 gap junctions and genetic modifiers and to elucidate the molecular and cellular mechanisms that regulate lens fiber cell morphogenesis, lens homeostasis and cataratogenesis.

Methods: Combined genetic mapping and phenotypic examinations were performed to identify genetic modifiers that modulate the cataractogenesis in Cx46KO mice. Lens vibratome sections were prepared for determine lens fiber cell morphogenesis using a laser confocal imaging analysis of various fluorescent labeling of lens membrane and cytoskeletal components. Lens proteins were characterized by different biochemical methods. Lens metabolites were analyzed by Mass-spec analysis.

Results: At least three genetic modifiers were mapped and two of them were identified as periaxin and CP49 genes. The third candidate was mapped to mouse Chr2. Confocal imaging data reveal that periaxin and CP49 regulate lens fiber cell shape or surface protrusions in Cx46KO lenses at the different backgrounds. 129-periaxin was colocalized with F-actin at abnormal elongated protrusions of cortical fibers. The levels of the disruption of F-actin and intermediate filaments were correlated with the severities of Cx46KO nuclear cataracts. Metabolite profiling reveals decreased antioxidants including glutathione (GSH) and cysteine, altered intermediates of glycolysis pathway in Cx46KO lenses. Glucose uptake and/or utilization seem not dependent on Cx46 gap junctions but obviously affected by mouse strain backgrounds.

Conclusions: Cx46 gap junctions, CP49 and periaxin synergistically regulate fiber cell morphogenesis to modulate nuclear cataract formation in Cx46KO lenses. Reduced metabolites such as GSH and cysteine and other energy metabolites and elevated calcium level cause increased degradation and aggregation of crystallins in inner mature fibers to facilitate nuclear cataract formation.[Supported by EY013849]

Session 8-4

Studies on TRPV1, NKCC1 and hydrostatic pressure responses in mouse lens

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Purpose: In porcine lenses the response to hyperosmotic shrinkage involves stimulation of an ion cotransporter NKCC1. Pharmacologic studies pointed to a mechanism that depends on activation of TRPV1 ion channels. Here we used a knockout strategy to examine and compare responses in lenses from wild type (WT) mice and mice that do not express TRPV1.

Methods: Lenses were obtained from TRPV1$^{-/-}$ and WT mice. Studies also were carried out in epithelial cells cultured from WT and TRPV1$^{-/-}$ lenses. NKCC1 phosphorylation was studied by Western blot. Rubidium (Rb^+) uptake was measured by atomic absorption spectrophotometry as an indicator of K^+ transport. Hydrostatic pressure (HP) in lens surface cells was determined in intact lenses using a manometer-coupled microelectrode approach.

Results: The TRPV1 agonist capsaicin (100 nM) caused a transient HP increase in WT lenses that peaked after ~30 min then returned toward baseline. Capsaicin did not cause a detectable change of HP in TRPV1$^{-/-}$ lenses. The NKCC inhibitor bumetanide prevented HP response to capsaicin in WT lenses. Capsaicin was shown to increase Rb^+ uptake and elicit rapid (<2 min) phosphorylation of NKCC1 in cultured WT lens epithelial cells but not TRPV1$^{-/-}$ cells. Bumetanide and the TRPV1 inhibitor A889425 prevented the Rb^+ uptake and NKCC1 phosphorylation responses to capsaicin in WT cells. Bumetanide also prevented HP recovery in WT lenses exposed to hyperosmotic solution. HP recovery was absent in TRPV1$^{-/-}$ lenses exposed to hyperosmotic solution.

Conclusions: A variety of responses to capsaicin and hyperosmotic solution point to a functional role for TRPV1 channels in mouse lens. Lack of NKCC1 phosphorylation and Rb^+ uptake responses in TRPV1$^{-/-}$ mouse epithelium reinforces the notion that a hyperosmotic challenge causes TRPV1-dependent NKCC1 activation. The results are consistent with a role for the TRPV1-NKCC1 pathway in lens osmotic homeostasis.

Session 8-5

The ciliary muscle and zonules of zinn modulate lens intracellular hydrostatic pressure through transient receptor potential vanilloid channels

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Purpose: Lenses have an intracellular hydrostatic pressure gradient to drive fluid from central fiber cells to surface epithelial cells that is regulated by a feedback control system that relies on TRPV1 and TRPV4 channels. The ciliary muscle transmits tension to the lens through the zonules of Zinn. Here, we have examined if ciliary tension influenced the lens intracellular hydrostatic pressure gradient.

Methods: We measured the ciliary body position and intracellular hydrostatic pressures in mouse lenses while pharmacologically causing relaxation, or contraction of the ciliary muscle. We also used inhibitors of TRPV1 and TRPV4, in addition to phosphoinositide 3-kinase (PI3K) p110α knockout mice and immunostaining of phosphorylated-Akt to determine how changes in zonular tension resulted in altered hydrostatic pressure.

Results: Ciliary muscle relaxation increased the distance between the ciliary body and the lens and caused a decrease in intracellular hydrostatic pressure that was dependent on intact zonules and could be blocked by inhibition of TRPV4. Ciliary contraction moved the ciliary body toward the lens and caused an increase in intracellular hydrostatic pressure and Akt phosphorylation that required intact zonules and was blocked by either inhibition of TRPV1, or genetic deletion of the p110α catalytic subunit of PI3K.

Conclusions: These results show that the feedback control system for hydrostatic pressure in lens surface cells was influenced by the tension exerted on the lens by the ciliary muscle through the zonules of Zinn. Modulation of the gradient of intracellular hydrostatic pressure in the lens could alter the water content, and the gradient of refractive index.

Session 8-6

Pharmacological regulation of the lens water transport and its effects on physiological optics of the bovine lens

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Purpose: The optical properties of the bovine lens have been shown to be actively maintained by circulating ionic and fluid fluxes that generate an internal microcirculation. In the mouse lens, this water transport through gap junction channels establishes a substantial intracellular hydrostatic pressure gradient, which is subjected to a dual feedback regulation that is mediated by the reciprocal modulation of the transient receptor potential vanilloid channels, TRPV1 and TRPV4. Here we determine whether a similar feedback regulation of pressure occurs in the bovine lens and whether pharmacological modulation of the pressure gradient can alter overall lens optics.

Methods: Hydrostatic pressure in bovine lenses was measured in the absence and presence of TRPV1 and TPRV4 activators and inhibitors using a microelectrode/pico-injector based pressure measurement system. Lens optics were measured in bovine lenses organ cultured in the absence or presence of those TRPV1 and TPRV4 activators and inhibitors using a laser ray tracing system.

Results: Bovine lenses have an intracellular hydrostatic pressure gradient that varied from 0 mmHg in the surface to 340 mmHg in the core. Activation of TRPV1 with Capsaicin caused a transient increase in surface pressure, while activation of TRPV4 with GSK1016790A caused a transient decrease in pressure. The transient alterations in pressure obtained by TRPV1 and TRPV4 activators were sustained if TRPV1 activation was performed in the presence of TRPV4 inhibitor HC-067047, or TRPV4 activation was done in the presence of the TRPV1 inhibitor A-889425. TRPV1 activation induced a transient change in lens power over a two-hour period of time, and small but significant changes in lens geometry, refractive index and spherical aberration.

Conclusion: These results confirm that the bovine lens also operates a TRPV1/4 mediated feedback control system to regulate lens hydrostatic pressure and that modulation of lens pressure alters the optical properties of the bovine lens.

Session 9-1

What the lens can tell us about aging and human age-related diseases

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Purpose: Presbyopia and cataract are due to age-related modification of long-lived human lens proteins. We still lack a complete understanding of the molecular bases for all these modifications. Recently it has become apparent that there are many long-lived proteins throughout the human body. Do all of these long-lived proteins decompose in similar ways and what are the consequences are of this deterioration?

Methods: Human lenses of varying ages were examined by proteomic methods. *In vitro* chemical reactions with peptides were employed to determine mechanisms of decomposition. Proteins from other tissues, such as the human brain, were also studied.

Results: Over time, proteins decompose. Racemisation, deamidation, truncation and crosslinking are the major processes and these are spontaneous i.e. no enzymes are required. Although not all of the events have been elucidated, it is clear that the consequences of protein decomposition for the properties of the lens are large.

Recent research has revealed that the lens is not unique; many organs and tissues in the human body contain long-lived cells and long-lived proteins. Over time it is probable that they, like lens proteins, also decompose. Almost nothing is known of the consequences for human health and disease.

For example, neurons are post-mitotic cells and show no turnover during our lives. The causes of major neurological diseases of man such as Alzheimers disease (AD), Parkinsons disease (PD), multiple sclerosis (MS) and motor neurone disease (MND also known as ALS) remain unknown. It is possible that age-related deterioration of the long-lived proteins in, or around, nerve cells contributes to, or is responsible for, these diseases. Two examples will be used to illustrate this: AD and MS. Researchers have been unaware of the existence of long-lived proteins and this factor may have contributed to the lack of progress in understanding these major health issues. Once it becomes widely known that long-lived proteins are ubiquitous and that they decompose, new avenues of research will become apparent.

Conclusions: It will be ironic, and remarkable, if a tissue with no nerve supply ends up providing fundamental molecular data that enables an understanding of the major human neurological diseases of old age

Session 9-2

Ageing, cataractogenic load and repair in the lens – an old dog still learning old tricks

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How proteins, lipid membranes and a few nucleotides conspire to fashion an eye lens capable of refracting light onto the retina is quite remarkable, but even more so how the lens retains function throughout its life. The cellular components must therefore be functional over decades, even centuries in the case of whales and sharks. Modern carbon dating suggest that protein turnover is minimal and yet the datasets are not without biological intrigue. For instance we are rather coy as a field to explain the apparent exchange of carbon between the nuclear and cortical lens regions. When it comes to explaining the ageing of cells and their component biomolecules there are also conceptual chasms and yet to me there is one fundamentally important biological detail – genetics/inheritance contributes far less than environment and circumstance. Of course accumulated oxidative damage is part and parcel of the ageing process and for the lens this means oxidation of both proteins and lipids as well as DNA damage. My attention is primarily in the use of ionizing radiation to effect oxidative damage in a precise and controlled fashion. The societal importance of understanding the lenticular response to ionizing radiation exposures is because medical imaging, air and space travel are all associated with increased cataract incidence. So we need to define "how safe is safe?". We have studied oxysterol formation in lens membranes after ionising radiation (X-ray) exposure and find that there is indeed a dose dependent increase in cholesterol based oxysterols. Cholesterol and dihydrosphingomyelin are both key to producing the very low oxygen tension in the lens nucleus and being resistant to oxidation. The questions we are addressing are not unique to ionising radiation induced cataract, rather they drive at the very heart of our understanding of lens biology – yes those same old tricks we are all still trying to learn as old or young dogs and what drives our Kona pilgrimage. Why do individuals develop cataract at different ages? Why are some people more or less susceptible to ionizing radiation induced cataractogenesis? I have rediscovered the concept of cataractogenic load, first proposed by Basil Worgul, and adapted this to explain how X-rays accelerate ageing for an individual. Our studies have also led us to rediscover another concept – that the lens lipids are protective. This concept I will re-adapt to explore the repair capacity of the lens. Our data help inform the debate on the proposed oxysterol reversal of cataract and the alpha-lipoic acid attenuation of presbyopia. The concept I prefer not to adddress is why it took this old dog so long to learn about these old tricks……..

Session 9-3

The molecular impact of aging in the lens and beyond

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Purpose: Lens protein modifications accumulate with age and play key roles in lens pathology. The objectives of this work are to identify novel age-related modifications to lens proteins, to determine their spatial localization, to elucidate the chemical mechanisms responsible for their formation, and to determine their presence in other non-lenticular long-lived proteins.

Methods: Human lenses of varying age were examined by spatially-resolved and quantitative proteomics methods, using microdissection/LC-MS/MS analysis and imaging mass spectrometry. *In vitro* chemical reactions with standard peptides were employed to determine mechanisms. Extracted protein from human brain tissue was also examined.

Results: In addition to previously reported truncation, deamidation, and thioether modifications to proteins by glutathione (GSH), recent proteomics analyses revealed the age-related formation of dehydroalanine (DHA) and four types of protein-protein crosslinks. Analysis of DHA containing peptides as a function of age and solubility suggests that, for some lens proteins, DHA decreases their solubility in older lenses. Major crosslinking mechanisms include: 1) thioether crosslinks through DHA intermediates, 2) crosslinks between lysine and asparagine/aspartic acid residues via an internal succinimide intermediate, 3) crosslinks between lysine and C-terminal aspartic acid (Asp) residues formed by protein truncation via an Asp anhydride, and 4) crosslinks between amine groups and C-terminal asparagine residues via a terminal anhydride. Imaging mass spectrometry revealed zones of protein expression and modification that correspond to zones of cytoskeleton remodeling and barrier formation. Analysis of human brain protein indicated the presence of thioether-linked GSH.

Conclusions: Lens protein modifications such as truncation, deamidation, and irreversible GSH adducts, accumulate with fiber cell and lens age. New evidence suggests multiple mechanisms are responsible for protein-protein crosslink formation. These age-related modifications are predicted to alter protein function in the lens and in other tissues with long-lived cells/proteins.

Session 9-4

Crystallin proteins: post-translational modifications and amyloid fibrils

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Purpose: To determine the effects of a crowded environment on the structure of aB-crystallin and oxidation on the structure of gS-crystallin. The rationale is that these conditions and modifications mimic alterations that occur in the ageing, including cataractous, eye lens.

Methods: X-ray crystallography, circular dichroism and fluorescence spectroscopy, dynamic light scattering, X-ray and neutron scattering and transmission electron microscopy were used to monitor the structure of aB-crystallin in the presence and absence of an inert crowding agent and upon dimer formation of gS-crystallin via disulphide bond cross-linking. The effects of crowding on aB-crystallin chaperone activity were also assessed.

Results: High concentrations of crowding agent lead to significant alterations in all levels of aB-crystallin structure, including unfolding and larger-scale aggregation to form amorphous or fibrillar-type species. Dimeric gS-crystallin is less stable to stresses such as elevated temperature.

Conclusions: The results provide insight into the great malleability and adaptability of crystallins to age-related changes. In a relative sense, despite major alterations in crystallin structure, their solubility and chaperone function are maintained. Thus, lens protein proteostasis operates in a very different manner to that of normal, metabolically active cells in order to maintain lens transparency.

Session 9-5

Age-related opacity changes in lenses of healthy centenarian humans and old wild-type mice

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Purpose: Crystallins comprise ~90% of lens protein in mature lens fiber cells and undergo various post-translational modifications during aging that disrupt the normal functioning of the proteins, facilitating aggregation, insolubilization and light scattering. We investigated the effect of age on light scattering and lens opacities in very old (over 100 years old) healthy humans and wild-type mice (over 27 months old).

Methods: Human post-mortem lenses were collected from 2 patients over 100 years old as part of the New England Centenarian Study: 1 male aged 100, 1 female aged 108. Wild-type mice were bred and maintained at Boston University. Aged mice were sacrificed and perfused with phosphate buffer saline and the lenses were isolated. Human and mouse lenses were imaged using a D70 digital Nikon camera and a custom-adapted Zeiss stereophotomicroscope.

Results: Human post-mortem lenses collected from two patients over 100 years old showed age-related brunescence ('yellowing') as expected with age. A cortical cataract was observed in each case, however no nuclear cataracts were observed. Aged mice also demonstrated light scattering increases in the cortical regions of the lens but not the nucleus at 27 months+ compared to younger mice.

Conclusions: Aging affects light scattering in humans and mice lenses. In centenarian humans who have lived a healthy lifespan, lenses may still be present and not needed to have been surgically removed. Opacities were observed in the cortical region but not in the nucleus of the post-mortem lenses in both centenarian humans and aged wild-type mice.

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Session 10-1

Increased aggregation and light scattering of human γS-crystallin due to cataract-associated deamidations

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Purpose: Deamidation is a major age-related modification in the human lens that is highly prevalent in crystallins isolated from the insoluble fraction of cataractous lenses. The purpose of these studies was to identify structural changes due to deamidation associated with this insolubilization.

Methods: Donor eyes were procured with approval from our Institutional Review Board. The nuclear region of lenses was isolated, homogenized and the water-soluble and insoluble fractions separated. Proteins were digested with trypsin and analyzed using an Orbitrap Fusion mass spectrometer (Thermo Scientific). Recombinant proteins were expressed with Asp replacing Asn in order to mimic *in vivo* deamidation sites at Asn 14, 76, and 143. Aggregation was determined using dynamic and static light scattering. NMR spectroscopy was used to measure amide hydrogen exchange on a Bruker 800 MHz spectrometer.

Results: The deamidation mimics, N14D, N76D, and N143D had increased light scattering compared to wildtype-γS and had increased aggregation during heating that □A-crystallin did not rescue. Aggregation was enhanced by oxidized glutathione. These changes were correlated to changes in protein dynamics. The hydrogen/deuterium exchange with NMR showed significant increases in solvent accessibility at the surface loops in both the N- and C-terminal domains, particularly for N76D.

Conclusion: The NMR findings combined with the *in vivo* insolubility and *in vitro* aggregation findings support a model that deamidation drives changes in protein dynamics, particularly at surface loops, that facilitate protein aggregation associated with cataracts. The likelihood of deamidation-induced aggregation would be enhanced in the oxidizing cytoplasm of the aged and cataractous lens.

Session 10-2

a-crystallins, oxidative stress, and zebrafish lens development: recent confounding observations

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Session 10-3

Protein condensation and cataract: an overview

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Purpose: Cataract is generally viewed as a disease in which lens opacity occurs primarily due to the unfolding and aggregation of the lens crystallins. Here we present an overview of our studies that show that cataract is also a member of a group of diseases designated as "Molecular Condensation Diseases", in which normally soluble proteins undergo a phase transition to form dense, usually insoluble phases that lead to light scattering and opacity, without significant change in protein structure and stability.

Methods: Condensed phases were characterized by measuring thermodynamic phase diagrams. Protein structure and stability were measured by using CD, Fluorescence, IR, Raman, and NMR spectroscopies, X-ray crystallography, and Differential Scanning Calorimetry.

Results: The majority of protein-derived condensed phases in cataract are formed as a result of genetic mutations or post-translational modifications, predominantly in the gamma crystallins. Using *in vitro* model systems, we have identified distinct molecular mechanisms underlying protein condensation that highlight the formation of light scattering elements such as, dense liquid droplets, amorphous aggregates, crystals, clusters, and rod-like fibers. In many instances, the formation of pathological condensed phases occurs essentially with the retention of native protein structure and stability, and without significant conformational change even in the insoluble phase. We discuss our results in light of other pathologies in which native structure is retained.

Conclusions: Our results show that there are important distinctions between protein condensation diseases, and protein conformational diseases in which native structure is not retained. These distinctions are likely to be important in guiding the development of specific therapeutic intervention strategies that are potentially relevant to either case.

Session 10-4

The crystal structure of the disulfide-linked γS-crystallin dimer provides insight into an aggregation-prone oxidation product associated with cataractous lenses

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Purpose: Oxidation of lens crystallin proteins is the major contributor to their destabilization and deleterious aggregation that scatters visible light, obscures vision, and ultimately leads to cataract. However, the molecular pathways for oxidation-induced aggregation is largely unknown. We have characterized an oxidation product (a disulfide-linked dimer) of human γS-crystallin and provided a molecular basis for its role in age-related cataract.

Methods: Human γS-crystallin was dimerized under ambient, oxidative conditions, and separated from the monomer via size-exclusion chromatography (SEC). The abundance of γS-crystallin dimer with varying levels of reduced glutathione was quantified by SEC and mass spectrometry. The structure of γS-crystallin dimer was determined by X-ray crystallography and verified by small-angle X-ray scattering (SAXS). Thermal stability was measured by intrinsic fluorescence, circular dichroism, ANS binding and solution turbidity. Thermally induced aggregation was monitored via solution turbidity and further characterized by dynamic light scattering and transmission electron microscopy.

Results: The crystal structure showed the γS-crystallin dimer adopts an extended V-shaped structure linked via an intermolecular disulfide bond at cysteine 24. Also evident was an intramolecular disulfide between cysteine 22 and 26. SAXS confirmed that the extended configuration was the in-solution, biological assembly. No gross conformational changes were observed upon oxidation relative to the monomer. The dimer was found to be stable in a reducing environment commensurate with that found in aged and cataractous lenses. Disulfide-linked dimerization of γS-crystallin significantly increases the protein's propensity to form large insoluble aggregates owing to non-cooperative domain unfolding, as is observed in crystallin variants associated with early-onset cataract.

Conclusions: These findings strongly suggest that this oxidative modification occurs in the glutathione-depleted, aging lens and, moreover, it contributes to cataract. The mechanism outlined here may apply more broadly to other crystallin proteins that undergo oxidative modification and thereby participate in the oxidation-driven aggregation cascade that underlies age-related cataract.

Session 10-5

Catalysis of aggregation by interface opening and disulfide exchange in cataract-associated variants of human γD crystallin

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Purpose: Destabilizing mutations and post-translational modifications in γ-crystallins are linked to onset of light-scattering aggregation that causes cataract disease. The highly stable wild-type human γD-crystallin HγD greatly accelerates aggregation of its cataract-related W42Q variant, without itself aggregating. Understanding the mechanisms behind this unusual phenomenon will reveal new biochemistry contributing to cataract formation and resistance.

Methods: We combined *in vitro* biophysical experiments with computational modeling, mathematical analysis, and mass spectrometry. Native, untagged HγD and its single- and multisite variants were expressed recombinantly. Oxidative aggregation was monitored by solution turbidity at physiologically relevant temperature, pH, and concentration. Mass spectrometry and mutagenesis mapped the disulfide bonds. Stability was probed by differential scanning calorimetry, as well as by a PEGylation gel-shift assay reporting on the solvent accessibility of buried Cys residues in the N-terminal domain. An analytical kinetic model of the aggregation process was developed and parametrized by fitting to experimentally observed turbidity curves.

Results: Transfer of disulfide bonds from an oxidoreductase site on the WT protein's C-terminal domain to form a non-native disulfide bond in the mutant N-terminal domain locked in an aggregation prone conformational intermediate, leading to non-amyloid aggregate assembly. A distinct, transient and non-covalent interaction between the full-length WT or its isolated C-terminal domain promoted misfolding of cataract-associated variants by opening up the native domain interface that otherwise resists aggregation of variants with a destabilized N-terminal domain. A detailed kinetic model predicted universal power-law scaling relationships for lag time and rate of the resulting aggregation, which were in excellent agreement with the data.

Conclusions: Oxidation/reduction chemistry and conformational opening of the native domain interface together explain the unusual phenomenon of WT-catalyzed aggregation of cataract-associated HγD variants. These mechanisms expand the space of possibilities for therapeutic intervention in cataract progression.

Session 11-1

Prevalence of and visual impairment by waterclefts and retrodots

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Purpose: To examine prevalence of and visual impairment by waterclefts (WC) and retrodots (RD) frequently observed in aging lenses.

Methods: Right eyes (N=3,588, 61.1±10.9 yrs) in ocular epidemiological studies of general residents in 6 regions (Sanya, Taiyuan, Taichung, Reykjavik, Mkurange, and Wajima) with different climate conditions and races were enrolled. Under mydriasis, WC were classified as WC-1 (opacities outside ⌀ 3-mm zone of the pupil center), WC-2 (within the ⌀ 3-mm zone), and WC-3 (both), and RD into four grades (RD-1 to -4) by area of opacity within this zone. Among Monzen Eye Study participants and Kanazawa Medical University Hospital patients, visual function was evaluated in 70 eyes with only WC (68.1±6.9 yrs) and 54 eyes with only RD (70.0±8.2 yrs) and compared with 77 transparent eyes (66.7±5.0 yrs). Corrected-distance visual function (CDVA), straylight as forward light scattering, higher-order aberrations (HOA), and backward light scattering (BLS) were measured.

Results: Prevalence rates of WC and RC in subjects aged 50+ years were: Sanya 5.0%, 38.4%; Taiyuan 3.0%, 22.4%; Taichung 18.3%, 22.9%; Reykjavik 22.1%, 23.5%; Mkuranga 10.0%, 42.3%; Wajima 36.8%, 35.0%, respectively. Adjusted for age, prevalence of WC was significantly lower in Reykjavik, Taiyuan, Sanya, and Mkuranga compared to Wajima. RD was lower in Reykjavik and higher in Sanya and Mkuranga compared to Wajima. WC showed higher risk with age, female, short-axis or hyperopia, similarly RD with age, and myopia. CDVA decreased and straylight increased with WC-2, WC-3, and RD-3+. HOA increased with WC-3. BLS did not differ among groups.

Conclusion: WC and RD have high prevalence in mid-elderly-age people impairing vision, so their diagnosis is as important as the three main types of cataract to evaluate the efficacy of anti-cataract drugs. The main causes of decreased visual function were straylight and HOA with WC, and straylight with RD.

Session 11-2

Loss of transparency and opacification of the lens

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Purpose: Multiple biomedical and biophysical factors involved in lens transparency represent potential mechanisms for treatment of opacification

Method: Transparency is the cellular characteristic that distinguishes the biological lens from other mammalian tissues. Development of transparent lens cells is the result of unique specializations involving genetics, metabolism, physiology and cell structure in response to the physical properties of light.

In humans, loss of lens transparency is a normal function of aging that accounts for approximately 50% of global blindness. Similar to other aging processes, loss of transparency is slow, progressive and multifactorial. A strategy for a solution to human cataract needs to consider collectively the fundamental parameters and kinetics of the modifications of lens cells with age.

This session seeks to be an interactive discussion of approaches to understand molecular mechanisms that govern loss of transparency and provide innovative treatments to prevent or treat loss of transparency in humans.

Conclusion: Loss of lens transparency is progressive and multifactorial involving complex interactions between and within cells that can lead to innovation in identification of a novel intervention to address cataract, the leading cause of blindness in the world.

Session 11-3

The process of developing an FDA approved anti-cataract drug

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Purpose: Describe the steps required for FDA approval of a drug for cataracts.

Methods: The procedures for FDA drug approval for humans and animals are very similar. Required steps for obtaining the initial Investigative New Drug Application (INDA) or Investigative New Animal Drug Application (INADA), concurrence on the Efficacy and Safety Protocols, as well as concurrence on the procedures for developing the Chemistry Manufacture and Controls (CMC) section were followed. These protocol procedures complied with Good Manufacturing Practice (GMP), Good Laboratory Practice (GLP) and Good Clinical Practice (GCP) principles. The study required specific documentation for each study participant which included dosing diaries, specific training records for each trial site personnel, and specific documentation and disclosure records for the trial site principal investigators and subinvestigators. Procedures for the dispensing and inventory of all study materials were also complied with. Specific data management procedures for each study site were utilized along with defined procedures for the statistician. This included the duplicate entry of all study data into a central data base with defined security and backup.

Results: For the final approval of Kinostat®, the drug sponsor Therapeutic Vision, Inc. must submit a New Animal Drug Application (NADA) along with supporting data, including all adverse effects associated with the drug's use. The NADA includes the results of the Safety and Efficacy studies which had been separately approved by the FDA during the submission process. The NADA also includes the CMC which provides information on the drug's chemistry, composition and component ingredients, manufacturing methods, facilities, and controls, proposed labeling, analytical methods for residue detection and analysis if applicable, an environmental assessment, and other information. The sponsor of the new drug is responsible not only for submitting all of this data to the FDA, but for also paying annual user fees, submission fees and manufacturing fees.

Conclusion: As illustrated by Kinostat®, the process of obtaining FDA approval for an anti-cataract drug takes years and costs millions of dollars to complete. Marketing the drug must take these factors into consideration for determining the sales price of the drug.

Session 11-4

Approaches to controlling chemical protein aging in the loss of transparency in lens

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Purpose: Define the role of crystallin modifications underlying the loss of transparency in the lens.

Methods: The most common risk factor for human cataract is age. The strategy for a solution to the cataract problem can be based on protection against the fundamental biochemistry and kinetics of the modification of lens proteins with age. Similarities between lens cell opacification and age-related diseases involving extracellular matrix, joints, lungs and the cardiovascular system suggest the molecular processes that can govern loss of transparency.

Results: In the field of glycation, skin collagen levels of advanced glycation end products predict the very long-term risk of diabetic retinopathy, neuropathy and nephropathy in type 1 diabetes, despite adjustment for mean glycemia. This indicates a direct relationship between the protein modification and tissue damage. In contrast, while glucose is the major precursor molecule in the extracellular matrix, ascorbic acid in the lens appears to be an important factor in destabilization of beta and gamma crystallins upon oxidation, leading to protein insolubilization, aggregate formation and opacification. These two paradigms suggest strategies for use of basic research results in diagnosis, prediction and mitigation of age-related tissue damage in loss of lens transparency.

The results achieved over many years of experimental research now can be considered with respect to commercialization for human application. Successful development of therapeutics requires scientific thinking outside the box and pragmatism. The speaker will summarize his experience working with biotech start-ups and established companies in the pursuit of druggable targets applicable to the prevention of protein aging and its biological consequences.

Conclusion: Control of loss of transparency is a complicated biomedical problem requiring multiple and innovative approaches that are in reach of lens investigators. The druggable target might not be far, if we, lens researchers, are willing to think translating our findings to the clinic.

Session 12-1

Redox imbalance results in the early onset of age related cataracts in cystine/glutamate antiporter knockout mice

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Purpose: The cystine/glutamate antiporter (CGAP) is responsible for maintaining extracellular cystine/cysteine redox balance and controlling glutathione (GSH) homeostasis in other tissues. Since GSH is the principal antioxidant in the lens and redox imbalance and increased oxidative stress lead to cataract formation, we used a global CGAP KO mouse to determine the effects of loss of CGAP on redox balance, GSH homeostasis and lens transparency.

Methods: Lenses were examined *in vivo* using slit-lamp microscopy followed by cryosectioning and labelling to visualize lens morphology. Plasma and aqueous humour cystine/cysteine and lens GSH/GSSG levels in WT and KO mice of ages ranging from 6 weeks to 12 months, were measured using mass spectrometry. Oxidative stress in the lens were measured using markers of lipid peroxidation and protein carbonyl content.

Results: Slit lamp microscopy revealed an anterior localised cataract at 3 months that were more frequent in KO lenses relative to WT, but which normalised by 12 months of age. Morphological analysis revealed no smooth muscle actin labelling or plaque formation suggestive of an atypical anterior subscapular cataract. Plasma and aqueous cystine levels were significantly greater in the KO for all age groups indicative of an oxidative shift of the cystine/cysteine redox balance. GSH levels were maintained in the lens at all groups, except at 9 months, where GSH was decreased in the epithelium in the KO relative to WT which coincided with significantly increased lipid peroxidation markers levels in KO lenses.

Conclusions: While an age-dependent anterior cataract develops in WT lenses, the development and progression of this cataract is accelerated in KO lenses, suggesting that loss of CGAP induces accelerated aging due to loss of extracellular redox imbalance, localised GSH depletion and enhanced oxidative stress. This animal model may be useful to further study dysfunction of redox signaling pathways

Session 12-2

Connexin50D47A alters the redox status and causes oxidation in the lens

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Purpose: Mutants of the lens fiber cell gap junction protein, connexin50 (Cx50), cause congenital cataracts. We have been studying mice expressing Cx50D47A to elucidate the mechanism by which such mutants lead to disease. Lenses of these mice are small and have cataracts and impaired differentiation. This study was undertaken to investigate whether the low connexin levels and reduced gap junctional conductance in Cx50D47A mice leads to an oxidizing environment in the lens.

Methods: The concentrations of GSH and GSSG were measured using a luminescent-based glutathione-S-transferase assay. Lens proteins were detected by immunoblotting. The distribution of S-nitrosylated proteins was assessed by immunofluorescence microscopy, and its intensity was quantified using ImageJ.

Results: The GSSG/GSH ratio was increased in heterozygous and homozygous Cx50D47A lenses. Levels of glutathione synthetase and glutathione reductase were increased in homozygous Cx50D47A lenses. Carbonylation of lens proteins was increased in both heterozygous and homozygous mice compared to wild-type animals. Immunoreactivity to S-nitrosylated proteins was detected in lens epithelium and cortex; it was more abundant in homozygotes than in wild-type animals.

Conclusions: These results demonstrate oxidation of glutathione and an increase in oxidized proteins in the Cx50D47A lenses, implying that the loss of lens intercellular communication and the disruption of the lens internal circulation lead to a more oxidizing environment. Increased levels of glutathione synthetase and glutathione reductase could represent compensatory responses to the altered redox status.

Session 12-3

Emerging functional crosstalk between the glutaredoxin system and Nrf2 antioxidant pathway: evidence from ultraviolet radiation-induced cataract formation

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Purpose: To determine the function of glutaredoxin (Grx) system, both glutaredoxin 1 (Grx1) and glutaredoxin 2 (Grx2), in protecting the lens against ultraviolet (UV)-induced cataract formation by using *Grx1/Grx2* double knockout (DKO) mice as a model.

Methods: *Grx1/Grx2* DKO mice were generated by intercrossing *Grx1* knockout (KO) and *Grx2* KO mice. One-month old *Grx1/Grx2* DKO and age-matched wild-type (WT) mice, half male and half female, 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. Glutathione (GSH), free protein thiol (PSH), and protein glutathionylation (PSSG) levels were measured as general markers of oxidative damage. 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.

Results: We found that UV radiation caused more severe anterior subcapsular cataract in *Grx1/Grx2* DKO than that of WT mice. The opacity of the lenses in DKO mice, appeared to extend deeper into the cortical and even nuclear regions. Lenses of *Grx1/Grx2* DKO mice contained significant lower levels of GSH and PSH. On the other hand, the accumulation of PSSG, a marker for protein thiol oxidation, was much higher in *Grx1/Grx2* DKO group. Deletion of *Grx1* and *Grx2* also decreased the expression of antioxidant enzyme transcription factor regulator, Nrf2, and its downstream antioxidant genes, including catalase, superoxide dismutase (SOD), and another redox regulator of thioredoxin (Trx). These changes were especially extensive in the lens after UV exposure.

Conclusions: With combined *Grx1* and *Grx2* deletion, the Nrf2-dependent antioxidant response is severely impaired, causing elevation of oxidative stress that may increase the lens susceptibility to UV-induced damage.

Session 12-4

Nox4 is required but not essential for TGFβ-induced cataract

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Purpose: Transforming Growth Factor-β (TGFβ) can induce an epithelial to mesenchymal transition (EMT) in lens that results in fibrotic cataract. We and others have previously shown that the reactive oxygen species (ROS) producing enzyme, NADPH oxidase 4 (Nox4), is upregulated in response to TGFβ-signalling in lens, and that pharmacological inhibition of Nox4 can abrogate aspects of this EMT. To better elucidate the role(s) of Nox4 in lens EMT *in situ*, the present study characterised TGFβ-induced cataract formation in the absence of Nox4.

Methods: We crossed mice overexpressing TGFβ in the lens, that develop anterior subcapsular cataract, with Nox4-deficient mice. The eyes of resultant progeny were grossly examined, collected and processed for histological evaluation. Eye sections were stained with Periodic-acid Schiff, and immunofluorescence was used to assess changes in epithelial and EMT/cataract markers. Western blotting for pERK1/2, pSmad2/3 and Nox4, and qPCR for Nox subunits were performed on lens epithelial explants from different mice (wild-type-WT, Nox4-deficient and/or TGFβ-overexpressing). Additionally, lens epithelial explants from WT or Nox4-deficient mice were treated with exogenous TGFβ2 (200pg/ml) and stained for reactive oxygen species using DHE and Mitosox.

Results: Mice overexpressing TGFβ deficient for Nox4, did not present anterior subcapsular cataracts by postnatal day 30, with the lens remaining transparent. Despite this transparency, histology and immunolabelling of lenses revealed the presence of anterior subcapsular plaques devoid of many EMT markers. Labelling of Nox4-deficient lens epithelial explants and mouse sections revealed elevated pSmad2/3 and pERK1/2 signalling. qPCR analysis revealed compensatory upregulation of Nox2 in mice deficient for Nox4. Moreover, TGFβ-treatment induced elevated but delayed ROS labelling in Nox4-deficient explants.

Conclusion: These results indicate that in mice at least, Nox4-induced ROS plays a role in the development of TGFß-induced EMT leading to cataract. In the absence of Nox4, there are other compensatory sources of ROS, such as Nox2 and mitochondrial ROS.

Session 12-5

Hypoxia regulation of lens fiber cell differentiation

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Purpose: The lens contains a decreasing oxygen gradient from surface to core. Based on the presence of this oxygen gradient, we hypothesize that decreasing levels of oxygen in the lens regulate the expression of lens fiber cell structural genes and genes required for the elimination of lens fiber cell organelles through transcriptional activation by the master regulatory of the hypoxic response, transcription factor hypoxia inducible factor 1 (HIF1) and by oxygen-dependent chromatin remodelling.

Methods: To test our hypothesis, we mapped the genomic-level expression of genes expressed by organelle-containing nascent lens fiber cells relative to undifferentiated lens epithelial cells and organelle-free mature lens fiber cells of the chick lens by RNA- sequencing, we tested the effect of hypoxia on the expression levels of these genes, we identified the genomic positioning of nucleosomes at the promoters of hypoxia-regulated genes by ATAC-sequencing and we examined the activity and spatial localization patterns of histone deacetylases 1 and 2 (HDACs 1 and 2) that could regulate chromatin remodelling of lens fiber cells under hypoxic conditions.

Results: Our data demonstrate that a diverse array of genes required for fiber cell structure and formation of the lens organelle-free zone (BNIP3L) are dependent on the hypoxic environment of lens fiber cells and that their transcription is regulated by the oxygen- dependent activation of transcription factor HIF1. Our data also show that the fiber cell- specific expression of these genes is dependent on nucleosome repositioning that we propose to be regulated by hypoxia-dependent activity of key HDACs.

Conclusions: Our results demonstrate that hypoxia is an essential requirement for the formation and function of mature lens fiber cells and they identify novel oxygen-dependent transcription control pathways essential for lens growth and transparency.

Session 13-1

A tale of two serines

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Purpose: Although many human connexin50 (Cx50) mutants have been linked to cataracts, most of them localize to the first half of the protein. We studied two cataract-linked mutants of Cx50 that alter serines in its carboxyl terminus (S258F and S259Y). These serines are phosphorylated in the lens. To understand the mechanism of cataract formation, we determined the cellular and functional behavior of these mutants and investigated the role of phosphorylation at these positions using phosphorylation-mimic mutants (S258D and S259D).

Methods: Wild-type and mutant Cx50 were stably expressed in HeLa cells. Cx50 was localized by immunofluorescence, and gap junction size and abundance were quantified. Intercellular communication was assessed by voltage clamp studies in *Xenopus* oocyte pairs and Neurobiotin microinjection in HeLa cells. Levels of Cx50 were determined by immunoblotting.

Results: Unlike wild-type Cx50, S258F and S259Y rarely formed gap junction plaques. Gap junction abundance and size were greatly increased for S258D and S259D compared to their cataract-linked counterparts. While S258F and S259Y induced small junctional conductances and supported little Neurobiotin transfer, S258D and S259D supported extensive intercellular communication. Cycloheximide treatment induced a much faster decay of S258F and S259Y levels than their aspartate-substitution counterparts. Chloroquine treatment significantly increased S258F and S259Y levels, implying that the lysosome was responsible for their increased degradation. However, aspartate-substitution at the two positions had differing impacts on channel function and resistance to lysosomal degradation.

Conclusion: Both S258F and S259Y support greatly reduced intercellular communication, likely because of decreased Cx50 at appositional membranes. In the lens, this would disrupt the internal circulation of water and ions leading to cataracts. The restoration of gap junction abundance and intercellular coupling by the aspartate mutants suggests that phosphorylation of these residues in the normal lens is important for gap junction stability at the plasma membrane.

Session 13-2

Creation and analysis of isomers-containing lens αA-crystallin

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Purpose: Recent studies have shown that many aspartyl residues (Asp) in lens αA-crystallin (αA-Crys) were inverted to isomers. However, direct evidence of the isomerization for protein structure, stability and function have never been reported. It is due to lack of the method for creation of iso-Asp-containing crystallin. In order to solve the problem and investigate the contribution of Asp isomers, we used Expressed Protein Ligation (EPL). The contributions of Asp isomers were evaluated using a series of ligation products.

Methods: Truncated human lens αA-Crys 1-141 (αA-Crys 1–141) was recombinantly expressed in *E.coli*. The segment of αA-Crys (residues 142–173), containing four different Asp isomers at Asp151, was synthesized by Fmoc-based peptide synthesis. Using with EPL, the αA-Crys 1–141 and each of four different Asp isomers containing peptide (L-α-Asp, L-β-Asp, D-α-Asp and D-β-Asp) were ligated. The inversion of Asp151 was confirmed by D/L analysis based on mass spectrometry equipped with nano-scale liquid chromatography (LC-MS/MS). Those oligomer size, stability and chaperone function were compared with recombinantly expressed wild type αA-Crys.

Results: The LC-MS/MS analysis identified the isomers in ligation products. The substitution of Asp151 into Asp isomers did not alter αA-Crys oligomer assembly and structure. The formation of β-Asp increased hydrophobicity of molecule. The lens specific αA-Crys chaperone like function was altered depending on each Asp isomers.

Conclusions: This study is the first report for inserting Asp isomers into the αA-Crys, and elucidating their contributions. The result would suggest that the isomerization of Asp151 in αA-Crys induced abnormal crystallin subunit-subunit interaction in lens. Those would imply one of the reasons for senile cataract formation in aged lens.

Session 13-3

Human γ-crystallin mutations affecting aromatic Greek key pairs and N-terminal tyrosine corner impairs tight packing and causes congenital nuclear cataract

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Purpose: The Greek key folds of lens crystallins provide the stability, compact packing, and short-range order necessary to maintain lens transparency. A "tyrosine corner" at the distal torque of the Greek Key motifs and six aromatic pairs in the β-sheet runs are critical for their stability. We investigated how mutations in the Greek key aromatic pairs (F10_Y11delinsLN, Y46D) and in the N-terminal domain tyrosine corner (Y67N) alter βγ-crystallin structure and stability leading to autosomal dominant congenital nuclear cataract.

Methods: Human crystallin c-DNAs were cloned into a pET-20b (+) vector and F10_Y11delinsLN, and Y67N γS-crystallins and Y46D γC-crystallin were generated by site-directed mutagenesis. Mutant and wild-type crystallins were overexpressed in *E.coli* BL-21(DE3)PLysS cells, purified by ion-exchange and size-exclusion chromatography, and their structures, stability and aggregation properties under thermal and chemical stress were characterized by fluorescence and circular dichroism methods.

Results: The mutations (FL10,11YN, Y46D and Y67N) do not distort the backbone under physiological conditions but alter its tryptophan microenvironment and expose the hydrophobic core. Mutants are less stable under thermal stress, undergoing two-state (F10_Y11delinsLN, Y67N) or three-state (Y46D) transitions. Mutants had lower thermodynamic stability under GuHCl induced stress and exhibited a three-state transition with clear intermediates whereas the wild-types show a two-state transition. Mutants also show different unfolding kinetics and an increased tendency to self-aggregate upon heating at 60°C.

Conclusion: Replacing Greek key pairs (F10_Y11delinsLN, Y46D) and N-terminal tyrosine corner (Y67N) in human γS- and γC crystallin impairs the tryptophan microenvironment, expanding the Greek key structure, and the effect is more severe because it affects the requisite tyrosine corner and aromatic Greek key pairs, corresponding to the clinical severity of the cataract. Decreased stability under thermal and chemical induced stress leads to self-aggregation and cataract. Hence aromatic Greek key pairs and tyrosine corners are essential in keeping γ-crystallin fold intact.

Session 13-4

Detection of early Alzheimer's Disease-linked molecular changes in the lens by quasi-elastic light scattering ophthalmoscopy in young subjects with Down Syndrome

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Purpose: Alzheimer's disease (AD) is characterized by age-dependent amyloidβ (Aβ) deposition in brain. In AD, Aβ also accumulates in the equatorial supranuclear subregion of the lens (Goldstein et al., Lancet, 2003). We identified identical AD-linked Aβ lens pathology in Down Syndrome (DS), a chromosomal disorder associated with early-onset AD neuropathology (Moncaster et al., PLoS One, 2010). Aβ accumulates as hetero-oligomeric cytosolic aggregates in AD and DS lens fibers. Aβ-containing aggregates act as Raleigh scattering centers that ultimately manifest as supranuclear lens opacities.

Methods: We used an investigational quasi-elastic light scattering (QLS) scanning laser ophthalmoscope to evaluate scattering properties in the supranuclear lens subregion of young subjects with DS (n=8; mean age: 13.6±2.1 years) and normal controls (n=15; mean age: 15.5 ± 1.9 years). The study was approved by the Institutional Review Board, Boston Children's Hospital.

Results: Our results reveal significant group differences in average scattering intensity (p=0.04) and average τ decay time (p=6.5x10^{-5}).

Conclusions: QLS ophthalmoscopy can be used to non-invasively detect and discriminate AD-linked molecular changes in lenses of DS subjects. These findings are consistent with our finding of early AD-linked Aβ lens pathology in DS and extend these results to living subjects evaluated by QLS ophthalmoscopy *in vivo*.

Session 13-5

Imaging mass spectrometry-based approaches to support lens biomolecular mapping and anti-cataract therapy development

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Purpose: To visualise nutrient and drug transport and metabolism in the lens and whole eye using MALDI imaging mass spectrometry.

Methods: MALDI (matrix-assisted laser desorption/ionisation) IMS (imaging mass spectrometry) has been applied to the lens and eye of a variety of species. Cryosections from bovine lenses organ cultured in artificial aqueous humour containing isotopically-labelled glucose (0-24hrs) were analysed by negative ion mode MALDI-FT-ICR imaging mass spectrometry (100um spatial resolution) to map nutrient transport in the normal lens. To investigate lenticular drug penetration, porcine lenses were organ cultured in a solution containing 16 different small molecule drugs for 4hrs, before being analysed by positive ion mode MALDI IMS. Finally, small molecule distributions in sections from normal whole eyes (fish, mouse, rat and rabbit) were mapped at up to 10um spatial resolution using negative ion mode imaging mass spectrometry.

Results: In normal bovine lenses, isotopically-labelled glucose uptake in the cortex was visualised, and its metabolism to isotopically-labelled metabolites such as sorbitol detected. Labelled sorbitol was first detected in the lens epithelium, before being detected in the cortex, and nucleus over time. In the porcine lens, 10 small molecule drugs were detected, that penetrated to the same distance in the lens, irrespective of their physicochemical properties. Drug concentrations were higher in the posterior lens. In whole eye experiments, a technique was developed to maintain the spatial integrity of endogenous small molecules in both ocular tissues and humours. Fish eyes showed a variety of spatially-distinct small molecule distributions in the ocular humours, while distributions in rodent humours were more uniform.

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

Session 15-1

Feasibility of bringing anti PCO therapeutics to clinical practice; physician acceptance and identification of a drug target

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Purpose: Posterior capsular opacification (PCO) is the most prevalent post cataract surgery (PCS) complication. However, PCO rates have decreased due to surgical and IOL innovations, while laser therapy is an effective treatment. This created controversy about the need for anti- PCO therapeutics. Further, while anti-PCO agents have been tested, their non-specific toxicity and/or lack of clinical efficacy has suggested that pharmacological PCO prevention is not a viable approach. Here, we assess the need for new PCO preventatives by surveying practicing cataract surgeons, and evaluate a potential drug target for the prevention of fibrotic PCO.

Methods: Fifty cataract surgeons practicing worldwide were interviewed. Mice lacking β8-integrin from the lens (β8ITG cKO) and wildtype controls were subjected to lens fiber cell removal surgery which mimics cataract surgery. The effect of b8-integrin deletion on the response of lens epithelial cells (LCs) to cataract surgery was evaluated by RNA sequencing and immunolocalization.

Results: The cataract surgeons interviewed felt that PCO is one of their major patient management concerns with 86% of adult, and 100% of veterinary and pediatric, cataract surgeons surveyed agreeing that effective anti-PCO therapeutics would improve clinical care. β8ITG cKO LCs proliferated less, and did not undergo a qualitative fibrotic response PCS. RNAseq found that β8ITG cKO LCs exhibit reduced upregulation of key fibrotic and inflammatory markers at 24 hrs. PCS compared to control. This was associated with highly attenuated canonical TGFβ signaling PCS while addition of active TGFβ rescued both the TGFβ signaling defect and fibrotic response.

Conclusion: As we previously found that aV-integrin is also essential for PCO, our data suggest that αVβ8 integrin is a "druggable" target that could ameliorate TGFβ activation by LCs PCS. As most of cataract surgeons surveyed are interested in new PCO preventatives, this study suggests a potential market for anti-αVβ8 therapeutics to prevent fibrotic PCO.

Session 15-2

Assessing the therapeutic potential of resveratrol to inhibit posterior capsule opacification

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Purpose: Posterior capsule opacification (PCO) is a common complication of cataract surgery. In addition to better IOL design and surgical approaches, it is likely that agents will also be required to improve outcomes for patients. No pharmacological agent is in clinical use to prevent PCO. In the current study we investigated the potential of resveratrol (RESV), a naturally occurring polyphenol, as a therapeutic agent.

Methods: The human lens epithelial cell line FHL124, a human lens capsular bag model and central anterior epithelium were used as experimental systems. Standard culture was in 5% FCS EMEM. 10ng/ml transforming growth factor-β2 (TGFβ2) was used to induce fibrotic changes. FHL124 cell migration was assessed using a scratch-wound assay and matrix contraction by the patch assay. In capsular bags, cell growth across the posterior capsule and capsular wrinkling were determined by image analysis. Immunocytochemistry and western blot were used to determine protein expression and QRT-PCR for gene expression in all model systems.

Results: Addition of 30μM RESV to FHL124 cells significantly slowed cell migration in a wound-healing assay. RESV significantly suppressed TGFβ2-induced expression of the myofibroblast marker alpha smooth muscle actin (αSMA) at both the message and protein level and significantly inhibited TGFβ2-induced matrix contraction. In human capsular bags, cell growth was significantly inhibited by 30μM RESV. RESv treatment also significantly inhibited TGFβ2-induced αSMA expression and capsular wrinkling. TGFβ2-induced genes associated with fibrotic disease were significantly suppressed in all experimental systems with RESV treatment.

Conclusions: RESV can suppress PCO related physiological events in three human model systems. RESV is consequently a putative therapeutic agent for the prevention of PCO, which could potentially improve the lives of millions of cataract patients.

Session 15-3

Identifying the mechanisms regulating sulforaphane induced lens cell death in PCO prevention

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Introduction: Posterior capsule opacification (PCO) can cause secondary visual loss following cataract surgery. Improved management of this problem is important. The isothiocyanate, sulforaphane (SFN) is reported to exert cytotoxic actions and this could be exploited to prevent PCO. The current study aimed to demonstrate the cytotoxic actions of SFN on lens cells and elucidate the mechanisms governing this physiological outcome.

Method: The human lens epithelial cell line FHL124 and lens epithelium generated by capsulorhexis on human donor lenses. The CellTiterGlo assay and LDH assay were employed to assess cell viability and death. Immunoblotting and real-time PCR were used to detect protein levels and gene expression for autophagy and endoplasmic reticulum stress (ERS) markers. Immunofluorescence was utilised to assess DNA damage (γH2AX) and mitochondrial networks. TMRE dye was used to detect mitochondrial membrane potential. Luciferase assays were employed to measure the ATF6 activity and GSH/GSSG ratio. N- acetyl cysteine (NAC) was used as a ROS scavenger.

Results: 50μM SFN was found to significantly reduce cell viability and increase cytotoxicity in both experimental models. Moreover, in FHL124 cells SFN promoted ERS responses, autophagy, disrupted the mitochondrial network, depolarised mitochondrial membrane potential and increased DNA damage. To determine the role of ROS in these SFN-induced responses, the ROS scavenger was used. Pre-treatment of NAC significantly prevented all SFN induced changes. In addition, the effect of SFN on glutathione homeostasis was investigated and was found to deplete GSH. Interestingly, NAC pre-treatment could not prevent SFN-induced GSH depletion.

Conclusion: SFN could serve as a therapeutic agent for PCO management. SFN is able to kill lens epithelial cells through activation of multiple stress pathways. ROS appear to play a key role in these events. A possible trigger for the cascade of SFN-induced events is depletion of GSH, which could increase susceptibility to oxidative stress.

Session 15-4

Lens epithelial cells adaptation to oxidative stress and its relevance to posterior capsule opacification

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Purpose: Posterior capsule opacification (PCO) is a common post-cataract surgery complication attributed to residual lens epithelial cell (LEC) adaptation and transformation, i.e., fibrosis. LECs after cataract surgery face a hostile growth microenvironment compared to healthy cells and the sustained stimulation from the microenvironment is expected to play a pivotal role in cell adaptation, survival, and invasion. We have discovered that the ROS-mediated Wnt/β-catenin signaling pathway is playing an important role in LECs adaptation and fibrosis (Am. J. Path, IOVS, and Free Radical Biol Med, 2017). Now, we are testing the mechanisms and its pathogenic role in PCO formation using cell culture, mouse, and rabbit PCO models.

Methods: Lens epithelial cells (FHL124), wild type, and glutathione *de novo* synthesis deficient (Gclc and Gclm) mice, and New Zealand albino rabbits were used for this study. Mouse cataract surgery was performed, and eye tissue was collected from 1 to 7 days postoperatively. A longitudinal study of rabbit cataract surgery with IOL implantation was carried out for 5-time points, i.e., at 0, 5day, 1m, 3m, and 6m. The PCO condition and LECs fibrosis, oxidation, and Wnt signaling markers were determined.

Results: FHL124 cells treated by Wnt10a conditioned medium readily promoted epithelial-mesenchymal transition (EMT) with increased expression of aSMA, vimentin, fibronectin, and Wnt signaling markers, which was also seen in mouse cataract PCO model. A sustained oxidative stressed environment was observed in the entire course of the rabbit study. Various inflammatory cytokines, including TGFβ1 and TGFβ2 were significantly increased at the day 5 postoperatively, then normalized relative to baseline. Intriguingly, a sustained Wnt/β-catenin signaling, was seen up to 6 months after surgery. This study points to a critical role of Wnt/β-catenin singling pathway in the pathogenesis of PCO formation, particularly at the chronic stage.

Conclusions: These results imply that survival signaling pathways are involved in oxidative stress adaptive response.

Session 15-5

Fabrication of implantable IOLs using 3D printing technology

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Purpose: Cataracts is an ophthalmic disease in which a healthy clear eye lens develops cloudiness or opaqueness, resulting in deteriorating vision. The lens of the eye is responsible for focusing light and producing a clear image on the retina. Cataracts is typically an ongoing process of healthy aging and is responsible for more than 50% of the world's blindness. In 2010, the WHO estimated that 20 million people were suffering from cataracts and that the prevalence is estimated to double to 40 million in 2020. At present, the only method to treat a cataract is by surgical intervention, in which the clouded lens is removed and replaced with a new artificial intraocular lens (IOL) and thus result in restoring a high-quality vision. Currently to manufacture an IOL either a moulding or lathing technique is used, but are associated with some drawbacks and prototype development require a considerable amount of time and may delay the life-cycle for new IOL development.

Method: We developed a new fabrication tool for prototyping intraocular lens and devices using 3D printing technology. A series of new polymeric material formulations were produced, establishing a new fabrication method for the 3D printing of IOLs. CAD designs of the IOLs were designed detailing the high resolution and accuracy of IOLs. The IOLs were then 3D printed and characterised for morphology, composition, thermal behaviour and biocompatibility.

Results: All the formulation resulted in highly transparent, foldable and biocompatible IOLs. Moreover, the 3D printed IOLs were also implanted in human lens capsular bag model using a standard injection method.

Conclusion: This new fabrication tool has the potential to be used for a wide range of varying IOL types, be more readily accessible, can reduce product development life-cycle and reduce the cost by eliminating the need for extensive machining processing.

Session 16-1

Genetic coding of lens differentiation, -omics and emerging regulatory models

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Purpose: The DNA genetic code operates at multiple levels, including *cis*-regulatory code of transcription, *cis*-regulatory code of 5'- and 3'-UTRs in mRNAs, and 3D-organization of the nucleus. Recent progress in technologies to probe chromatin structure, transcriptomes (i.e. mRNAs and ncRNAs) and proteomes allow genome-scale analyses of lens development and differentiation to generate novel insights into the molecular mechanisms of lens morphogenesis and their underlying gene regulatory networks (GRNs).

Methods: Mouse lens cells were microdissected into lens epithelium and lens fibers. "Open" chromatin domains were visualized using ATAC-seq using cells from embryonic (E14.5) and newborn (P0.5) lenses. RNA-seq studies were conducted using E14.5, E16.5, E18.5 and P0.5 stages. Primary analyses are focused on DNA-binding transcription factors and RNA-binding proteins expressed in differentiating lens fibers. Promoter-enhancer looping of the □A-crystallin locus and nascent transcription were studied using the 3C method and RNA FISH, respectively.

Results: We identified dynamics of open chromatin changes by defining differentially accessible regions during mouse lens fiber cell (Path1: epiE14.5→fibE14.5→fibP0.5) and epithelium (Path2: epiE14.5→epiP0.5) differentiation. Unbiased *cis*-motif analysis of "open" regions, corresponding to promoters and enhancers, revealed the *cis*-regulatory logic of lens differentiation via known (e.g. AP-1, Etv, Maf, Pax6, and Sox) and novel (e.g. CTCF, Tead, and NRF1) motifs. Novel abundant RNA-binding proteins in lens include Carhsp1, Ddx5, Ddx39b, Fxr1, Rbm38, Ybx1 and Ybx3. The 5'- (3'-) distal enhancer DCR1 (DCR3) forms a major (minor) loop with the □A-crystallin promoter in lens but not in ES cells, respectively.

Conclusions: A combination of –omics is a powerful approach to elucidate the genetic basis and molecular mechanisms governing lens differentiation. Current studies further reveal novel levels of genetic code complexity and stimulate follow up mechanistic studies of the individual cellular and molecular processes of lens morphogenesis and their underlying GRNs.

Session 16-2

Foxe3 and downstream genes in lens development

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Purpose: *FOXE3* encodes a forkhead/winged helix-containing DNA-binding transcription factor that is critical for lens development. Murine models of loss of Foxe3 function have been generated, but the pathogenesis of the lens defects remains incompletely understood and Foxe3-deficient lens transcriptome data is not available. We hypothesized that studying the gene regulatory networks (GRNs) disrupted by loss of the orthologous zebrafish gene, *foxe3*, would improve our understanding of lens biology.

Methods: We used CRISPR/Cas9 to target the foxe3 homeodomain and characterized the phenotype of the mutant larvae. We performed RNA-Seq on RNA extracted from pooled whole larvae and pooled eye tissue obtained from wildtype larvae and *foxe3* mutant larvae with lens defects. These results were compared to gene expression in the embryonic murine lens from the iSyTE2.0 lens database.

Results: Larvae that were homozygous for an indel variant, c.296_300delTGCAG, predicting p.(Val99Ala*fs**2) in *foxe3* exhibited small or absent lenses The eye phenotype demonstrated autosomal recessive inheritance and was fully penetrant. RNA-Seq on whole larvae identified 584 upregulated and 1586 downregulated differentially expressed genes (DEGs), including that have human orthologues associated with cataracts and eye defects, such as *cryba2a*, *cryba1l1*, *mipa* and *hsf4*. 181 of the genes that were dysregulated in the *foxe3* mutant larvae showed enriched expression in the iSyTE2.0 data for the murine E12.5 lens. Among the *foxe3* downregulated genes that were also enriched in iSyTE2.0 data were candidates for eye development, such as *grifin*, *mipb*, *crygmx* and *cryba2b*. RNA-Seq performed on eye tissue confirmed downregulation of several of the DEGs observed in whole larvae, including *cryba2b*, *mipb* and *grifin*.

Conclusion: Our findings show that the zebrafish *foxe3* mutant model is highly relevant to vertebrate lens development. We have identified candidate genes for the GRNs involved in eye development downstream to *foxe3* and are currently studying these genes using CRISPR/Cas9.

Session 16-3

iSyTE: a systems resource for gene discovery in lens development and cataract

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Purpose: Thus far, lens biology has been investigated in detail using cellular, biochemical and molecular genetics-based approaches. This has led to major advances in our understanding of the regulatory processes in lens development and homeostasis. However, the following challenges remain in lens research: (1) the identification of new genes linked to lens development and cataract remains difficult, and (2) a global picture of the myriad of complex interactions between the various lens regulatory factors and their targets, over developmental time, is not readily comprehensible or accessible. Here, we describe the application of systems-level approaches to address these challenges.

Methods: Genetic perturbation-based experimental data on mouse lens from the published literature was systematically recorded and analyzed using custom-algorithms for derivation of "evidence-based" gene regulatory networks (GRNs) in lens development. Based on the reported age of analysis, lens GRNs were defined for different stages, namely, lens initiation, primary fiber cell differentiation, secondary fiber cell differentiation and post-natal development.

Results: To expedite the process of lens gene discovery, we developed and updated the bioinformatics tool iSyTE (integrated Systems Tool for Eye gene discovery; https://research.bioinformatics.udel.edu/iSyTE/) by meta-analysis of lens gene expression microarray profiles. This was extended by including high-throughput RNA-sequencing profiles at embryonic lens stages and is presently being further expanded to include lens proteome data. We analyzed the wealth of molecular functional data in the lens literature to derive "evidence-based" GRNs, defined as a comprehensive circuit map of the regulator-target interactions. The updated iSyTE has expedited lens gene discovery and led to prediction/identification of several new cataract associated genes.

Conclusion: High-throughput lens expression data and lens molecular functional data were analyzed to derive stage and/or genetic perturbation-specific GRNs that inform on regulatory interactions and predict key regulators in lens development and disease. This rich information is made freely accessible as an interactive web-resource.

Session 16-4

The TRPM3 locus in lens development and cataract

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Purpose: Mutations in the human genes encoding transient receptor potential cation channel subfamily M, member 3 (TRPM3, melastatin 2) and its intronically hosted micro-RNA, miR-204, underlie inherited forms of early-onset cataract ± glaucoma with anterior eye defects or retinal dystrophy with ocular coloboma ± cataract, respectively. Both the genes for TRPM3 and miR-204 are co-regulated by the paired-box transcription factor, PAX6, during eye development and miR-204 is required for lens development in mice. However, the role of TRPM3 in lens biology is unclear. Here we have characterized the lens phenotype of mice harboring a human cataract-associated mutation in TRPM3 (*Trpm3*-mutant) with that of mice lacking TRPM3 (*Trpm3*-null).

Methods: Mutant and null gene-targeted mice were generated using CRISPR/Cas9 editing or standard homologous recombination techniques, respectively. Lenses were characterized by light microscopy, histochemistry, and (immuno-)fluorescence confocal microscopy techniques.

Results: Despite strong expression of TRPM3 in lens epithelial cells, neither heterozygous nor homozygous *Trpm3*-null mice developed cataract. By contrast, heterozygous *Trpm3*-mutant mice developed progressive anterior cataract, whereas, homozygous *Trpm3*-mutants developed severe anterior cataract and microphthalmia. Histochemical staining revealed progressive lens degeneration in heterozygous *Trpm3*-mutants coupled with accumulation of abnormal nucleated cells and calcium phosphate-like deposits in homozygous *Trpm3*-mutant lenses. Immuno-fluorescent labeling revealed disruption of AQP0-positive lens fiber cell membranes and localization of αSMA-positive and CD68-positive cells to the anterior and posterior regions of *Trpm3*-mutant lenses.

Conclusions: Collectively, our mouse data support an ocular disease association for TRPM3 mutations in humans and suggest that TRPM3l dysfunction leads to disturbed lens calcium homeostasis, progressive cataract development and lens fiber cell degeneration, and an ocular immune-cell response.

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Session 16-5

Transcriptional profiling of single cells – insights into lens fiber cell morphogenesis

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Purpose: A post-natal day two (PND02) mouse lens presents a complete paradigm for investigations into the study of the progression of an incipient epithelial cell to a terminally differentiated elongated fiber cell state. We have initiated investigations through transcriptional profiling of individual fiber cells derived from three different states of differentiation, the equatorial fiber cell, the cortical fiber cell and the central (nuclear) fiber cell.

Methods: We have employed three different methodologies for transcriptional profiling: (a) Microfluidic qRT-PCR (quantitative reverse transcription-polymerase chain reaction) in Biomark (Fluidigm Inc.), (b) single-cell RNA sequencing of individual fiber cells and (c) whole lens RNA sequencing of the fiber mass of single lenses. For isolation of intact single fiber cells, we modified a previously published procedure (Srivastava et al, IOVS 38, 2300, 1997). A temporally controlled dissociation of fiber cells from a pliable PND02 lens allows us to collect fiber cells spatially derived from the equatorial, cortical and nuclear regions of the lens.

Results: The population profiles of transcription characteristically reflect the average expression of crystallins, membrane proteins and transcription factors. A comparison with collective single cell data brings about significant differences between these two sets of data. This is further enhanced by computational analysis, which separates the data into clusters, which spatially correspond to expression profiles of groups of fiber cells expressing specific gene(s). This is highly significant.

Conclusion: The progression in cellular and morphological differentiation is attended by molecular expression, which reveals deterministic gene activity of specific crystallins in terminally differentiated fiber cells. The heterogeneity of the expression profiles also explains the potential molecular basis for the remarkable heterogeneity in cataract phenotypes; we are seeking to address whether this heterogeneity is regulated and how?
(This work is supported by NEI/NIH grants to SPB)

Poster 1

Expression of Grx2 in the capsule tissue of age-related cataract patients

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Purpose: Glutaredoxin2 (Grx2) is an important isozyme of glutaredoxin1 (thioltransferase) against oxidative stress. We wanted to test whether Grx2 plays a role in the development of human age-related cataracts. And to explore whether changes in Grx2 expression of age-related cataracts are associated with diabetes.

Methods: The capsule tissue that was torn off during phacoemulsification was collected and stored in liquid nitrogen. According to the LOCS III classification, the capsule tissue was divided into three groups, I and II, III and IV, V and VI. Protein and mRNA were extracted separately, and the expression level of Grx2 was detected by Western blot and qPCR. The capsules of grades II to III were sorted out and divided into two groups according to the patient's lack of diabetes and diabetes over 15 years. Western blot and qPCR were used to detect the protein level and mRNA level of Grx2, respectively.

Results: With the increase of human cataract level, the expression level of Grx2 protein and mRNA is also gradually increased. There was no significant difference in the expression levels of Grx2 protein and mRNA between the diabetic and non-diabetic patients.

Conclusions: In the human body, Grx2 acts as a protective molecule and increases with the severity of cataracts. However, with or without diabetes, there is no difference on Grx2. It is suggested that the expression of Grx2 in age-related cataract is not related to diabetes. [Supported by National Natural Science Foundation of China (No. 81570823) and Xi'an No. 4 Hospital Innovation Project (No. LH-6)]

Poster 2

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

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Abstract
The absence of clinical tools to evaluate individual variation in the pace of aging represents a major impediment to understanding aging and maximizing health throughout life. The human lens is an ideal tissue for quantitative assessment of molecular aging *in vivo*: long-lived proteins in lens fiber cells are expressed during fetal life, do not undergo turnover, accumulate molecular alterations throughout life, and are accessible *in vivo*. Using quasi-elastic light scattering (QLS), we measured age-dependent signals in lenses of healthy human subjects. Age-dependent QLS signal changes detected *in vivo* 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*. Our findings demonstrate that QLS analysis of human lens proteins provides a practical technique for assessment of molecular aging *in vivo*.

Poster 3

Species-dependent changes in lens crystallin protein solubility during aging

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Purpose: A known feature of lens aging is the decrease in water-soluble crystallin protein levels. The relationships of crystallin solubility and chaperone-like activity to cataractogenesis and severity are not fully understood, but are critical to understand in order to develop and characterize translational animal models of cataracts. To investigate lens protein changes during aging, we used biochemical and analytical techniques to characterize crystallin proteins in the lens water-soluble and water-insoluble (urea-soluble) fractions across multiple species.

Methods: Lenses from mouse, rabbit, dog, and human at multiple ages were dissected and homogenized. Water-soluble and urea-soluble protein fractions were characterized by Western blot. Water-soluble fractions were further analyzed by SEC-MALS. Additionally, chaperone-like activity of unclarified and clarified homogenates was measured. Briefly, heat-induced aggregation of recombinant αB-crystallin harboring the destabilizing R120G mutation was monitored in the presence of lens homogenate and compared to assays with recombinant αA- or αB-crystallin.

Results: Consistent with literature reports, water-soluble alpha-crystallin levels in the nucleus decrease dramatically as a function of age, and gamma-crystallin levels similarly decrease throughout the lens in every tested species. Further, the degree of soluble high molecular weight aggregate formation and loss of native proteins positively correlates with animal age. Despite this, whole lens homogenates retain surprisingly high levels of chaperone-like activity throughout aging, exceeding those of recombinant αA- and αB-crystallin at matched concentrations. Homogenate fractionation experiments by size exclusion chromatography show this chaperone-like activity can be attributed primarily to native alpha-crystallin. These results suggest that native alpha-crystallins may have post-translational modifications and/or structural differences from their recombinant counterparts that enhance chaperone capability.

Conclusion: Herein, we systematically compared water-soluble crystallin levels in multiple therapeutically-relevant species as a function of age. Notably, aged lens homogenates demonstrated increased chaperone activity compared to recombinant αA-crystallin or αB-crystallin.

Poster 4

A cytosolic phospholipase-mediated organellar degradation in the lens in zebrafish and mice

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Purpose: During terminal differentiation of lens fiber cells, all membrane-bound organelles including the ER and mitochondria are degraded, though its underlying mechanism remains largely unknown. We previously reported that macroautophagy is not required for this process in mice, suggesting the presence of a yet-unknown macroautophagy-independent mechanism of organelle degradation in the lens.

Methods: We established a live-imaging and CRISPR/Cas9-based knockout systems using zebrafish to reveal the precise process and molecular mechanism of organelle degradation. We also generated and analyzed mice deficient for candidate genes.

Results: By a live-imaging analysis using zebrafish, we reveal that organellar membranes are ruptured during terminal differentiation in the lens. A microarray and founder CRISPR/Cas9 knockout screens using zebrafish identified a PLA2-family phospholipase that is essential for degradation of organelles, but not nuclear DNA. The lipase is critical for achieving optimal lens transparency. The lipase translocates from the cytosol to various organelles just before organelle degradation and its predicted transmembrane domain in a C terminus is required for its organelle targeting and subsequent organelle degradation. The lipase is conserved among vertebrates and, in mice, one of its homologues is highly expressed in the lens and essential for organelle degradation. In contrast to the case in zebrafish, the degradation of nuclear DNA partially requires the lipase in mice. The lipase-mediated degradation of the nuclear envelope may be important for efficient degradation of the nucleoplasm in mice.

Conclusions: Based on these findings, we propose a novel conserved macroautophagy-independent mechanism of organelle degradation; cytosolic phospholipases localize to target organelles to degrade their membranes.

Poster 5

Cataract-inhibitory effects of water chestnut and lutein in Shumiya cataract rat

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Purpose: Oxidative stress and Glycative stress is a risk factor for age related cataract. Lutein (LU) and water chestnut (Trapa bispinosa Roxb) (TBE) has been reported to have various physiological functions such as antioxidant and antiglycation activity. In this study, the effects of LU and TBE on cataracts and the expression of antioxidant-related genes in lens epithelial cells Shumiya cataract rats (SCRs) were assessed.

Methods: Six- or nine-week-old cataractous SCRs and non-cataractous SCRs were administered LU+ TBE or castor oil (CO) as a control by a feeding needle for 4 or 3 weeks. 5-week-old SCRs were given ad libitum access to the solid regular chow containing LU, TBE, and LU+TBE or the same chow without LU and/or TBE for 3 weeks. At the end of the feeding, each rat's lenses were extracted and photographed. Rats' right eyes were processed for histological observation, and the left lens epithelial cells were used for total RNA extraction. The expressions of anti-oxidant protein, peroxiredoxin 6 (Prdx6), and catalase mRNAs were examined using real-time quantitative PCR.

Results: Lens opacity appeared in all cataractous SCRs administered LU+TBE or CO for 3 or 4 weeks from 6 or 9 weeks of age. However, compared to the CO group the lens opacity was decreased in the cataractous SCRs administered LU+TBE in all experiments. The expressions of Prdx6 and catalase mRNAs were increased after the administration of LU+TBE in lenses from the cataractous SCRs.

Conclusions: Our results highlight the anti-cataract and anti-oxidative effects of LU+TBE in SCRs. LU+TBE supplementation rather than LU or TBE alone may be useful to delay cataract progression.

Poster 6

The effect of aging on the antioxidative activity of astaxanthin in human aqueous humor

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Purpose: Astaxanthin (AX) is an anti-oxidative substance drawing recent attention. We have reported antioxidative effects of AX intake in the aqueous humor through the measurement of reactive oxygen species (ROS)-related parameters and other factors. This time, we analyzed our data based on the age of the subjects.

Methods: Subjects were 16 males and 19 females (average age 71.3 and 70.6, respectively) who underwent bilateral cataract surgery on one side before and the other side after the intake of AX (6 mg/day for 2 weeks). The O2•- scavenging activity, H2O2 level, and total hydroperoxides (TH) level in the aqueous humor were measured and the effect of aging on these parameters before and after the AX intake was analyzed.

Results: 1. Each of the ROS-related parameters had no relationship with age before the AX intake. After the AX intake, only TH level showed the relationship ($r=0.4$, $p<0.05$). 2. TH levels did not differ between the group of patients aged below 70 and that of patients aged 70 or older before the AX intake. After the AX intake, TH level decreased to a greater extent in the former group (-0.21± 0.18) than in the latter group (-0.05 ± 0.31) with a significance ($p<0.05$), resulting in a significantly lower level in the former group ($p<0.05$).

Conclusion: The subjects of this study were elderly patients who underwent cataract surgery. This time, it was shown that the decrease in TH levels observed after the AX intake that we had reported is likely to be attributable more to the response of subjects aged below 70. Since TH level reflects general antioxidative status, the possibility was suggested that AX intake would exert greater antioxidative effect in younger population. In the future, comparative studies involving younger subjects and studies employing different AX doses would be needed. scavenging activity, H2O2 level,

Poster 7

Experimental safety evaluation of intraocular lenses using rabbit cataract model

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Purpose: Glistening and sub-surface nano glistenigs (SSNG) are well known as opacifications of intraocular lenses (IOLs) after cataract surgeries. However, it is difficult to analysis it in eyes. In this study, the opacifications were evaluated using rabbit eyes.

Methods: Eyes of 8 weeks albino rabbits weighting 2 kg had phacoemulsification in 2.4mm corneal insertion, after that, 5 types of hydrophobic acrylic IOLs (SN60WF before changing of IOL production process by Alcon (Q code), SN60WF after changing of IOL production process by Alcon (A code), ZCB00 Johnson and Johnson, YP2.2 Kowa, XY1 HOYA) were implanted. After 6 months post operation, the IOLs were extracted and cleaned to remove attachment proteins. The IOLs were placed in our model eye maintaining a constant temperature. The surface and inner light scatterings of the IOLs were imaged and analyzed with an anterior segment analyzer (EAS-1000, NIDEK). The intensity of light scattering was quantified in a selected area of surface or inner of IOL using densitometry. To measure the increasing rate of the light scatterings, these unused IOLs were also quantified.

Results. The ZCB00V, YP2.2 and XY1 developed a few glistening but no SSNG. However; the Q code developed glistening and 2 moderate and 2 mild SSNGs and the A code developed glistening and 3 mild SSNGs.

Conclusions. The long periods implantation in rabbits are useful experiment to evaluate glistening and SSNG in hydrophobic acrylic IOLs. The resent IOLs are designed to prevent water phase separations.

Poster 8

Effect of capsular tension ring to prevent posterior synechia after cataract surgery using rabbit models

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Purpose: It is well known that the rabbit cataract model is useful to study secondary complications after cataract surgery. However, the model causes strong inflammation and the posterior synechia between iris and lens capsule. It disturbs the observations of intraocular lens (IOL) and posterior capsule. In this study, we investigated the effect of capsular tension ring (CTR) to prevent posterior synechia after cataract surgeries.

Methods: We prepared CTR130A1 (HOYA), hydrophobic acrylic IOLs 255 (HOYA) and XY1 (HOYA). Japanese white rabbits of 10-week old (around 2.5kg) were also prepared. After general anesthesia, cataract surgeries were performed. The rabbits were divided into 4 groups with 5 eyes (1: 255 only, 2: 255+CTR, 3: XY1 only, 4: XY1+CTR). After the surgeries, we applied 0.1%Rinderon®, 0.5%Cravit®, Midrin-P® and Bromfenac Sodium® twice a day to control inflammation and infection. After 1, 2 and 4 weeks, we observed the anterior segments by slit-lamp microscope. The pictures were graded according to the degree of synechia (grade 0: no synechia, 1: within 1/4 lap, 2: 1/4 to 1/2, 3: 1/2 to 3/4, 4: over 3/4) and they were compared. And histological analysis was performed using hematoxylin-eosin staining.

Results: There are no significant changes were observed until 2 weeks after the operation. After 4 weeks, significant synechia were observed on no CTR group (Group 1: 3.4 and Group 3: 2.4). However, the CTR suppressed the synechia (Group 2: 0.8 and Group 4: 0).

Conclusion: Posterior Synechia occurred between 2 and 4 weeks easily using rabbit models after cataract surgery. The CTR prevents the synechia by extending lens capsule. The CTR will be a important item to observe IOLs, anterior capsule contraction and secondary cataract using rabbit models.

Poster 9

Hydrogen prevents corneal endothelial damage in cataract surgery

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Purpose: In phacoemulsification, free radicals cause corneal endothelial damage. H_2 acts as a free radical scavenger. We have already reported that H_2 dissolved in ocular irrigating solution prevented corneal endothelial damage during phacoemulsification in an animal model. In this study, we examined the effect of H2 gas in clinical cases.

Methods: Phacoemulsification was performed in 64 eyes of 32 patients (Age: 75.4±7.68, Male:17, Female:15) using H2 dissolved solution in one eye and conventional solution in oppsite eye. The endothelial cell density (ECD) was measured at the center of the cornea using noncontact specular microscopy preop, 1 day, 1 weeks and 3 weeks postop. This study was approved by the institutional review board of Nippon Medical School Hospital.

Results: The reduction rate of ECD was 11.6±2.2% at 1 day, 14.3±2.6% at 1 week and 16.8±3.0% at 3 weeks in the control group, while 6.0±1.9% at 1 day, 7.8±2.0% at 1 week and 7.8±1.9% at 3 weeks in the H_2 group. The rate was significantly small in the H2 group at all time points.

Conclusion: H_2 dissolved in the ocular irrigating solution evidently reduced corneal endothelial damages during phacoemulsification, suggesting that the considerable part of the damages associated with phacoemulsification is an oxidative stress.

Poster 10

Comparison of visual outcomes after implantation of Symfony® and FINE VISION®

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Purpose: To investigate visual outcomes after implantation of Symfony® and FINE VISION®.

Methods: Symfony® was implanted in 71 eyes of 45 patients and FINE VISION® was implanted in 83 eyes of 51 patients at Nippon Medical School Musashikosugi Hospital between June 2014 and July 2019. Uncorrected and corrected visual acuity for far（5m）, intermediate （60cm） and near（33cm） distance, contrast sensitivity, and visual defocus curve were measured, and patient satisfaction were evaluated 1 months after surgery.

Results: The mean uncorrected visual acuity in Symfony® was 0.31(0.49) for near, -0.14(1.36) for intermediate, 0.03(0.94) for distance. FINE VISION® was0.16(0.70) for near, 0.12(0.75) for intermediate, -0.02(1.05) for distance Log Mar (snellen). Near visual acuity were better for FINE VISION®, intermediate visual acuity were better for Symfony® （$p<0.05$）.The average of contrast sensitivities for Symfony® cases were within the range of standards under40 years old. However, those for FINE VISION® cases were out of range in the high frequency resion.

Conclusion: Near visual acuity for FINE VISION® cases was better than those for Symfony® cases. Both IOLs provided good visual acuity at intermediate and distance.

Poster 11

Clinical evaluation of surface modified intraocular lenses

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Purpose: Surface modification is a useful technology to modify the characteristics of materials. UV/Ozone modification increase the adhesion of intraocular lenses (IOLs) and producing the prevention of PCO. In this study, we evaluate the stability and preventive effects for secondary cataracts using the surface modified IOL.

Methods: Subjects comprised 30 patients, 60 eyes that underwent phacoemulsification. The mean age of patients is 70.8±8.6. Surface modified hydrophobic acrylic IOL (SP2, HOYA) or multiple pieces IOL (FY-60AD, HOYA) were randomly implanted. Images were taken using an EAS-1000 (NIDEK) after 1, 2, 3, 4 and 5 year postoperatively, to analyze the light scattering and posterior sub-capsular opacification (PCO). The images were photographed from 2 directions (0 and 90 degree) and the densities on surface (sub-surface nano glistening), center (glistening) and PCO of IOLs were measured and compared. The probability of survival ratio about YAG laser capsulotomy were also measured at 9 years.

Results: The densities on surface of SP2 and FY-60AD were 2.1 CCT and 5.5 CCT after 5 years. The center was 1.4 CCT and 2.2 CCT. The posterior was 6.5 CCT and 11.4 CCT respectively. There were statistically significances. The survival rates of YAG laser capsulotomy were 74.8% in SP2 and 13.7% in FY-60AD 9 years postoperatively.

Conclusions: Surface modified hydrophobic acrylic IOL (SP2) is prevented PCO for more than 9 years. And the material is also stable during the same periods.

Poster 12

More than just a reactive oxygen species scavenger: grapes prevents UV radiation-induced cataract by upregulating anti-apoptotic protein XIAP

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Purpose: To investigate if grapes may protect against *in vivo* ultraviolet (UV) radiation-induced cataract and to study its mechanism of action by identifying its molecular targets in the lens.

Methods: The grape powder was provided by the California Table Grape Commission (CTGC). C57BL/6J mice were fed with the regular diet, or the grape diet (regular diet supplemented with 5%, 10%, and 15% grape powder) for 3 months. The animals were then exposed to 20.6 kJ/m^2 UV radiation for 15 min to induce cataracts. The degree of the cataract was evaluated under a dissecting microscope. Glutathione (GSH), free protein thiol (PSH), and protein glutathionylation (PSSG) levels were measured to reflect the oxidative damage. To explore its anti-cataractogenic mechanism, agilent literature search (ALS) was used to pull the protein targets of grape powder.

Results: We found that 10% and 15% grape powder diet significantly inhibited the onset as well as the severity of UV-induced cataracts. In the 15% grape powder diet group, the majority of lenses remained largely transparent. The GSH and PSH levels were much higher in the 15% grape powder diet group compared with that of the regular diet control group. The accumulation of PSSG, a marker for protein thiol oxidation, was largely inhibited in the grape powder diet groups. For target prediction, a total of 145 proteins regulated by grapes were identified through ALS and were visualized by protein network. Among these protein targets, X-linked inhibitor of apoptosis (XIAP) was correlated with all of the active ingredients of grapes, indicating anti-apoptotic protein XIAP might be one of the most critical molecular targets of grapes.

Conclusions: Grape powder dose-dependently protected the lens from UV-induced cataract formation. Its protective effects may involve not only directly scavenging free radicals but also activating the XIAP-mediated anti-apoptotic pathway.

Poster 13

Effects of Grx2 on the oxidative damage under hyperoxia in human lens epithelial cells

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Purpose: High concentration of oxygen is an important risk factor for the formation of nuclear cataract. Glutoredoxin 2 (Grx2) plays an important role in regulating redox balance and repairing oxidative stress injury. To observe the influences of hyperoxia on the oxidative damage of human lens epithelial cells (HLECs) and the role of Grx2 in this process.

Methods: The HLECs were cultured in 80% oxygen mixture for 2 days to establish the oxidative damage model induced by hyperoxia *in vitro*. The Grx2 low expression cell model was constructed by plasmid transfection. The differences of Grx2 mRNA level after different culture times were detected by QPCR. The concentrations of GSH and GSSG were detected by microenzyme labeling. Cell survival was detected by CCK-8 assay.

Results: Grx2 mRNA level changed dynamically with culture time, which was increased to 1.67±0.12 times at 6 h, peaked to 3.47±0.35 times at 18 h, and then decreased gradually. After down-regulation of Grx2, the level of GSH decreased by 52.7%, and the level of GSSG increased significantly to 1.28 times under hyperoxia. Cell survival showed that the number of Grx2 low expression cells decreased by 83.0% after 5 days culture under hyperoxia, which was significantly higher than that of the negative control (70.8%).

Conclusions: This study revealed that hyperoxia could induce an increase of oxidative stress level and a decrease of cell viability in HLECs. Down-regulation of Grx2 could aggravate this degree of oxidative damage. Grx2 may play a protective role in the function of HLECs under hyperoxia.

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Poster 14

Inhibitory effects of topical administration of a ROCK inhibitor on in vivo secondary cataract formation

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Purpose: Secondary cataract is the most common complication after cataract surgery. ROCK pathway affects cell migration, proliferation, and differentiation of various kind of cells. To examine whether ROCK inhibiter affects secondary cataract formation, Rho-kinase inhibitor were topically administered in vivo after cataract surgery.

Methods: Phacoemulsification with intraocular lens (IOL) implantation was performed in the right eye of an 8-week-old Japanese rabbits (JW weight approximately 2 kg; n = 7). Y-27632 was topically instilled in the right eye of three rabbit twice daily (Group Y) and normal saline solution was applied as a control to the right eye of four other rabbit (Group C). Three weeks after cataract surgery, sections of the eyes were made, and the area occupied with the proliferated lens epithelial cells and differentiated lens fiber was measured with image analysis software (Image J).

Results: The areas between posterior lens capsule and posterior surface of IOL were 1.21 ± 0.65 and 2.92 ± 0.72 mm2 in Groups Y and C, respectively (p=0.02). The maximum thicknesses in this area were 6.40 ± 2.12 and 13.10 ± 2.82 μm in Groups Y and C, respectively (p=0.02). Furthermore, the total areas in whole lens bag were 3.24 ± 0.91 and 6.54 ± 1.39 mm2 in Groups Y and C, respectively (p=0.01).

Conclusions: These findings suggest that topical administration of ROCK inhibitors may suppress secondary cataracts formation.

Poster 15

Similar structural perturbation in two distinct cataract-associated mutants, Y66N and V41M, of human gammaS–crystallin

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Purpose: Structural studies of cataract-associated crystallin mutants provide compelling rationale for the molecular mechanisms of cataract. We compare the structural perturbations caused by the Y66N mutation with those of the V41M mutation [1] in human gammaS-crystallin (HGS).

Methods: Recombinant proteins were expressed in E. coli [1]. NMR spectra were recorded at 298K in a Bruker Avance 700 MHz spectrometer, equipped with a z-axis gradient TX1 cryoprobe. Backbone amide protons were assigned using 2D [^{1}H, ^{15}N] Heteronuclear Single Quantum Coherence (HSQC) NMR [1].

Results: Y66N has similar overall structure to HGS. Tyr66, in the 2nd Greek key motif (residues 44-86) is integral to the "tyrosine corner". Chemical Shift Perturbation (CSP) data from NMR-HSQC reveal that the main perturbation is localized within the same strand of Greek key 2, and in the neighboring strand of Greek key 1 (residues 5 – 43). Trp 72 (Greek key 2) is perturbed, probably leading to a change in Trp-fluorescence emission.

Conclusions: CSP data for Y66N and V41M [1], show striking similarities: Local structural perturbation seems limited to Greek key motifs 1 & 2. In V41M, Met41 causes a large perturbation in the first strand of Greek key 1, and a smaller perturbation in Greek key 2 due to a new S-pi interaction between Met41 and Trp 46. In Y66N, Asn66 in the first strand of Greek key 2 disrupts the "tyrosine corner", causing a large perturbation in the adjoining strand of Greek key 1. Therefore, two very different cataract-associated HGS mutants produce similar structural perturbations.

[1] Bharat, SV, Shekhtman, A., and Pande, J. (2014) BBRC 443, pp 110-114

Poster 16

Does water content in the lens nucleus change during accommodation?

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Purpose: During accommodation, the crystalline lens gains additional power by becoming thicker and rounder. Since the change in lens geometry has been shown to be predominantly driven by changes in the shape of the nucleus, an increase in its volume should occur during accommodation, however, this has not yet been observed. Alterations in water state (free vs. bound) could explain this change in shape but not volume. To test this hypothesis, we developed *in vivo* magnetic resonance imaging (MRI) protocols to measure changes in nuclear free and total (free + bound) water content during accommodation.

Methods: 7 young, healthy emmetropes underwent MRI with high resolution structural scan and T1 quantitative mapping with a 3T clinical scanner (Skyra, Siemens). During the scan, participants were instructed to focus on a word target with stimuli-to-accommodation (STA) of 0-, 1-, 3- and 5-Dioptres (D). Scans were repeated under pharmacological cycloplegia (1.0% Tropicamide) at a subsequent visit. Lens geometry and nuclear ($r/a \leq 0.4$) free and total water content were extracted from MR-images using custom-written software. Changes with increasing STA were assessed and compared between normal and cycloplegic conditions.

Results: In normal accommodation, lenses became significantly thicker and rounder with increasing STA, while lenses under cycloplegia underwent minimal shape change. Total water content of the lens nucleus did not change with increasing STA in either condition but free water increased more under cycloplegia than in normal accommodation.

Conclusions: MRI can be used to detect changes in lens water content in response to an accommodative stimulus, however, these changes were only observed under cycloplegia. We believe the absence of a shape change allows changes in water content to be observed as a localized increase in free, but not total water, whereas the shape change observed during normal accommodation results in a redistribution of free water.

Poster 17

Cataract surgery triggers remnant lens epithelial to initiate the immediate early response

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Purpose: Posterior capsular opacification (PCO) is a common complication following cataract surgery. While fibrotic PCO pathogenesis is driven by transforming growth factor beta (TGFβ) signaling, it is not known how surgery initiates this cascade. We recently reported that lens epithelial cells (LECs) remaining post cataract surgery (PCS) globally activate the expression of inflammatory mediators a full day prior to the onset of TGFβ signaling, however this study did not reveal the underlying mechanisms.

Methods: Lens fiber cells were removed from adult wild-type (WT) mice to model cataract surgery. RNA was isolated from remnant LECs at 0 and 6 hours PCS, and RNAseq analysis conducted. Transcriptomic changes were validated by immunofluorescence and the function of EGR1 in LECs PCS was investigated in mice homozygous for an EGR1 deletion mutation.

Results: RNAseq revealed that 828 genes were differentially expressed in LECs by 6 hours PCS. The topmost upregulated gene was FosB (244 FPKM) along with other immediate early genes (IEGs) such as Fos (431 FPKM) and EGR1 (364 FPKM). Pathway analysis revealed that these genes were highly enriched in inflammatory signaling components, while few fibrotic markers were upregulated. However, several Wnts were elevated consistent with the onset of canonical WNT signaling in LECs at 12 hours PCS. The role of IEGs in the inflammatory burst was supported by an inhibition of inflammatory mediator expression PCS in EGR1 null mice.

Conclusions: The initial drivers of PCO pathogenesis are unclear. However, LECs drastically alter their transcriptome within 6 hours post cataract surgery, and many of these changes, particularly the induction of IEG transcription factors, have the potential to set the stage for ocular inflammation and fibrotic PCO. This idea is supported by the role for the IEG, EGR1, in regulating a subset of the inflammatory response of lens cells to cataract surgery.

Poster 18

Lens epithelial cell heterogeneity in lens growth and cataract formation

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Purpose: To test a novel hypothesis that the intrinsic heterogeneity of lens epithelial cells regulates lens growth and homeostasis, and to gain insight into the heterogeneity and functional states of lens epithelium in Gja8 or connexin 50 mutant mice.

Methods: Single-cell RNA-sequencing (scRNA-seq) analysis of lens epithelial cells from one-month-old wild-type and Cx50 or Gja8 knockout (Cx50KO) mice was performed. Based on the scRNA-seq date, immunostaining and western blot were conducted to verify the protein expression changes.

Results: In comparison to wild-type control, the Cx50 knockout mice exhibit microphthalmia and smaller lenses with reduced lens epithelium proliferation. The single cell transcriptomic data of postnatal day 21 lenses reveal the expression profiles of several thousand genes and at least six distinctive cell clusters of wild-type lens epithelium but 8 distinctive cell clusters of Cx50KO epithelium. There are many upregulated and downregulated genes between wild-type and Cx50KO epithelial cells, including upregulated genes encoding growth inhibitory transcriptional factors in the small Cx50KO lenses.

Conclusions: The scRNA-seq data indicate that lens monolayer epithelium from the anterior pole to the equator consists of distinctive cell clusters, they are precisely organized for mediating external signals of surrounding ocular tissues and environmental stimuli to regulate the lens growth, size, shape and transparency. Disruptions of specific cell cluster(s) impair the regulation of lens growth and homeostasis, leading to various lens and/or eye pathological outcomes including cataracts and microphthalmia in Cx50KO mice.

Poster 19

Pax6 directly regulates Birc7 to control apoptosis of lens epithelial cells

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Purpose: Pax6 is a master regulator for brain and eye development. Through control of different transcription factors and differentiation related genes, it regulates lens development. Whether Pax6 is implicated in control of lens apoptosis remains to be further studied. Here we show that pax6 can regulate apoptosis through direct control of Birc7, a member of the inhibitors of apoptosis protein gene family.

Methods: Birc7 overexpression αTN4-1 cell line was generated with G418 selection. CRISPR-Cas9 technology was used to silence Pax6 and Birc7. CellTiter-Glo® luminescent cell viability assay and Hoechst staining were used to detect apoptosis. ChIP and EMSA were used to analyze Pax6 control of Birc7 gene expression.

Results: RNAseq analysis revealed that the expression levels of Birc7 in the lenses of wildtype and Pax6 mutant mice were significantly different. ChIP assay and EMSA demonstrated that Pax6 directly controls expression of Birc7 gene. Overexpression of Birc7 in wildtype mouse lens epithelial cells promotes obvious apoptosis, which is in contrast with its canonic function reported in most studies.

Conclusions: Pax6 regulates lens epithelial cell apoptosis through control of Birc 7 gene, and different from its canonic function in most cells, Birc 7 prevents apoptosis in lens epithelial cells (Supported by grants from National Natural Science Foundation of China, 81570824, 81770910, 81970787, and 81900842, and the Fundamental Funds from the State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University).

Poster 20

The SUMOylation E3 ligase PIAS1 regulates apoptosis and inflammatory response in the ocular lens

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Purpose: The protein inhibitor of activated STAT-1 (PIAS1) is one of the well-known SUMOylation E3 ligases. PIAS1 has been shown implicated in different biological processes including control of apoptosis and repression of innate immunity. We previously demonstrated that apoptosis of lens epithelial cells (LECs) induced by stress factors acts as the common cellular basis for non-congenital cataractogenesis. Whether PIAS1 is implicated in the stress-induced lens pathogenesis remains elusive. Moreover, whether PIAS1 can regulate cytokine expressions in LECs remains to be studied.

Methods: Mouse lenses were treated with glucose oxidase (GO) to induce cataract formation. QRT-PCR and western blot were used for analyzing gene expression. PIAS1 expression construct was genrated to establish PIAS1 expression stable line. CellTiter-Glo® luminescent cell viability assay was used to detect apoptosis. CRISPR-Cas9 technology was used to knockout PIAS1.

Results: In the GO-induced cataract model, expression of PIAS1 was significantly downregulated. While overexpression of PIAS1 in mouse LECs accelerates apoptosis, its knockout enhanced viability. The mRNAs for CLL2 and CXCL10 were upregulated in mouse LECs under PIAS1 knockout, but downregulated with PIAS1 overexpression. TGFβ1 and IL1RA mRNA expression showed similar changes. Mechanistically, PIAS1 regulates both interferon and NF-κB pathways.

Conclusions: PIAS1 is implicated in stress-induced pathogenesis of the ocular lens. More importantly, PIAS1 can regulate cytokine expressions in mouse lens epithelial cells through modulating interferon and NF-κB pathways. (Supported by grants from National Natural Science Foundation of China, 81570824, 81770910, 81970787, and 81900842, and the Fundamental Funds from the State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University).

Poster 21

Aquaporin 8 in the lens

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Purpose: Aquaporin 8 (AQP8) is reported to facilitate the diffusion of hydrogen peroxide across cell membranes. The mRNA expression of AQP8 and the localization of AQP8 in the lens were first investigated in our previous reports.

Methods: Lenticular anterior capsule samples, including LECs, were collected during cataract surgery after informed consent. Immunohistochemical staining showed AQP8 was distributed throughout the whole area of the anterior capsulotomy. For investigating the localization of AQP8 in other areas of lens, lens samples were collected from male C57BL/6J mice at 14 weeks of age and observed with a light microscope after immunostained with antibodies against mouse AQP8.

Results: AQP8 labeling was observed surrounding the cytoplasm of LECs. RT-PCR and gel electrophoresis also revealed the presence of AQP8 mRNA in the lenticular anterior capsule. The results of immunohistochemical staining were comparable to those of RT-PCR and gel electrophoresis. The localization of AQP8 in anterior central part of the human lens were confirmed.
In mice, AQP8 labelling was distributed in LECs throughout the central anterior surface to the germinal zone and was detected in the cell membrane and cytoplasm as relatively homogenous small granules. AQP8 was also observed in the cell membrane of fiber cells in the outer cortex while not detected in the deep cortex and posterior pole of the lens. Further, the whole lenses of mice were observed with a scanning electron microscope after immunostained. Homogenous small particles were detected on the anterior through to the equator of the lens surface, while not on the posterior surface. The same particles were detected on the surface of fiber cells in superficial cortex while not in deep cortex nor those near to the posterior pole.

Conclusion: The results indicate the distribution of AQP8 in the lens. This is the first investigation confirming the distribution pattern of AQP8 in the lens.

Poster 22

bA3/A1-crystallin effects on the pathogenesis of persistent fetal vasculature (PFV) disease by regulating EGFR/MTORC1/autophagy signaling pathway

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Purpose: Persistent fetal vasculature (PFV) is a human disease that results from failure of the fetal vasculature to regress normally. The regulatory mechanisms responsible for fetal vascular regression remain obscure, as does the underlying cause of regression failure. Nuc1 is a spontaneous mutation in the *Cryba1* gene (encoding βA3/A1-crystallin). In Nuc1 rat, normal regression of the hyaloid artery is inhibited and astrocytes abnormally ensheath the retained artery. Moreover, the Nuc1 astrocytes show atypical migration and increased proliferation, compared to controls. In this study we have investigated the molecular mechanisms that control proliferation and migration of astrocytes in Nuc1 rats.

Methods: Astrocytes were isolated and expanded in culture from the optic nerve of Nuc1 and wild type (WT) rats. MTS and wound healing assays were performed to investigate proliferation and migration respectively. GFP-RFP-LC3 construct was used to study lysosomal degradation of autophagosomes. Western blotting was used to study autophagy flux, MTORC1 activation, epidermal growth factor receptor (EGFR) expression and.

Results: Our studies in Nuc1 rat indicate that the autophagy pathway is severely impaired in Nuc1 ocular astrocytes. Further, we show that CRYBA1 interacts with EGFR and that loss of this interaction in Nuc1 astrocytes increases EGFR levels. Moreover, our data also show a reduction in EGFR degradation in Nuc1 astrocytes compared to control cells that leads to over-activation of the MTORC1/mechanistic target of rapamycin complex 1 pathway. The impaired EGFR/MTORC1/autophagy signaling in Nuc1 astrocytes triggers abnormal proliferation and migration. Interestingly, gefitinib (EGFR inhibitor) treatment not only rescued abnormal proliferation, migration, and autophagy flux in Nuc1 astrocytes, but significantly reduced the retention of the hyaloid artery in Nuc1 rats.

Conclusion: Herein, we demonstrate *in vivo* that gefitinib (EGFR inhibitor) can rescue the PFV phenotype in Nuc1 and may serve as a novel therapy for PFV disease by modulating the EGFR/MTORC1/autophagy pathway.

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Poster 23

Analysis of tissue interaction involved in lens glutathione homeostasis in chicken embryo lens ex vivo models

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Purpose: The lens epithelium and immature differentiating fiber cells are thought to be responsible for providing most of the lens glutathione (GSH) via GSH de novo synthesis. In addition, several reports suggest that adjacent ocular tissue such as vitreous may also help regulate glutathione homeostasis of lens. Since the synthesis ability of GSH in lens is known to decrease with aging, the regulation of GSH levels in lens by surrounding ocular tissue will take a more important role in aged human lenses due to impaired de novo synthesis. In this study, we examined the effect of surrounding tissues such as iris and vitreoretinal on GSH levels in lens using chicken embryo lens ex vivo models.

Methods: Lens explants were obtained from 20-embryonic day-old chickens. We prepared lenses, iris-attached lenses and lenses with iris and vitreous. Chicken lenses were placed in 48-well plate containing serum-free medium M199, supplemented with antibiotics. We confirmed the %LDH release values were similar, suggesting that the degree of lens membrane damage during enucleation was comparable between lenses with or without iris.

Results: After ex vivo culture, lens opacity was observed in lens without iris, and GSH concentration in the lens was prominently decreased within 24 hours of ex vivo culture. There was no distinction in GSH efflux between lens and iris-attached lens. GSH/GSSG ratio was also found to reduce in opacified lens compared to transparent lens. The tissue adhesion of iris tended to maintain lens transparency compared to lens without iris, and GSH concentration in the iris-attached lens was also significantly higher than lens alone.

Conclusion: Our results suggested tissue interaction between lens and iris may be involved in maintenance of GSH levels in lens. The mechanism is under consideration, but GSH synthesis may be transiently maintained by iris adhesion.

Poster 24

Lens filament proteins CP49 and filensin are required for cold cataract formation in young mouse lenses

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Purpose: To investigate the molecular and cellular mechanism of cold temperature induced cataract in young lenses of the wild-type C57BL/6J (B6), the CP49 knockout (KO) and the filensin KO mice. To determine how lens specific intermediate filament proteins, CP49 and filensin, are involved in cold cataract formation in interior lens fibers.

Methods: Cold cataract formation was examined and imaged at different temperatures using dissecting microscopic imaging. Lens vibratome sections were immunostained and confocal imaged for characterizing fiber cell morphology and membrane/cytoskeleton structures.

Results: B6 wild-type lenses from postnatal day 14 mice under 25℃ and 4℃ treatment show the hollow-shaped cold cataracts in inner fibers about 245-420 μm (zone II) and 420-575 μm (zone III) from lens surface, intermediate regions between lens cortex and lens nucleus. In contrast, both the CP49 KO and filensin KO lenses show no cold cataract at 25℃, and a reduced cold cataract in zone II at 4℃. Immunofluorescent staining data reveal CP49 and filensin proteins accumulate or aggregate at fiber cell boundaries only in cold cataract zones.

Conclusions: CP49 and filensin are essential for cold cataract formation in zone III inner fibers of B6 wild-type lenses, their accumulation and/or aggregation at fiber cell boundaries might contribute to the light scattering. Cold cataract in zone II fibers is independent of CP49 and filensin. Cold cataract formation of inner fibers results from alterations of distinct high molecular weight complexes which included intermediate filaments, in addition to γ-crystallin aggregation/precipitations reported previously.

Poster 25

An unexpected role for aldose reductase in regulation of lens regeneration

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Purpose: After cataract surgery, residual lens epithelial cells (LEC) adhering to the capsular bag can respond along divergent pathways, generating mesenchymal cells leading to fibrosis or alternatively differentiating into lens fiber-like cells. The fibrotic response can lead to posterior capsular opacification (PCO), while the differentiation response can lead to regeneration of lens material. The purpose of our study was to explore a possible role for aldose reductase (AR) in the LEC response to lens extraction.

Methods: Lenses were removed from C57BL/6 (WT), AR-transgenic (AR-Tg), and AR null (ARKO) mice using an extracapsular lens extraction (ECLE) procedure. A subset of WT mice were then maintained on the AR inhibitor Sorbinil. Eyes were harvested 8 weeks postoperatively and examined with either H&E staining or immunostaining for the lens fiber cell marker γ-crystallin. Histological sections were digitized to produce three-dimensional reconstructed lens models, facilitating comparison of both morphology and size across experimental groups. Unoperated eyes were used as size controls.

Results: Eight weeks after ECLE, ARKO mice eyes contained a regenerated intracapsular, lens-like structure, exhibiting a circular shape and bow region with elongated, nucleated cells that were immunopositive for γ-crystallin. Although WT and AR-Tg eyes also produced an intracapsular, γ-crystallin-positive mass after ECLE, their structures were smaller, were less lens-like in shape, and lacked recognizable lens-like cellular organization. WT mice treated with Sorbinil also exhibited significantly more signs of lens regeneration compared to WT and AR-Tg mice, as assessed by the size and 3D morphology of the regenerated material. Studies of LEC cultures showed higher levels of regeneration markers Bmi1 and Pax6 in ARKO and sorbinil-treated WT cells as compared with WT controls.

Conclusion: These results are consistent with the idea that AR plays a critical role in the post-operative response of LECs, and suggest that AR inhibition may result in enhanced lens regeneration.

Poster 26

In vivo density change of lens epithelial cells induced by UVR exposure

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Introduction: Our research group focuses on the mechanism of ultraviolet radiation (UVR) induced lens damage. Posterior capsule opacification (PCO) is the most common complication for cataract surgery. PCO results from the growth and abnormal proliferation of lens epithelial cells (LECs) on the posterior capsule. Lens epithelium is the first target for toxic factors such as ultraviolet radiation. UVR-300 nm (UVR-B) is especially an important risk factor for cataract development. Our previous findings show that exposure to UVR-B can damage the lens epithelium, affect the expression of proteins, induce apoptosis and lead to cataract formation. Therefore, it is possible to prevent PCO by sufficient dose exposure of ultraviolet radiation to residual lens epithelial cells after cataract surgery.

Purpose: To determine the effect of different doses of UVR on the distribution of lens epithelial cells (LECs).

Methods: Altogether, 40 Sprague Dawley rats were unilaterally exposed in vivo to 1, 3, 6 and 8 kJ/m^2 UVR-300 nm for 15 minutes. One week after UVR exposure, exposed and contralateral non-exposed lenses were removed. One midsagittal section from each lens was stained with DAPI for fluorescence microscopy in order to determine the spatial distribution of LECs.

Results: The LECs density difference between exposed and contralateral non-exposed eyes at exposure dose of 1, 3, 6 and 8 kJ/m^2 was estimated as a CI(95%), -0.1±2.1, 0.1±3.0, -4.1±1.4 and -2.9±2.7
$Cell \bullet \mu m^{-1} \bullet 10^{-2}$, respectively. Data was fit to a linear model considering the initial density difference at 0. The inclination coefficient was estimated as CI(95%), -0.4 ± 0.1 $Cell \bullet \mu m \bullet kJ^{-} \bullet 10^{10}$.

Conclusion: UVR-300 nm induces significant LECs density decrease at exposure dose ≥ 6 kJ/m^2. With fitting to a linear model the evolution of dose- cell density dependence can be determined.

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Poster 27

Modeling congenital cataract in vitro using mouse-specific induced pluripotent stem cells in a reproducible, controlled manner

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Purpose: Pathological mechanisms of inherited and early developmental cataractogenesis remain largely unknown in part due to lack of appropriate cellular models. The aim of this study is to develop a stable culture system for mouse lens development using pluripotent stem cells.

Methods: Isolated mouse lens epithelia cells were reprogrammed with four Yamanaka factors to generate mouse induced pluripotent stem cells (iPSCs), which were further engineered to carry knockin alleles of fluorescent lens-specific reporters: EGFP and mCherry at the *FoxE3* and *Cryaa* loci respectively. The iPSCs were induced to differentiate into (lens progenitor cells and) lentoid bodies (LBs) using the (classical) three-step procedure by Yang et. al., (2010) employing sequential treatment of growth factors, including Noggin, BMP4, BMP7 and FGF2. We tested if addition of exogenous Wnt5a protein at the third step could accelerate the differentiation process to produce larger LBs . Expression of lens-specific markers was examined by real-time PCR, immunostaining, and Western blotting during the in vitro developmental process. The structure and optical features of LBs were investigated using optical and transmission electron microscopy.

Results: The iPSC-derived LBs exhibited transparent crystalline lens-like structures and expressed lens-specific markers including CRYAA, CRYAB, BFSP1, and MIP. During LBs differentiation, the placodal markers SIX3, PAX6, and the specific early lens markers SOX2, PROX1 were observed at appropriate time points. Microscopic examination revealed the presence of lens epithelial cells adjacent to the lens capsule.

Conclusion: The employment of cell type-specific reporters for establishing and optimizing differentiation in vitro seems to be an efficient and generally reproducible approach for developing differentiation protocols for desired cell populations. Our study provides a robust system for research into pathological mechanisms of early developmental cataractogenesis and screening of drug candidates to treat cataracts.

Poster 28

Epidemiological study of nuclear emergency workers at Tokyo Electric Power Company Fukushima Nuclear Power Plant: findings from cataract study 3-6 years after the nuclear accident

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The lens is highly sensitive to radiation. Radiation cataract is lens damage and excessive proliferation of epithelial cells by radiation exposure. The 2007 Recommendations of the International Commission on Radiological Protection (ICRP) indicates dose limits in Japan for regular exposed workers as 100 mSv/5years but less than 50 mSv/year, and for nuclear emergency workers as 100 mSv/year. In response to the Fukushima nuclear accident in 2011, the Ministry of Health, Labour and Welfare (MHLW) temporarily increased emergency dose limits from 100 to 250 mSv from March 14 to December 16, 2011. During this period 20,000 emergency workers engaged in decontamination operations at Tokyo Electric Power Company (TEPCO) Fukushima Nuclear Power Plant (NPP).
We started a longitudinal cataract study of nuclear emergency workers at TEPCO in 2013. About 700 workers with more than 50mSv radiation dose in Fukushima NPP, Kashiwazaki NPP, TEPCO Fukushima NPP receive annual health examinations are enrolled in our study. Findings from 2013 to 2016 (3-6 years after the nuclear accident) revealed a slightly increased presence of vacuoles, which are often precursors of *posterior subcapsular* cataracts. There was a significant correlation between lens radiation transmittance and age, with older workers showing higher radiation transmittance. After adjusting for age, lens radiation transmittance levels were 86.62±1.82mSv for eyes with transparent lenses, 99.9±41.08mSv for eyes with cortical opacity and 69.81±8.45mSv for eyes with vacuoles.
There was no significant correlation between exposure dose and opacity type. Logistic regression analysis was used to compare workers with less than (low exposure group) and more than (high exposure group) average exposure dose 80mSv. The latter group showed no significant increased risk of any opacity type.
Few studies have reported correlations between low dose radiation exposure and lens opacification at the early stages of radiation exposure. Continued longitudinal investigation is required to monitor any correlations between ocular exposure radiation dose and opacity development.

Poster 29

Lens, zonules and ciliary processes

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Purpose: There have been numerous studies on the mechanisms involved in accommodation. However information on simultaneous changes in all components is scarce because of limitations in available techniques. The iris prevents imaging of the whole lens and associated structures and optical and sonic corrections are needed, reducing confidence in results obtained. Finite Element Models have been constructed using assumptions where data are unavailable, such as the length and location of the zonular fibres. In some cases these appear to be inconsistent with known properties. The present study was undertaken to explore other sources of information and test some of the assumptions.

Methods: Measurements were made on 25 published MRI images of accommodated or disaccommodated eyes and converted to dimensions by reference to the known globe diameters. Although resolution could have been better, it was possible to discern trends and obtain reasonable estimates of simultaneous changes in dimensions and shapes. The size and orientation of the ciliary processes, the location of the lens relative to these and the position of the zonular insertion ring were used to examine the lengths and orientation of the anterior zonules.

Results: Lens dimensions and anterior chamber depths were consistent with published data, providing confidence in the other measurements. The locations of the lens equator as well as the anterior and posterior surfaces indicated that growth in lens thickness was equal on both sides of the equator but most of the increase during accommodation occurred in the anterior and reduced anterior chamber depth. The posterior of the lens moved very little. Circumlental space decreased with lens growth but the distance between the ciliary process and the zonular insertion ring on the lens appeared constant.

Conclusions: Lens thickness change during accommodation is predominantly in the anterior. Questions are raised about the presence and/or function of equatorial zonules.

Poster 30

Pharmacological modulation and changes in osmolarity alter the subcellular distribution of TRPV1 and TRPV4 channels in the mouse lens

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Purpose: The transient receptor potential vanilloid (TRPV) channels, TRPV1 and TRPV4 have been shown to pharmacologically regulate lens hydrostatic pressure and lens volume. Here we show that the subcellular distribution of TRPV1 and TRPV4 can be regulated both by pharmacological modulators of channel activity and changes in extracellular osmolarity.

Methods: Eyes from 6 - 8 week old C57BL/6 mice were extracted and the lenses were either left in the globe with their zonules attached, or removed from the eye by cutting the zonules. In both settings lens were organ cultured in Artificial Aqueous Humor (AAH) of either different osmolarities, or in the absence or presence of specific activators and inhibitors of TRPV1 and TRPV4. Lenses were then sectioned, labelled with either TRPV1 or TRPV4 specific antibodies and wheat germ agglutinin and DAPI to visualise cell morphology and nuclei, respectively, and visualised with confocal microscopy.

Results: In peripheral fiber cells in the outer cortex of the mouse lens, TRPV1 is located predominately in a cytoplasmic pool, but upon incubation in either hypertonic AAH, or the TRPV4 activator GSK1016790A (GSK101), TRPV1 translocated to the plasma membrane. In contrast, in peripheral fiber cells TRPV4 is predominately located in the membrane, but upon incubation in hypertonic AAH, or the TRPV4 activator GSK101, TRPV4 labelling shifted to the cytoplasm.

Conclusions: These results show that in peripheral fiber cells the subcellular location (membrane versus cytoplasmic) of TRPV1 and TRPV4 can be dynamically and reciprocally regulated by pharmacological activation of the two channels and by changes in extracellular osmolarity. These results suggest that TRPV1/4 mediated signaling pathways are strong targets for the development of therapies to regulate lens function and prevent the onset of cataract.

Poster 31

Etv transcription factors functionally diverge from their upstream FGF signaling in lens development

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The signal regulated transcription factors (SRTFs) control the ultimate transcriptional output of signaling pathways. Here, we examined a family of FGF-induced SRTFs – Etv1, Etv 4, and Etv 5– in lens development. Contrary to FGF receptor mutants that displayed loss of MAPK signaling and defective cell differentiation, Etv deficiency augmented ERK phosphorylation without disrupting the normal lens fiber gene expression. Instead, the transitional zone for lens differentiation was shifted anteriorly as a result of reduced Jag1-Notch signaling. We also showed that Etv proteins suppresses mTOR activity by promoting Tsc2 expression, which is necessary for the nuclei clearance in mature lens. These results revealed the functional divergence between Etv and FGF in lens development, demonstrating that these SRTFs can operate outside the confine of their upstream signaling.

Poster 32

Inhibition of gamma crystallins aggregation as a paradigm for the development of anti-cataract drugs

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Purpose: Gamma crystallins are monomeric cysteine-rich proteins thought to be involved in age-related nuclear cataract. This is supported both by genetic and chemical data showing that γ-crystallin mutants are associated with cataract, while oxidative stress destabilizes and precipitates oxidized γ-crystallins, respectively. Gamma crystallins are protected by glutathione and α-crystallins, both levels of which decline in the aging lens. Moreover, ascorbylation alters protein charges and oxidizes the protein, thereby further contributing to destabilization. Based on the concept that α-crystallins protect γ-crystallins via chaperone activity, we hypothesized that it should be feasible to find "α-crystallin mimetic" small molecules/drugs that inhibit γ-crystallin aggregation exposed to H_2O_2 and or heat stress.

Methods: Bovine lens crystallins were extracted and purified over Sephadex G-200 to isolate Pool 1 (a-crystallin polymers), Pool 2 (alpha-crystallin oligomers), Pool 3 beta-H and beta-L crystallins, and Pool 4 (monomeric gamma crystallins). Pools 1-3 and a library of 2560 drugs (500-50 uM) were tested for their ability to protect bovine γ-crystallins (Pool 4, 3 mg/ml) aggregation upon exposure to oxidative (OS) and/or heat shock stress (HSS). Absorbance at 600 nm was used as index of turbidity.

Results: Aggregation of γ-crystallins by HSS/OS was potently inhibited by Pool 1 proteins (α-crystallins) at sub-stoichiometric ratio of 0.5:1 confirming earlier findings. Among 2560 FDA approved and experimental drugs tested at 1:1 drug: protein ratio, 63 had turbidity protecting activity. 4 had antioxidant activity, 31 chaperone activity and 26 had dual antioxidant & chaperone activity. At 0.5: 1 ratio, 7 compounds were obtained that had strong protective activity against γ-crystallin destabilization. When tested against human recombinant γD crystallins exposed to heat shock, overall efficacy was similar to that observed for bovine lens crystallins, implicating thereby a common protective mechanism.

Conclusions: Current studies are aimed at clarifying the mechanism of action and in vivo efficacy of these prototypic drugs.

Poster 33

Heat shock factor 4 regulates lysosome activity by modulating the αB-crystallin-ATP6V1A-mTOR Complex in ocular lens

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Purpose: Germline mutations in heat shock factor 4 (HSF4) cause the congenital cataracts. Previously, HSF4 is involved in regulating lysosomal pH in mouse lens epithelial cell line *in vitro*. However, the underlying mechanism remains unclear.

Methods: The HSF4-deficient mouse lens epithelial cell lines and zebrafish were used in this study. The immunoblotting and quantitative RT-PCR is used for gene expression. The protein-protein interaction was tested with GST-pull downs. The lysosomes were fractioned by ultracentrifugation.

Results: The deficiency of HSF4 or knocking down αB-crystallin by siRNA elevates lysosomal pH and increases the ubiquitination and degradation of ATP6V1A by the proteasome. αB-crystallin localizes partially in the lysosome and interacts with solely the ATP6V1A protein of the V1 complex of V-ATPase. Furthermore, αB-crystallin can co-precipitate with mTORC1 and ATP6V1A in GST pull down assay. Inhibition of mTORC1 by rapamycin or siRNA can dissociate αB-crystallin from the ATP6V1A and mTORC1complex, shortening the half-life of ATP6V1A and increasing the lysosomal pH. Mutation of ATP6V1A/S441A (the predicted mTOR phosphorylation site) reduces its association with αB-crystallin. In the zebrafish model, deficiency of HSF4 reduces αB-crystallin expression and elevates the lysosomal pH in lens tissues.

Conclusion: HSF4 regulates the lysosomal acidification by controlling the association of αB-crystallin with ATP6V1A and mTOR and regulating ATP6V1A protein stabilization.

Significance: This study uncovers a novel function of αB-crystallin of which αB-crystallin can regulate lysosomal ATP6V1A protein stabilization by complexing to ATP6V1A and mTOR. This highlights a novel pathway in which HSF4 regulates the proteolytic process of organelles during lens development.

Poster 34

Morphological changes of lens tissues due to different IOL materials on early cataract postoperatively

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Purpose: Intraocular lens (IOL) materials vary, and postoperative lens tissue reactions such as anterior capsule contraction and subsequent cataract are also different. In this study, we investigated the early histological response after cataract surgery according to IOL materials using rabbits.

Method: Japanese white rabbits of 10-week old were anesthetized and phaco surgeries were performed. Three types of IOLs with different cell adhesiveness on surface were prepared and implanted. Hydrophobic acrylic with posterior surface was UV/Ozone treated XY1 (HOYA), XY1 with the front surface was also UV/Ozone treated XY1+ group, and silicone IOL (Q-310Ai, Star Japan) were implanted randomly. One week after surgery, those eyes were observed by slit-lamp microscope and were extracted for making pathological specimens. Next the eyeball was enucleated after euthanasia, fixed with 4% paraformaldehyde, and embedded in Technovit 8100® (kulzer). The obtained tissue section was stained with hematoxylin-eosin, and the degree of fibrosis of the remaining tissue was graded into 4 grades of level 0-3.

Result: There is no significant difference in opacity of the anterior capsular edge of all eyes. Wrinkle degree in the silicone group was strong and averaged at grade 2.83, whereas the XY1 group had grade 1.5 and XY1+ group had 0.83.

Discussion: The tissue reactions of lens epithelial cells on the surfaces are difference in materials and surface treatments. It may be possible to control complications after cataract surgeries by clarifying the tissue reaction due to IOL materials and properties.

Poster 35

Effects of North American ginseng extracts on lens health and plasma in streptozotocin diabetic rats using early and late treatment

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Purpose: To investigate the effects of aqueous ginsenoside extracts from North American ginseng on blood lipid profile, lens antioxidant status and lens heat shock proteins in Streptozotocin (STZ) type 1 diabetic rats.

Methods: Sprague-Dawley rats were divided into five groups of 16 rats each: 1. 12-weeks normal, 2. 12-weeks diabetic, 3. 12-weeks diabetic ginseng-treated, , 4. 6-weeks diabetic, followed by 6-weeks ginseng treatment (6WG), 5. 6-weeks diabetic. Animals were made diabetic (blood glucose ≥18 mM) by injecting (IP) with 20 mg/kg STZ for 4 consecutive days. North American ginseng aqueous extract (250 mg/kg in drinking water) was administered daily to 6-week diabetic rats. The general pathology of diabetes was assessed using body weight, blood lipid profile, HbA1c level, cataract formation, glutathione (GSH), plasma malondialdehyde (MDA) and total antioxidant level in various tissues.

Results: Lens GSH content was decreased in the diabetic-control group. 6-week ginseng treatment (6WG) significantly increased GSH concentration relative to diabetic group. Plasma glucose, triglycerides, cholesterol/HDL and HbA1c were elevated, and animal weight was decreased in diabetic rats. Ginseng treatment generally reduced triglycerides in diabetic rats. Both early and late treatment with ginseng decreased MDA, triglycerides and cholesterol in diabetic rats. Total antioxidant level in diabetic lenses and plasma decreased compared to normal rats. Cataract grade, measured by ScantoxR, was not significantly changed by treatment. Focusing ability measured by NIH contrast “ImageJ” although decreased in diabetes, was lower if only treated with 6 weeks diabetic ginseng.

Conclusions: Dietary ginsenoside extracts from North American ginseng started immediately or even delayed for 6-weeks has beneficial effects on preserving more normal GSH levels in the lens and triglyceride levels in plasma in a type 1 diabetic rat, even without insulin treatment.

Poster 36

Validating novel, lens signal pathway-mediated gene expression using human pluripotent stem cell-derived lens cells

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Purpose: The ability to predict how gene expression is controlled by signaling pathways will aid our understanding of both tissue and disease development. We recently developed an algorithm that predicts tissue-specific signaling pathways based on transcriptomic and protein-protein interaction data. We used this algorithm to create a universe of signaling pathways predicted to control lens epithelial cell gene expression. Here, we hypothesized that cell culture testing of predicted lens signaling pathways would reveal new details of how lens gene expression is controlled.

Methods: Lens signaling pathways were predicted using the SPAGI algorithm. A subset of predicted pathways was tested using human pluripotent stem cell-derived lens epithelial cells. Cells were treated: with four different growth factor treatments (control, 10 ng/mL FGF2, 100 ng/mL FGF2, 10 ng/mL PDGF); without or with specific chemical inhibitors of ERK1/2 or AKT1; at each of two time-points (20 minutes and 24 hours). Protein and RNA samples were collected from the 24 treatments for reverse-phase protein arrays and RNA-seq analysis.

Results: In total, 72 protein samples were analyzed using 18 antibodies, yielding information on both the expression level and phosphorylation status of predicted signal pathway members. These data support correct prediction of both the relative position of kinases within the signaling pathways, and also the phosphorylation status of kinases and transcription factors. Analysis of the corresponding 96 RNA-seq libraries identified differentially-expressed genes for each treatment. Gene ontology analyses of the differentially-expressed genes identified biological processes that match both the treatment used and known lens biology. Promoter analyses support the involvement of algorithm-predicted transcription factors in regulating the treatment-specific differentially-expressed genes.

Conclusions: These combined, large-scale immunoblotting, RNA-seq and bioinformatics analyses support the SPAGI-predicted lens gene expression networks tested here. This includes the prediction that the transcription factor ELK1 is involved in regulating lens gene expression.

Poster 37

The safety and cytokine levels in the aqueous humor after delayed sequential bilateral cataract surgery

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Purpose: To assess the differences in cytokine levels in the aqueous humor (AH) of bilateral eyes receiving sequential phacoemulsification and intraocular lens implantation.

Methods: The levels of 33 cytokines in AH samples collected from separate single-eye operations of 26 age related cataract patients who experiencing sequential cataract surgery were compared between the first-eye and second-eye groups.

Results: The AH level of transforming growth factor beta 2 (TGF-β2), an immunosuppression regulator, in the second-eye group was signifificantly higher than that in the fifirst-eye group ($p = 0.002$). No differences in the concentrations or detection rates of other cytokines were observed between the first- and second-eye groups.

Conclusion: During bilateral sequential cataract surgery, the AH of the second eye had a higher level of TGF-β2 but not of proinflflammatory cytokines or chemokines compared with those in the first eye, implying a protective mechanism preventing the sympathetic immune reaction induced by the first-eye cataract surgery. (Supported by National Natural Science Foundation of China. No. 81873674)
Key words: bilateral cataract surgery, endophthalmitis, aqueous humor, cytokine