US-Japan Cooperative Cataract Research Group Meeting

日米白内障研究会

December 1-5, 2007
Kailua-Kona, HI

A joint effort of
The National Foundation for Eye Research and
The Japanese Society for Cataract Research

PROGRAM & ABSTRACTS

US – Japan
Cooperative Cataract Research
Group Meeting

December 1 – 5, 2007
Kona, Hawaii

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Abstracts .... 1 – 60

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PROGRAM COMMITTEE:

Peter Kador
University of Nebraska Medical Center

Hiroyuki Matsushima
Dokkyo Medical University

John Clark
University of Washington

Melinda Duncan
University of Delaware

Shelley Golard
University of Washington

Richard Mathias
SUNY at Stony Brook

Thomas White
SUNY at Stony Brook

Acknowledgement

We thank the following for their financial support of this meeting

National Foundation for Eye Research

The National Foundation for Eye Research Congratulates

Jin H. Kinoshita Lecture

Frank Giblin, Ph.D
Eye Research Institute, Oakland University.

Frederick A. Bettelheim Travel Award

Julie Lim, Ph.D.
University of Auckland

Henry Fukui Travel Award

Peter Lauf, Ph. D
Wright State University.

Alvira Reddy Travel Award

Masami Kojima, Ph.D.
Kanazawa Medical University

W. Gerald Robinson Travel Award

Yin Wang, Ph.D.
University of Nebraska Lincoln

Young Investigator Travel Awards

Zeynep Firtina, Ph.D.
University Of Delaware

M. Rachel Kirker, Ph.D
Duquesne University.

Takeshi Tomomatsu MD
University of Fukui

Ayanobu Saitoh MD
Dokkyo Medical University

PRESENTATION GUIDELINES

1. **For Paper Presenters**

- 20 minutes are allowed for each paper: (15 minutes presentation and 5 minutes discussion). Please be punctual with your presentation time.
- Computer projection will be used for the presentations. Your computer can be directly connected to the projector via a standard 15 pin video cable, or you can bring your presentation (preferably in PowerPoint) on a CD or USB flash drive.
- Send questions about computer presentations to sgolard@u.washington.edu now.
- Be sure to check your presentation prior to your session.

2. **For Poster (Hot Topics) Presenters**

- Poster board will be available at the conference.
- The size of the poster board is 64” (160 cm) wide by 48” (120 cm) high. You can use one board per presentation.
- Presenters mount their posters with the indicated number on **Sunday before 8:00 a.m.**
- Posters will remain on display through the meeting and be taken down on Wednesday.
- CCRG committee is not responsible for poster materials left at the close of the meeting.
- Poster presenters should be available during their assigned times on **Sunday** through Wednesday for questions. Request comments and try to bring out discussion with floor participants for your fruitful experiences and improvement of your research.
- You are encouraged to prepare a maximum 3 minute presentation with 2 or 3 PowerPoint slides for introduction of your poster during the “Hot Topics” sessions Sunday, 9:40 a.m. and 3:00 p.m.

2007 CCRG At-A-Glance

Saturday December 1

Registration .... 4:00 p.m.
Welcome Reception .... 7:00 p.m. - 9:00

Sunday December 2

I. Developmental Biology .... 8:00 a.m. – 9:40
Break/Hot topics (HT-1 through HT-8) .... 9:40 a.m. - 10:20

II. Ion & Water Transport, Accommodation and Presbyopia .... 10:20 a.m. – 12:00
Lunch .... 12:00 p.m. - 1:00

III. Structure/Function, Accommodation & Scattering .... 1:00 p.m. – 3:00
Break/Hot topics (HT-9 through HT-18) .... 3:00 p.m. – 4:00

IV. Junctions and Lens Function .... 4:00 p.m. – 6:00

Monday December 3

V. Cell Biology & Cataracts .... 8:00 a.m. - 10:00
Break/Hot topics .... 10:00 a.m. – 10:20
VI. Intracellular Homeostasis, Oxidation & Aging..................... 10:20 a.m. – 12:20

Free Afternoon

Tuesday December 4

VII. On the Pathway to an Accommodating Lens for Cataract Replacement
.... 8:00 a.m. - 10:00
Break/Hot topics .... 10:00 a.m. – 10:20

VIII. Oxidation and Cataracts .... 10:20 a.m. - 11:40
Lunch/Hot topics .... 11:40 a.m. – 1:00

Perspectives from Industry.. 1:00 p.m. – 1:30
IX. Biophysics and Cataract .... 1:30 p.m. – 3:10
Break/Hot topics .... 3:10 p.m. - 3:30

X. Crystallins and Cataracts .... 3:30 p.m. – 4:50

Kinoshita Lecture. .... 5:30 p.m. – 6:30
Cocktails and Banquet .... 7:00 p.m. – 9:00

Wednesday December 5

XI. Amyloid and Cataracts... 8:00 a.m. – 9:00

XII. Cataract Risk Factors .... 9:00 a.m. - 10:20

PROGRAM

(Abstract # is the page number for the full abstract)

Saturday, Dec 1, 2007

Registration ....4:00 p.m.

Welcome Reception ....7:00 – 9:00 p.m.

Sunday, Dec 2, 2007

Session I. (8:00 a.m. – 9:40)
Developmental Biology

Moderators: Tiffany Cook and Richard Lang

1. Transcriptional Regulation of Early Lens Development
Hisato Kondoh,
Yuka Saigou, Yusuke Kamachi and Masanori Uchikawa
Graduate School of Frontier Biosciences, Osaka University

2. A Genetic Analysis of Lens Development and Cataractogenesis in Zebrafish
Jeffrey M. Gross,
Ryan Sze, Julie Hayes, Richard Nuckels
Section of Molecular Cell and Developmental Biology, Institute for Neuroscience and Institute for Cell and Molecular Biology, The University of Texas at Austin, Austin TX 78712, jmgross@mail.utexas.edu

3. Conserved role for Pros/Prox1 during fly and mouse lens development
Tiffany Cook
Mark Charlton-Perkins, S. Leigh Whitaker
Cincinnati Children's Hospital Medical Center

4, Modulators of Prox1 function
Melinda K. Duncan
Xiaoren Chen, Tapan P. Patel, Jennifer R. Taube, Vladimir I. Simirskii
Department of Biological Sciences, University of Delaware, Newark, DE, USA

5. Pygopus2 has a Critical, Catnb-Independent Function in Lens Induction
Ni Song[1,2,4,5],
Kristopher R. Schwab[7], Larry T. Patterson[3], Terry Yamaguchi[6], Xinhua Lin[2,5], Steven S. Potter[2,5], and Richard A. Lang[1,2,4,5]
[1]Divisions of Pediatric Ophthalmology, [2]Developmental Biology, and [3]Nephrology, [4]Children's Hospital Research Foundation, Department of Ophthalmology, and [5]Graduate Program of Molecular and Developmental Biology, [6]College of Medicine, University of Cincinnati, Cancer and Developmental Biology Laboratory, [7]Cell Signaling in Vertebrate Development Section, National Cancer Institute ,Department of Pathology, University of Michigan

Break and Hot Topics
(9:40 a.m. – 10:40)

Moderators: Hiroyki Matsushima, Suraj Bhat, John Clark

HT-1. Slit lamp and histological detection of huntingtin polyglutamine repeats in mouse lenses
Judy M. Clark
Ernest E. Arnett, Teri M. Greiling, Hidayat R. Djajadi, and John I. Clark
University of Washington, Seattle,WA

HT-2. Contrast sensitivity of high value-added intraocular lenses.
Norihito Gotoh [1)]
Hiroyuki Matsushima [1)], Shinichiro Yoshida [2)], Tadashi Senoo [1)]
[1)] Department of Ophthalmology, Dokkyo Medical University (Japan)
[2)] Yoshida Eye Hospital (Japan)

HT-3. Identification of Estrogen-Regulated Genes in the Lens Using the ERΔ3 Transgenic Mouse Model with Inducible Cataracts
M. Rachel Kirker
Sheri L. Rea, Paula Witt-Enderby, Vicki L. Davis
Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, PA

HT-4. Crosslinks of pentosidine and pyridinoline in human diabetic cataract lenses
Hirotaka Hashimoto 1,2
Kiyomi Arai 2, Makoto Chikuda 2, Yoshitaka Obara 3
1,Tsukuba Hashimoto Optical Clinic 2,Department of Ophthalmology, Koshigaya Hospital, Dokkyo University School of Medicine 3,International University of Health and Welfare

HT-5. Change of Light Scattering Intensity in Eyes with Posterior Vitreous Detachment, Monzen Eye Study
Kota Nagai[1)]
H. Sasaki[1,3)] R. Honda[2)] Y. Kawakami[1),3)] Y. Sakamoto[1),3)] J. Qu[1)] K. Sasaki [)]
Kanazawa Medical University (Dept. of Ophthalmology) Kanazawa Medical University (Dept. of Hygiene) Kanazawa Medical University
(Division of Vision Research for Environmental Health)

HT-6. A Common Mechanism for Age-related Cataract in 4 Species
Wolf, N.S.
Pendergrass, W.R.
University of Washington

HT-7. Quantitative Analysis of TGF-β_2 in Human Diabetic Cataractous Lenses
Masaya Nishio[1)]
Takehiko Ise[1)], Kiyomi Arai[1)], Yukihiro Matsumoto[1)], Makoto Chikuda[1)], Yoshitaka Obara[2)]
1) Dokkyo Medical University Koshigaya Hospital
2) International University of Health and Welfare

HT-8. The Biology of Transparency: Non-crystallin Function of αB-crystallin Suggests a Physiological Basis for the Distribution of Crystallins in the Ocular Lens
Suraj P. Bhat
Rajendra K. Gangalum

Jules Stein Eye Institute, Geffen School of Medicine, Brain Research Institute and Molecular Biology Institute @ UCLA, Los Angeles, CA 9007

Session II. (10:40 a.m. – 12:00)
Ion and Water Transport, Accommodation and Presbyopia

Moderators: Jim Hall and Oscar Candia

6. Volume and surface changes of the mammalian lens during accommodation

Oscar A. Candia,
Rosana Gerometta, Aldo C. Zamudio and Chi-wing Kong
Mount Sinai School of Medicine, NY USA

7. Characterization of two Novel Zebrafish AQP0s, Zeb1-AQP0 and Zeb2-AQP0

Alexandrine Froger
Karin Németh-Cahalan, Katalin Kalman, James E. Hall.
Department of Physiology and Biophysics, University of California Irvine, Irvine, CA, 92697-4561.

8. Regulatory volume decrease by intermediate conductance K channels and role of electroneutral cation-chloride cotransporters in human lens epithelial cells.

Peter K Lauf[1,2],
Ameet A. Chimote[1], and Norma C. Adragna[1,3].
[1]Cell Biophysics Group, [2]Department of Pathology, [3]Department of Pharmacology and Toxicology, Wright State University Boonshoft School of Medicine, 3640 Col Glenn Hwy, Dayton, OH, 45435, USA. peter.lauf@wright.edu

9. Regulation of AQP0 water permeability

James E Hall
Karin Németh-Cahalan, Katalin Kalman, Alexandrine Froger
Department of Physiology and Biophysics, UC Irvine, Irvine CA 92697

10. Water, Stiffness and Age

Roger Truscott*
Karl Heys, Michael Friedrich
*Save Sight Institute, University of Sydney and Chemistry Department, University of Wollongong, NSW, Australia

Lunch & Hot Topics **..**12:00 – 1:00

Session III. (1:00 p.m. – 3:00)
Structure/Function, Accommodation & Scattering
Moderators: Guido Zampighi and Tamir Gonen

11. The structural basis for the dynamics of accommodation
J.R. Kuszak[1]
Mike Mazurkiewicz[1] and Rebecca K. Zoltoski[2]
[1]Department of Ophthalmology, Rush University Medical Center; [2]Basic and Health Sciences, Illinois College of Optometry, Chicago, IL, USA

12. Three dimensional structure of a human connexin26 gap junction channel reveals a plug in the vestibule
Atsunori Oshima[1]
Kazutoshi Tani[1], Yoko Hiroaki[1], Yoshinori Fujiyoshi[1], Gina E. Sosinsky[2]
[1] Department of Biophysics, Faculty of Science, Kyoto University, Oiwake, Kitashirakawa, Sakyo-ku, Kyoto 606-8502, Japan
[2] National Center for Microscopy and Imaging Research, Department of Neurosciences, University of California San Diego, La Jolla, CA 92093

13. Conical Tomography and Density Segmentation of Lens Fiber Cells
G. A. Zampighi
Department of Neurobiology and Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles CA

14. The thin junctions of lens fiber cells
Tamir Gonen
Department of Biochemistry, University of Washington, Seattle, WA, 98155, USA

15. Spatially Resolved Ocular Proteomics: Where are we?
Kevin L. Schey
Angus C. Grey, Danielle Thibault, Zhen Wang
Department of Cell and Molecular Pharmacology, Medical University of South Carolina

16. Human Age-Related Nuclear Cataracts: Bridging Ultrastructure and Theory to Understand Sources of Scattering
Costello, MJ
Johnsen, S, Metlapally, S, Gilliland, KO
Dept. Cell and Developmental Biology, Univ. North Carolina, Chapel Hill, NC and Dept. Biology, Duke University, Durham, NC

Break and Hot Topics
(3:00 p.m. – 4:00)
Moderators: Hiroyki Matsushima, Suraj Bhat, John Clark

HT - 9. Superoxide-dependent NADH photo-oxidation enhanced by lambda-crystallin
Masayasu Bando[1]
Mikako Oka[2], Kenji Kawai[1], Hajime Obazawa[3], Makoto Takehana[2]
[1]Department of Ophthalmology, Tokai University School of Medicine, [2]Department of Molecular Function and Physiology, Kyoritsu University of Pharmacy, [3]Eye Research Institute of Cataract Foundation

HT - 10. Transparency in Zebrafish Lens and Cornea
Teri M.S. Greiling
Earnest Arnett, Scott S. Houck, John I. Clark*

HT - 11. Clinical Evaluation of Micro Incision Cataract Surgery (MICS)
Ayanobu Saitoh
Norihito Gotoh, Hiroyuki Matsushima, Tadashi Senoo
Department of Ophthalmology, Dokkyo Medical University

HT - 12. Na-Cl Cotransport (NCC) in Human Lens Epithelial Cells (LECs).
Norma C. Adragna[1,3],
Ameet A. Chimote[1], and Peter K. Lauf [1,2]
[1]Cell Biophysics Group, [2]Department of Pathology, [3]Department of Pharmacology and Toxicology, Wright State University Boonshoft School of Medicine, 3640 Col Glenn Hwy, Dayton, OH, 45435, USA.

HT - 13. Cell-Autonomous Involvement of *Mab21l1* is Essential for Lens Placode Development
Naoki Takahashi[1,2],
Ryuichi Yamada[1], Yoko Mizutani-Koseki[3], Noriko Osumi[4], Haruhiko Koseki[3]
[1]Graduate School of Biological Sciences, Nara Institute of Science and Technology
[2]Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo
[3]Department of Molecular Embryology, Graduate School of Medicine, Chiba University
[4]Department of Developmental Neurobiology, Tohoku University Graduate School of Medicine

HT - 14. Roles for Heat Shock Proteins and Chaperones in Modeling Dietary Strategies for Astronaut Cataract Risk Reduction.
John Trevithick[1,2]
Tomasz Dzialoszynski[1,2], Carmen Carrasquilla[2], Heather Thomas[2]
1.Department of Pathology, Schulich School of Medicine and Dentistry, and 2. School of Kinesiology, University of Western Ontario, London, Ontario, Canada N6A3K7

HT - 15. Influence of the tilting of aspheric intraocular lens on the ocular higher order aberration and the contrast sensitivity function
Yasuo Sakamoto [1,2)]
Hiroko Nakaizumi [1)], Nami Yamamoto [1)], Mayumi Sakamoto [1)], Hiroshi Sasaki [1,2)], Kazuyuki Sasaki [2)]
1) Department of Ophthalmology, Kanazawa Medical University / Japan
2) Division of Vision Research for Environmental Health, Institute of Kanazawa Medical University / Japan

HT - 16. In Vivo Detection of Alzheimer's Disease-Linked Aβ Peptide Accumulation in the Lens
Juliet A. Moncaster[1]
Robert D. Moir[2], Anca Mocofanescu[1], Suqian Lu[1], Mark Burton[1], Ling Fu[1], Joy Ghosh[1], Weilan Xu[1], Maria Ericsson[3], Ernest Arnett[4], Martin Sadowski[5], Thomas Wisniewski[5] Chester A. Mathis[6a], William E. Klunk[6b], John I. Clark[4], Rudolph E. Tanzi[2] and Lee E. Goldstein[1]

HT - 17. Effect of 5-S-GAD (Eye drops) on UV-B-Induced Cataract in Rats
Hiroyoshi Kawada[1,2]
Masami Kojima[1,3,4], Yoko Yamashiro[3], Masamichi Fukuda[1], Takahito Kimura[2], Shunji Natori[5], Kazuyuki Sasaki[3], And Hiroshi Sasaki[1,3]
1Department of Ophthalmology, Kanazawa Medical University, Uchinada, Ishikawa, Japan; 2Teika Pharmaceutical Co., Ltd., Toyama, Japan; 3Division of Vision Research for

Environmental Health, Medical Research Institute, Kanazawa Medical University, Uchinada, Ishikawa, Japan; 4School of Nursing, Kanazawa Medical University, Uchinada, Ishikawa, Japan; 5National Institute of Agrobiological Science, Tsukuba, Ibaragi, Japan.

Session IV. (4:00 p.m. – 6:00)
Junctions and Lens Function

Moderators: Tom White and Lisa Ebihara

17. Regulation of Connexin43 gap junctions in lens homeostasis.

Charles F. Louis[1]
Monica M. Lurtz[11], Yubin Zhou[2], & Jenny Yang[2]
[1]Department of Cell Biology, University of California Riverside, Riverside, CA 92521; [2]Department of Chemistry, Georgia State University, Atlanta, GA 30302.

18. Roles of Gap Junction Communication in Lens Core

Xiaohua Gong
Catherine Cheng, Chun-hong Xia
Vision Science and Optometry,UC Berkeley/UCSF Joint Graduate Program in Bioengineering, University of California, Berkeley, CA, USA

19. Identification and characterization of gap junctional hemichannels from dissociated mouse lens epithelial cells and newly differentiating fiber cells.

Lisa Ebihara
Jun-Jie Tong, Xiaoqin Liu
Department of Physiology and Biophysics, Rosalind Franklin University of Medicine and Science, North Chicago, IL

20. Optimal lens epithelial cell proliferation is dependent on the connexin isoform providing gap junctional coupling.

Miduturu Srinivas
Yang Gao, Leping Li, Caterina Sellitto, Thomas W. White
Department of Physiology and Biophysics, Stony Brook University and Department of Biological Sciences, SUNY College of Optometry,

21. Interactions between Gap Junctional Communication and Mitogen Activated Protein Kinase Signaling in Lens Growth

Thomas W. White[1]
Teresa I. Shakespeare[1], Xiaohua Gong[2], Caterina Sellitto[1]
[1]Physiology and Biophysics, State University of New York; [2]Optometry, University of California, Berkeley, CA.

22. Lens abnormalities in Cx32 for Cx43 knockin mice

Peter R. Brink
Caterina Sellitto, Adam DeRosa, Leping Li, Virginijus Valiunas, Thomas W. White
Physiology and Biophysics, State University of New York, Stony Brook, NY

Monday, Dec 3, 2007

Session V. (8:00 a.m – 10:00)
Cell Biology & Cataracts

Moderators: Marjorie Lou and David Li

23. The effect of high glucose for the transdifferentiation of lens epithelium by transforming growth factor beta.

Takeshi Tomomatsu
Yoshihiro Takamura, Eri Kubo, Syousai Tsuzuki, Yoshio Akagi
Department of Ophthalmology, Faculty of Medical Sciences, University of Fukui

24. Protein Phosphatase-1 Dephosphorylates p53 and Attenuates p53-Bak Pathway to Promote Survival of Human Lens Epithelial Cells
David Wan-Cheng Li[1, 2, 3]
He-Ge Chen[1, 3], Qin Yan[1], Jin-Ping Liu[1], Dan Yuan[1, 3], Mi Deng[1], Lili Gong[1].
[1]Dept of Biochemistry and Molecular Biology, [2]Dept of Ophthalmology & Visual Sciences, College of Medicine, University of Nebraska Medical Center; [3]Key Laboratory of Protein Chemistry & Developmental Biology of National Education Ministry of China, College of Life Sciences, Hunan Normal University,

25. Activation of the unfolded protein response in the lens results in cataracts
Zeynep Firtina
Brian P. Danysh, Melinda K. Duncan
Department of Biological Sciences, University of Delaware, Newark,DE,USA

26. The novel function of thioredoxin as a growth factor in human lens epithelial cells
Marjorie F. Lou[123]
M. Rohan Fernando[1,] Yin Wang[2,] Jennifer Wyatt[2]
[1]Dept. of Veterinary & Biomedical Sciences, [2]Dept. of Biochemistry and [3]Dept. of Ophthalmology, University of Nebraska, NE, USA

27. The role of NADPH oxidase (NOX) in regulating platelet derived growth factor (PDGF) mitogenic signaling in Human Lens Epithelial Cells
Yin Wang[1]
Marjorie Lou[123]
[1]Department of Biochemistry, [2]Department of Veterinary and Biomedical Sciences, and [3]Department of Ophthalmology, University of Nebraska, NE, USA

28. Evaluation of Poly(ADP-Ribose)Polymerase (PARP) Inhibitors on Diabetic Cataract Formation
[1]Irina G.Obrosova,
[2] Weizheng Xu, [2] Jie Zhang, [2]Peter F. Kador, [1]Viktor R. Drel
[1]Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, L; [2]MGI Pharma, Baltimore, MD; [3]Department of Pharmaceutical Sciences and College of Pharmacy, University of Nebraska Medical Center, Omaha, NE

Break & Hot Topics **...**10:00 – 10:20

Session VI. (10:20 a.m. – 12:20)
Intracellular Homeostasis, Oxidation & Aging
Moderators: Rick Mathias and Dee Takamoto

29. Sensing Oxygen in the Lens: Roles for PKC and Gap Junctions
Dolores J. Takemoto
Michael Barnett, Vladimir Akoyev, Laura Grauer, Dan Madgwick, Satyabrata Das, Debarshi Banerjee, and Dingbo Lin
Department of Biochemistry, Kansas State University, Manhattan, KS, USA

30. Morphology, distribution and dynamics of mitochondria of the lens and corneal epithelium

[1,2]Vladimir Bantseev
[2]Jacob G. Sivak
[1]Covance Laboratories Inc., Madison WI USA (current affiliation); [2]University of Waterloo, Waterloo ON Canada

31. Metabolite mapping in the rat lens: new insights into amino acid uptake pathways

Julie Lim[1]
Ling Li[1], Marc Jacobs[1,2], Paul Donaldson[1]
Department of Physiology[1], Bioengineering Institute[2], University of Auckland, Auckland, New Zealand

32. The Effects of Aging and Oxidation on Homeostasis in the Lens

Richard T Mathias
Department of Physiology & Biophysics, SUNY at Stony Brook, NY

33. The use of Two-Photon Excited Flash Photolysis to study intercellular communication pathways in the mouse lens.

PJ Donaldson
AMG Sisley, MB Cannell, C Soeller
Department of Physiology, University of Auckland, Auckland, New Zealand

34. Mass Spectrometry Identification of Post-Translational Modifications in Lens Fiber Connexins

Gunnar Valdimarsson[1, 2]
Werner Ens[3], Ken Standing[3] and David Shearer[1]
Department of Biological Sciences[1], Department of Biochemistry and Medical Genetics[2], and Department of Physics and Astronomy[3], University of Manitoba, Winnipeg, Manitoba Canada

FREE AFTERNOON

Tuesday, Dec 4, 2007

Session VII. (8:00 a.m. – 10:00)
On the Pathway to an Accommodating Lens for Cataract Replacement

Moderators: Arlene Gwon and Hiroyuki Matsushima

35. Characteristics of corneal and sclerocorneal incision for micro incision cataract surgery (MICS)

Masamoto Aose[1)]
Hiroyuki Matsushima[1)], Kouichiro Mukai[2)], Yasuo Ishii[2)], Eiichiro Matsui[1)], Hideho Nobori[1)], Norihito Gotoh[1)], Tadashi Senoo[1)]
1) Department of Ophthalmology, Dokkyo Medical University, Tochigi, JAPAN
2) New Vision eye institute, Tokyo, JAPAN

36. Experimental *In Vivo* Anti-PCO Treatments: Status

Jean-Marie Parel[1,4,7],
Sonia Yoo[1,2], Yoshiko Takesue[3], Bernard Duchesne[4], Viviana Fernandez[5], Robert Augusteyn[6,7], Brien Holden[7,8].
[1]Ophthalmic Biophysics Center and [2]ABLEH, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine; [3]Fukuoka University Department of Ophthalmology [4]University of Liege CHU Sart-Tilman; [5]University of Cali Medical School; [6]University of Melbourne Department of Ophthalmology, [7]VisionCRC and [8]UNSW, Sydney, Australia

37. First Demonstration of Laser-Assisted, Aspiration-Only Crystalline Lens Extraction and Laser Created Capsulorhexis

Ruth Hill Yeilding[1],
Ramon Narnajo Tackman[2], Gary P. Gray[3], R. Ty Olmstead[3] and Randy W. Frey[3]
[1] UTMB Brackenridge Hospital, Austin TX, [2] Universidad Nacional Autonoma de Mexico City, [3] LensAR Inc, Winter Park, FL

38. Different surface modification and CCC (continuous curvilinear capsulorhexis) contraction after cataract surgery

Hiroyuki Matsushima[1]
Kouichiro Mukai[1], Hidetoshi Iwamoto[1], Yoko Katsuki[2], Mayumi Nagata[1] Yoshitaka Obara[3] and Tadashi Senoo[1]
[1]Department of Ophthalmology, Dokkyo Medical University, Tochigi, JAPAN
[2]HOYA Corporation Medical Division, Saitama, JAPAN[3]International University of Health and Welfare, Tochigi, JAPAN

39. Engineering the Crystalline Lens

Arlene Gwon, MD
Lawrence Gruber, B.S., A.H.T.
Advanced Medical Optics, Inc. University of California at Irvine

40. *In-situ* observation of dynamic accommodation

Rebecca K. Zoltoski[1]
Elizabeth Wyles[1], Mike Mazurkiewicz[2] and J.R. Kuszak[2]
[1]Illinois College of Optometry; [2]Department of Ophthalmology, Rush University Medical Center, Chicago, IL, USA

Break & Hot Topics **...** 10:00 – 10:20

Session VIII. (10:20 a.m. – 11:40)
Oxidation and Cataracts

Moderators: John Trevithick and Eri Kubo

41. Proteomic characterization of lens epithelial cells in mice null for the antioxidant protein, Peroxiredoxin 6

Eri Kubo[1],
Nilya Hasanova[1], Yukie Tanaka[2], Fatma Nigar[3], Dhirendra P Singh[3], Yoshio Akagi[1]
1. Department of Ophthalmology, University of Fukui. Fukui, Japan
2. Division of Research Laboratories, Centers for Advanced Research Support, University of Fukui. Fukui, Japan
3. Department of Ophthalmology and Visual Sciences, University of Nebraska Medical Center, Omaha, NE

42. Clarification of Mechanisms of Gas Induced Cataract after Vitrectomy and Gas Exchange

Eiichiro Matsui[1)]
Hiroyuki Matsushima[1)], Kouichiro Mukai[2)] and Tadashi Senoo[1)]
1) Department of Ophthalmology, Dokkyo Medical University School of Medicine, Tochigi, JAPAN.
2) New- vision eye institute, Tokyo, JAPAN.

43. Thioredoxin reductase, but not GSH or catalase, defends human lens epithelial cells against UVA light.

F.J. Giblin
V. A. Padgaonkar, V.R. Leverenz, A.V. Bhat, S.E. Pelliccia
Eye Research Institute, Oakland University, Rochester, Michigan, USA 48309

44. Dynamics of rat lens epithelial cells in sugar cataractogenesis. ~Possible association of basic fibroblast growth factor~

Yoshihiro Takamura
Takeshi Tomomatsu, Eri Kubo, Syousai Tsuzuki, Yoshio Akagi
Department of Ophthalmology, Faculty of Medical Sciences, University of Fukui

Lunch & Hot Topics **..**11:40 – 1:00

Perspectives from Industry......................................1:00 – 1:30

45. The features of HOYA IOL and IOLs trend in Japan

Soichiro Motono – HOYO Corporation

Session IX. (1:30 p.m. – 3:10)
Biophysics and Cataract
Moderators: H. Sasaki and J. G. Sivak

46. Difference of cataract development by different frequency in millimeter wave
Masami Kojima1, 2, 3
Yoko Yamashiro1, Yasuo Sakamoto1,2, Yutaka Kawakami1, 2, Hiroshi Sasaki1, 2, Kazuyuki Sasaki1
Division of Vision Research for Environmental Health, Medical Research Institute, Kanazawa Medical University1
Department of Ophthalmology, Kanazawa Medical University2
Department of Nu

47. Myopia Induced by Retrodots and Nuclear Cataract
Sasaki H[1,2]
Fujita N[1], Sakamoto Y[1,2], Kojima M[1,2], Qu J[1], Yamamoto N[1], Kawakami Y[1], Nagai K[1], Nakaizumi H[1], Sasaki K[2]
[1]Department of Ophthalmology, Kanazawa Medical University, Uchinada, Japan; [2]Division of Vision Research for Environmental Health, Medical Research Institute, Kanazawa Medical University, Uchinada, Japan.

48. “NASCA” – The NASA Study of Cataract in Astronauts. Baseline Analyses
Leo T. Chylack, Jr., M.D.[1]
Alan H. Feiveson, Ph.D.[6] , Leif Peterson, Ph.D.[3,4], F. Keith Manuel, O.D.[5] , Mary L. Wear, Ph.D.[7], William H. Tung, B.S.[1], Francis A. Cucinotta, Ph.D.[6],
1. Brigham and Women’s Hospital, Center for Ophthalmic Research, Boston, MA2. Department of Ophthalmology, Harvard Medical School 3. Baylor College of Medicine (BCM), Houston 4. The Methodist Hospital, Houston 5. Space Center Eye Associates (SCEA), Houston 6. NASA, Johnson Space Center 7. NASA, Wyle Laboratories

49. Quantitative Evaluation of Selective Diffusion within the Anterior Lens Capsule
Brian P. Danysh[1]
Tapan P. Patel[1], David A. Edwards[2], Kirk J. Czymmek[1,3], Melinda K. Duncan[1]
[1]Department of Biological Sciences, University of Delaware, Newark, DE
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[3]Delaware Biotechnology Institute, University of Delaware, Newark, DE

50. The Visual Environment Affects the Refractive Development of the Eye but Not the Lens –The Example of the Fish Eye
Sivak, J.G.
Shen, W.
School of Optometry and Department of Biology, University of Waterloo, Waterloo, Ontario, Canada

Break & Hot Topics **..**3:10 – 3:30

Session X. (3:30 p.m. – 4:30)
Crystallins and Cataracts

Moderators: K. Krishna Sharma and Joseph Horwitz

51. αA^{G98R} -Crystallin: Altered structure, stability and chaperone activity contribute to cataract

K. Krishna Sharma*#
Raju Murugesan* and Puttur Santhoshkumar*
Departments of Ophthalmology* and Biochemistry#, University of Missouri, Columbia, MO 65212

52. Alpha-Crystallins and their role in cataract and other diseases

Joseph Horwitz
Xiaohua Gong
Jules Stein Eye Institute, School of Medicine, UCLA, and School of Optometry, UC Berkeley

53. Identification of Microtubule Interactive Domains in αB crystallin

Scott A. Houck
Joy G. Ghosh, John I. Clark
Department of Biological Structure, University of Washington, Seattle, Washington, 98195-7420, USA

Kinoshita Lecture .... 5:30 p.m. – 6:30
Cocktails & Banquet .... 7:00 p.m. – 9:00

Wednesday, Dec 5, 2007

Session XI. (8:00 a.m. – 9:00)
Amyloid and Cataracts
Moderators: Juliet Moncaster and Yoshihiro Takamura

54. Kinetics of Amyloid Fibril formation in vitro by Human gamma Crystallins
Yongting Wang[a]
Sarah Petty[b], Jonathan King[a]
[a]Massachusetts Institute of Technology, Department of Biology, 31 Ames Street, Cambridge, MA 02139-4307, USA. [b]College of the Holy Cross, Department of Chemistry,1 College St. Worcester, MA 01610, USA

55. Indoleamine 2,3-dioxygenase Over-expression in the Lens
Maneesh Mailankot,
Magdalena Staniszewska, Heather Butler, Lixing Reneker and Ram H. Nagaraj
Case Western Reserve University, Cleveland, OH and University of Missouri-Columbia, MO

56. Metal Analysis in Human Alzheimer Disease Brain & Lens
Lee E. Goldstein[1]
Anca Mocofanescu[1], Suqian Lu[1], Mark Burton[1], Ling Fu[1], Chris Rosen[2], Kathleen A. Bjornstad[2], Eleanor A. Blakely[2], Matthew A. Marcus[3] and Juliet A Moncaster[1]
[1]Molecular Aging and Development Laboratory, Brigham & Women's Hospital, Harvard Medical School, Boston, MA 02115, [2]Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, [3]ALS Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

Session XII. (9:00 a.m. – 10:20)
Cataract Risk Factors
Moderators: Alan Taylor and Sam Zigler

57. Cataract Development in Estrogen Receptor Knock-Out and Transgenic Mouse Models
M. Rachel Kirker[1]
Chi Chao-Chan[2], Sheri Rea[1], Carmen M.H. Colitz[3], and Vicki L. Davis[1]
[1]Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, PA; [2]Immunopathology, National Eye Institute, NIH, Bethesda, MD; College of Veterinary Medicine, [3]Ohio State University, Columbus, OH and Animal Eye Specialty Clinic, West Palm Beach, FL

58. βA3/A1-crystallin is expressed in astrocytes and the Nuc1 mutation produces abnormalities in the retinal vasculature
J. Samuel Zigler, Jr
Debasish Sinha, Stacey Hose and Andrew Klise
Wilmer Eye Institute, Johns Hopkins University School of Medicine

59. Linking risk for nuclear and cortical lens opacities, dietary carbohydrate intake and glycemic index, and protein quality control

Allen Taylor
Chung-Jung Chiu, Fu Shang, Wangwang Jiao, Edward Dudek, Roy C. Milton, Gary Gensler,J.M.
U.S.D. A. Human Nutrition Research Center on Aging at Tufts University, Boston, MA 711 Washington Street, Boston, (CJC , FS, WJ, ED, AT); AREDS Coordinating Center, The EMMES Corporation, Rockville, Maryland (RCM and GG)

60. Multifuctional Antioxidants Delay Cataract Formation

Peter F. Kador,[a,b]
James Randazzo,[a] Hongxia Jin,[a] and Karen Blessing[a]
[a]College of Pharmacy, [b]Department of Ophthalmology, University of Nebraska Medical Center, Omaha, NE, U.S.A

CCRG gratefully acknowledges support for the 2007 meeting from the following corporations.

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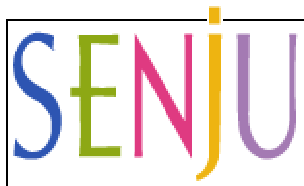

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ABSTRACTS

Transcriptional regulation of early lens development

Hisato Kondoh
Yuka Saigou, Yusuke Kamachi and Masanori Uchikawa
Graduate School of Frontier Biosciences, Osaka University

Purpose: Several transcription factors, such as Sox2, Pax6 and FoxE3, play pivotal roles in the regulation of early lens development. Two basic problems concerning their roles, namely (1) how these transcription factors are regulated and (2) what kind of target genes these factors regulate, must be answered to elucidate the molecular processes in which these factors are involved.

Methods: A focus of our research has been on the regulation of the *Sox2* gene in lens development. During the early stages of lens development, three enhancers having different spatio-temporal specificities appear to regulate the *Sox2* gene in the development of lens and retinal tissues. These *Sox2* enhancers have been subjected to intensive study.

Results and conclusion: The regulation of these *Sox2* enhancers was studied by analyzing interacting transcription factors. Individual enhancers of *Sox2* were knocked out in the mouse genome, and its consequences on lens and eye development were also investigated. The results indicate that auto-regulatory loops operating on *Sox2* and *Pax6* expression are central to early eye development, and that multiple parallel pathways of gene regulation reinforce normal lens development.

A Genetic Analysis Of Lens Development And Cataractogenesis In Zebrafish

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Ryan Sze, Julie Hayes, Richard Nuckels
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Purpose: Zebrafish have become increasingly useful as animal models of human visual system disorders as researchers can perform large-scale genetic screens and rapidly identify the affected loci. We have utilized zebrafish to identify gene products important for lens development and cataractogenesis.
Methods: Forward genetic screening has identified recessive mutations in the genes encoding laminin γ1 (*lamc1*), laminin β1 (*lamb1*), and a protein with Ubiquitin-like, PHD, SRA/YDG and RING finger domains (*uhrf1*) that result in lens abnormalities. Through a complementary reverse genetic screening approach with morpholino antisense oligonucleotides, we have identified a fourth locus required for lens development, *cugbp1*, which encodes a polyadenylated RNA-binding protein. Molecular, ultrastructural, biochemical and *in vivo* confocal imaging assays have been utilized to characterize these mutants and "morphants".
Results: *lamb1* and *lamc1* mutants possess defects in the formation of the lens capsule basement membrane, which lead to lens dysplasias and lens degeneration. *uhrf1* mutants also present with lens dysplasias but here it appears that they result from an overproliferation of cells within the anterior lens epithelium that leads to dysregulated lens fiber formation and rupture through the capsule. We hypothesize that uhrf1 functions as a chromatin binding E3 ubiquitin ligase that modulates cell cycle progression within the lens epithelium, and we are testing this hypothesis through additional molecular and biochemical assays. Loss of *cugbp1* function leads to cataracts, and we hypothesize that cugbp1 regulates cell cycle exit and differentiation of lens fibers.
Conclusion: These zebrafish mutants and "morphants" identify novel loci involved in lens development and cataractogenesis.

Conserved role for Pros/Prox1 during fly and mouse lens development

Tiffany Cook
Mark Charlton-Perkins, S. Leigh Whitaker
Cincinnati Children's Hospital Medical Center

Purpose: – In vertebrates, a functional lens requires the ability of lens fiber cells to differentiate, elongate, and express appropriate levels of refractive crystallin molecules. Factors which affect any of these processes are detrimental and can lead to the formation of cataract disease. This proposal is focused on developing a new genetic model that will allow us to rapidly identify new pathways that are necessary for lens development as a means to circumvent events that lead to cataract formation and blindness.
Methods: Prox1 is a transcription factor that has recently been shown to regulate mouse lens fiber elongation and crystallin gene expression. Unfortunately, Prox1 knockout animals die by embryonic day 13.5, making further analysis of this phenotype difficult. The fruit fly *Drosophila melanogaster*, like vertebrates, expresses a Prox1-related factor, Prospero (Pros), in cells that form the lens. Eye-specific loss- and gain-of-function experiments analyzed the role for Pros during fly lens cell development.
Results: Pros is essential for normal lens formation in the fly eye. Similar to Prox1, Pros affects lens cell differentiation and crystallin expression. We also found two transcription factors that genetically interact with Pros to regulate lens development.
Conclusion: This is one of the first reports that lens formation in invertebrates and vertebrates shares similar transcriptional regulatory pathways. These studies provide the framework necessary to perform more in-depth genetic studies aimed at identifying key regulatory pathways necessary for normal lens development as a means for understanding what aspects of these pathways are disturbed in patients suffering from congenital and age-related cataracts.

Modulators of Prox1 function

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Xiaoren Chen
Tapan P. Patel
Jennifer R. Taube
Vladimir I. Simirskii

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Purpose: –Prox1 is a transcription factor important for lens fiber cell differentiation which has been implicated in both transcriptional activation and repression of genes important for lens function. Here we explore the molecular basis of its diverse functions.
Methods: Prox1 binding sites were characterized by DNaseI footprinting, gel shifts, computer modeling, transfection analyses and chromatin immunoprecipitation. Prox1 interacting partners were identified by the yeast two hybrid assay and characterized functionally in transfection tests.
Results: Prox1 interacts with different sequences in the chicken βB1-crystallin promoter, notably, Prox1 transactivates via a relatively low affinity site while it represses gene expression through the high affinity sites. Prox1 interacts with proteins involved in cell cycle control such as PCNA and PA2G4, the RNA splicing factor SC35 as well as chromatin remodeling complexes and these interactions modulate Prox1 function as a transcription factor.
Conclusion: Prox1 function as a transcription factor is complex and can be altered by its precise molecular contacts with DNA and by interactions with a variety of different protein partners.

Pygopus2 has a critical, Catnb-independent function in lens induction

Ni Song[1,2,4,5]
Kristopher R. Schwab[7], Larry T. Patterson[3], Terry Yamaguchi[6], Xinhua Lin[2,5], Steven S. Potter[2,5], and Richard A. Lang[1,2,4,5]

Divisions of Pediatric Ophthalmology[1], Developmental Biology[2], and Nephrology[3], Children's Hospital Research Foundation, Department of Ophthalmology[4], and Graduate Program of Molecular and Developmental Biology[5], College of Medicine, University of Cincinnati, Cancer and Developmental Biology Laboratory[6], Cell Signaling in Vertebrate Development Section, National Cancer Institute ,Department of Pathology[7], University of Michigan

Drosophila Pygopus was originally identified as a core component of the canonical Wnt signaling pathway and a transcriptional coactivator through interaction with Catnb. Here we have investigated the microophthalmia that arises in mice with a germ-line null mutation of *Pygopus2*. We show that this phenotype is a consequence of defective lens development at inductive stages. Using a series of regionally limited cre recombinase transgenes for conditional deletion of *Pygopus2*^{flox} we show that Pygopus2 activity in pre-placodal presumptive lens ectoderm, placodal ectoderm and ocular mesenchyme all contribute to lens development. In each case Pygopus2 is required for normal expression levels of the critical transcription factor Pax6 by acting in parallel to Pax6^{preplacode} and N-cadherin. Finally, we provide multiple lines of evidence that although Pygopus2 can function in modulating the Wnt pathway its activity in lens development is independent of Catnb. Interestingly, ectopic lenses formed in the head surface ectoderm outside the presumptive lens ectoderm when Catnb but not Pygo2 was deleted in the OM, suggesting Catnb was involved in a lens suppression function of the OM. This study provides important in vivo data that will facilitate a further understanding of Wnt signaling pathway and lens development in the future.

Volume and surface changes of the mammalian lens during accommodation

Oscar A. Candia, Rosana Gerometta, Aldo C. Zamudio and Chi-wing Kong

Mount Sinai School of Medicine, NY USA

During accommodation mammalian lenses change shape between a rounder and a flatter configuration. This requires a change in volume, capsular surface area, or both.

In a human model, 10 diopters of accommodation resulted in a volume change of 2.6 % (AJP; 2007). We have now determined the change in surface corresponding to the volume change by using Pappus's theorem. In the human lens, an increase in surface of 2.0 % concomitant with a 2.6 % decrease in volume was calculated. The "accommodated" surface and volume were 1.72 cm^2 and 0.155 ml, respectively.

In the bovine model, the increase in surface was 4.7 % for a decrease in volume of 5.5% (n=7). The "accommodated" surface and volume were 7.79 cm^2 and 1.77 ml respectively. To determine if the lens could recover its volume within an accommodation time of 200 milliseconds, we photographed the anterior face of rabbit and bovine lenses so that their equatorial diameter could be measured. The lens with the intact zonulae and ciliary body was glued to a stretchable rubber washer. Eight hooks controlled by motors stretched the washer and the lens to an un-accommodated state. A software-controlled system photographed the lens in its accommodated state, after a controlled stretch and 200 milliseconds and 5 seconds after the stretching force was released. Rabbit lenses reverted 96 % of the equatorial diameter increase within 200 ms (n=9). Preliminary experiments with the bovine lens indicate similar recovery.

These results indicate that the accommodated lens can increase its volume and reduce its surface, in 200 ms or less.
Supported by NEI EY00160.

Characterization of two Novel Zebrafish AQP0s, Zeb1-AQP0 and Zeb2-AQP0

Alexandrine Froger

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Purpose: To determine the roles of AQP0 in lens function and development. Two functions have been proposed for Aquaporin 0 (AQP0) in the mammalian lens: water transport and formation of tight junctions. However, the involvement of AQP0 in lens development and function is not entirely understood. Zebrafish is an excellent model for studying ocular development, function and disease. Consequently we searched for a zebrafish AQP0 homologue to investigate the role of AQP0 in lens development and homeostasis.

Methods: MIPfun, the only functionally characterized fish AQP0 from killifish, was used as the query sequence in BLAST searches. Whole-mount in situ hybridization (WMISH) was performed with digoxigenin-labeled antisens RNA probes. cRNA were expressed in *Xenopus* oocytes and their water permeability measured in response to an osmotic challenge. Antisense morpholinos were microinjected into zebrafish embryos to inhibit their gene expression.

Results: The BLAST searches revealed that whereas mammalian genomes contain only one AQP0 gene, the zebrafish genome contains two, Zeb1-AQP0 and Zeb2-AQP0. By WMISH we detected both genes expression in the embryonic lens. When expressed in *Xenopus* oocytes Zeb1-AQP0 was a functional water channel. Disruption of Zeb1-AQP0 and/or Zeb2-AQP0 expression with morpholinos resulted in cataract formation.

Conclusion: Zebrafish is the first species known to express two different AQP0s. In zebrafish embryos both genes expression are lens specific. We find that Zeb1-AQP0 is a functional aquaporin and even if Zeb2-AQP0 function is still under investigation, the disruption of both genes by morpholino injection reveals that Zeb1-AQP0 and Zeb2-AQP0 have essential roles in lens development.

Regulatory volume decrease by intermediate conductance K channels and role of electroneutral cation-chloride cotransporters in human lens epithelial cells.

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Purpose: To understand volume regulation in human lens epithelial cells (LECs) through ion fluxes during hyposmotic regulatory volume decrease (RVD).
Methods: Atomic absorption spectrophotometry of ^{85}Rb uptake and cell K (K_c) and water by wet/dry weight. Exposure of primary human (FHL124) LECs to ouabain ± bumetanide and ion channel-specific inhibitors in varying osmolalities with Na, K or methyl-D-glucamine and Cl, sulfamate or nitrate defining total ion fluxes, Na/K pump, Cl-dependent Na-K-2Cl (NKCC) and K-Cl (KCC) cotransport, K channels and obligatory water loss. Western blots and immunofluorescence microscopy.
Results: In isosmotic (300 mOsM) media, ~90% of the total Rb influx was due to Na/K pump and NKCC, ~ 10% to KCC and a residual ptassium leak. Reducing osmolality to 150 mOsM lowered cell water and K_c by a 16-fold increased K permeability and failed to inactivate NKCC and activate KCC. Sucrose replacement or raising extracellular K but not Rb or Cs to >57 mM in 200 mOsM media prevented K_c and water loss. Rb influx equaled K_c loss, both blocked by clotrimazole (~50 μM), an inhibitor of the intermediate conductance channel (IK), $K_{Ca}3.1$. Other select blockers of K and anion channels, or connexin hemichannels failed to inhibit K_c loss. Western blots and immunocytochemistry revealed presence of IK channels, besides the big conductance (BK) $K_{Ca}1.1$ channel, and small conductance K (SK) channels $K_{Ca}2.1$, $K_{Ca}2.2$, $K_{Ca}2.3$.
Conclusion: $K_{Ca}3.1$ (IK) channels are main players in RVD of FHL124 LECs. Both NKCC activation and KCC inactivation are explained by post-RVD RVI (regulatory volume increase).

Regulation of AQP0 water permeability

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Purpose: –Investigate the regulation of AQP0 water permeability by pH, calcium and zinc and examine its possible role in lens homeostasis
Methods: We make mutations of wild-type aquaporins and inject the resulting cRNAs into *Xenopus* oocytes. We measure the water permeability under different ionic conditions by using video microscopy to record the rate at which injected oocytes swell in hypotonic solutions.
Results: Wild type AQP0 water permeability can be modulated by pH, calcium, and zinc. The modulation by pH depends on external histidines. Calcium modulations involves the cytoplasmic c-terminus and is dependent on the phosphorylation state of a number of serine residues. Zinc modulation is highly cooperative and requires two external histidines H40 and H122.
Conclusion: The water permeability of AQP0 can be altered by the concentrations of H+, Ca2+ and Zn2+,three ions, all present in the lens at concentrations which modulate water permeability in our experiments. Since non-lens aquaporins lack these modes of regulation, it seems probable that they are used by the lens as part of its normal development and maintenance machinery.

Water, Stiffness and Age

Roger Truscott*
Karl Heys, Michael Friedrich

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Purpose: To examine the relationship between the huge increase in human lens stiffness with age, that is the basis for presbyopia, and the state of water in the lens.
Methods: Stiffness was measured using DMA, Total water using TGA and Free water using DSC.
Results: Presbyopia affects everyone by age 50. There are several theories for presbyopia, however, as has been pointed out, the increase in lens stiffness is sufficient to explain its onset. So what leads to this massive age-dependent increase in lens stiffness? Compaction as judged by lens protein concentration is not responsible. Could water play a part? Suggestive evidence came from pig lens experiments where the time that the lens was stored frozen affected its stiffness. Fresh human lenses were slightly stiffer than age-matched frozen lenses.
The percentage of total water in the lens nucleus that was free, was found to increase steadily with age. For example at age 20, for each bound water molecule, there is one free molecule. By age 80 there are 2 free molecules for each bound water molecule.
Cataract lenses, even those with advanced nuclear cataract (Type IV), were not significantly different from age-matched normal lenses either in the total or free water content.
Conclusion: With age, there is a linear transformation of bound water to free water in the centre of human lenses. This parallels the increase in lens stiffness. The reason is still unclear but does not appear to be due to age-dependent aggregation of lens proteins.

The structural basis for the dynamics of accommodation

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Purpose: Restoring the dynamics of accommodation in human lenses with scale 4-D CADs (animations) suggests that the progressively more complex sutures formed after birth are an underlying basis for the mechanism of accommodation.
Methods: The shape, organization, and parameters (width, thickness, and length) of fibers from different age human lenses (20 – 85 years old, n=48+) were ascertained from LM, TEM, and SEM micrographs. The dynamics of accommodation were restored by tweening scale reconstructions of the above human lenses at known time points from both inter- and intra-lens analysis.
Results: Approximately 1,200 growth shells (GSs) are formed after birth and throughout young adulthood. While the middle segment of fibers in all GSs are aligned in the radial cell columns established during gestation, the arrangement of their end segments varies precisely and incrementally in successive GSs. A 3-6 branch suture evolves through infancy, subsequently a 6–9 branch suture is formed throughout adolescence, and a 9–12 branch suture is produced from young adulthood to middle age. Scale 4-D CAD reconstructions of specifically the evolving suture branches in successive GSs demonstrates that cumulatively their alignment throughout the lens produces ascending spiraling suture planes not unlike the blades of a propeller, a specialized screw.
Conclusion: The variable but closely related different suture patterns in successive GSs conveys upon the lens the mechanical properties of a specialized screw, a propeller. In this manner, the circumferential contractile forces of the ciliary muscles are translated into lift during accommodation and compression during unaccommodation.

Three dimensional structure of a human connexin26 gap junction channel reveals a plug in the vestibule

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Purpose: We focus on the structure of Cx26 gap junction channels as determined by electron crystallography, The short cytoplasmic tail of Cx26 makes it more amenable for the formation of two-dimensional crystals.
Methods: We over-expressed a hexahistidine tagged hCx26M34A using baculovirus recombinant expression system. Detergent solubilized and purified proteins were reconstituted into the synthetic lipid (DOPC) to produce two-dimensional orthorhombic crystals symmetry. Four cryo-electron microscopy untilted images were processed and merged at 7Å resolution. Total 254 images were combined for the 3D map at 10Å resolution.
Results: The projection map contained six-fold symmetric channels each of which shows a weaker but significant density in the channel pore. The 3D map revealed that this crystal form contained two sets of symmetry related intercellular channels and three lipid bilayers. Each channel is made up of two hexameric hemichannels docked at their extracellular surfaces. A prominent new density resided in each hemichannel pore was observed and it had contact with the inner most helices of surrounding subunits at the bottom of the vestibule, suggesting that the channel was closed physically and directly by a pore plug.
Conclusion: Our structure strongly suggests a new structural mechanism whereby two interacting Cx26 hemichannels are functionally independent of each other. This structure demonstrates that physical blocking may be an essential gating mechanism of gap junctions, such that the opposing hemichannels can regulate the channel activity autonomously by a pore plug.

Conical Tomography and Density Segmentation of Lens Fiber Cells

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Purpose: To apply conical tomography and density segmentation methods to fiber cells for determining the distribution of proteins in the lenses of normal animals as well as those in the early stages of cataract formation. Conical geometry was selected for its perfect anisotropy in the XY plane since every Fourier section shares a different line with every other section of the series.
Methods: The alignment and reconstruction of the conical series required fiduciary markers in conjunction with the weighted back projection algorithm. Specimen shrinkage from imaging was then corrected using refinement based on projection matching. Analysis of the maps was performed using the semiautomatic segmentation program, JUST, based on the watershed algorithm.
Results: We imaged monoclonal antibodies raised against filensin (CP95), a protein comprising the “beaded” filaments of the fiber cell cytoplasm. The antibody molecules, easily identified by their ‘y’-shaped structure, were bound to the junction between the ~12 nm diameter globular domain of filensin and the core of the filament. The attachment to the plasma membrane and to the fiber junctions, on the other hand, was through the globular domain.
Conclusion: Our results indicate that conical electron tomography in conjunction with density segmentation is an exemplary method for determining the distribution of proteins in their fiber cells. The resolution of ~3 nm allows the identification of molecules based on their dimensions and shape. We thus conclude that studies of lenses with conical tomography are ideally suited for identifying the structural changes underlying the early stages of cataract formation.

The thin junctions of lens fiber cells

Tamir Gonen

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Purpose: To understand how aquaporin-0 forms the lens thin junctions.
Methods: We used electron crystallography to determine the structure of the aquaporin-0 mediated membrane junction to 1.9Å resolution.
Results: Two AQP0 tetramers interact in a head-to-head fashion, through their extracellular domains. Proline residues that are conserved in AQP0 from different species, but which are substituted in other aquaporins, mediate these specific adhesive contacts. In the junctional form, AQP0 channels close and the channel ceases to function as a water channel. Annular lipids in our 2D crystals mediate crystal contacts; and are also involved in forming the thin junctions in vivo.
Conclusion: Our studies indicate that lens lipids may play a crucial role in forming the thin junctions that are abundant in lens fiber cells.

Spatially Resolved Ocular Proteomics: Where are we?

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Angus C. Grey, Danielle Thibault, Zhen Wang

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Purpose: to measure lens protein expression and modification with high spatial resolution
Methods: Laser capture microdissection (LCM) combined with proteomics methods and direct tissue profiling/imaging were employed to detect changes in the lens proteome in a spatially resolved manner. LCM captured tissue was washed and digested with trypsin prior to LC/MS/MS analysis. Lens sections were washed and either spotted or sprayed with MALDI matrix solution for direct tissue profiling or imaging, respectively.
Results: LCM coupled with LC-MS/MS experiments revealed spatial differences in posttranslational modifications including truncation of AQP0 and phosphorylation of AQP0 and phosphorylation of connexins. Direct tissue profiling of lens membrane proteins revealed a novel modification of AQP0 in a fiber cell age-specific locale. In addition, direct tissue imaging provided spatial resolution of major crystallin proteins and their modified forms.
Conclusion: Spatially resolved proteomics is a powerful new tool for measuring spatial distributions of lens proteins and their modifications, distributions that may be critical for proper lens development or for cataractogenesis.

Human Age-Related Nuclear Cataracts: Bridging Ultrastructure and Theory to Understand Sources of Scattering

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Purpose: To use ultrastructure to identify features of the lens nucleus that may contribute to excess scattering during cataract formation and to predict the amount of scattering theoretically.
Methods: Vibratome sections of fresh transparent and cataractous nuclei were fixed for transmission electron microscopy or confocal light microscopy. Confocal serial optical sections were used to examine large volumes for the distribution and size of scattering particles. Separate theoretical approaches were used to predict scattering from each distinct type of structural feature to explain the opacification of mature nuclear cataracts.
Results: Alterations to membranes with age included loss of membrane segments, enlarged extracellular space (ECS) and deposits in the ECS. These changes were enhanced in mature cataracts. The cytoplasm was smooth and homogeneous at low magnification and textured at high magnification, although no distinct high-molecular weight particles were observed. Advanced cataracts had sufficient texturing to contribute to opacification based on Debye-Bueche analysis of random fluctuations in refractive index (RI). Mie scattering theory predicted that large particles, called multilamellar bodies (MLBs), produced forward scatter in mild cataracts and total opacification in advanced cataracts from India with a high density of MLBs.
Conclusion: Regions of low mass density near membranes and dense ECS protein deposits on membranes produce RI fluctuations that contribute to high-angle (small particle) scattering characteristic of the aging lens nucleus. Texturing also increases high-angle scattering but can be pronounced in some advanced cataracts. MLBs increase scattering with age and cataract formation, and, at high MLB density, can render the nucleus opaque.

Regulation of Connexin43 gap junctions in lens homeostasis.

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Purpose: To determine the mechanism by which intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) regulates cell-to-cell communication mediated by Cx43, the major connexin in lens epithelial cell gap junctions.

Methods: Cell-to-cell communication was determined in Cx43-transfected HeLa cells in which ($[Ca^{2+}]_i$ was determined with the Ca^{2+} indicator Fura-2 and simultaneously cell-to-cell communication was determined by analyzing the number of cells to which the injected dye Alexa Fluor 594 transferred.

Results: Extracellular addition of Ca^{2+} in the presence of the Ca^{2+} ionophore ionomycin effected a sustained elevation in the intracellular $[Ca^{2+}]$ that resulted in an inhibition of the cell-to-cell transfer of the fluorescent dye Alexa Fluor594 (IC_{50} of 360 nM Ca^{2+}). The intracellular Ca^{2+}-mediated decrease in cell-to-cell dye transfer was prevented by an inhibitor of calmodulin action. In experiments using HeLa cells transfected with a Cx43 C-terminus truncation mutant ($Cx43^{\Delta 257}$), cell-to-cell coupling was similarly decreased by an elevation of the intracellular $[Ca^{2+}]$ (IC_{50} of 310 nM Ca^{2+}) and similarly prevented by the addition of an inhibitor of calmodulin. We predicted a potential CaM binding site with high predicative score in Cx43 and have applied a variety of biophysical approaches to demonstrate a high affinity Ca^{2+}-dependent binding of CaM to this peptide sequence.

Conclusion: Our data indicate that physiological concentrations of intracellular $[Ca^{2+}]$ regulate the permeability of Cx43 in a calmodulin-dependent manner that does not require the major portion of the C-terminus of Cx43. Our findings also strongly suggest a Ca^{2+}-dependent interaction between CaM and the lens-specific gap junction protein Cx43.

Roles of Gap Junction Communication in Lens Core

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Purpose: To understand how gap junction communication contributes to the formation and maintenance of lens fiber cells in the lens core.
Methods: Lenses of mice with null and/or point mutations of alpha3(Cx46) and alpha8(Cx50) connexin genes were characterized by histology, immunohistochemistry and biochemistry. Living lenses of compound mutant animals of connexin knockouts and GFP transgenic mice were used to evaluate cellular structures and protein distribution.
Results: Loss-of-function mutants of alpha3 and/or alpha8 connexins, due to knockout or point mutation, show sustained and abnormally distributed fiber cell nuclei and/or degeneration of fiber cells in the lens core. A combination of S50P mutant and wild-type alpha8 prevents lens primary fibers to fully elongate to contact anterior epithelium and abolishes actin-filaments in the lens core. Similar to wild-type lenses, alpha3 and alpha8 knockout lenses displayed a mosaic pattern of GFP protein expression in epithelial cells and in peripheral differentiating fibers but a uniform distribution of GFP proteins in lens core. Surprisingly, lenses lacking both alpha3 and alpha8 connexins displayed mosaic pattern of GFP protein expression in all fiber cells.
Conclusion: Inhibition of gap junction communication perturbs fiber cell maturation and/or homeostasis needed for the survival of lens core fiber cells. Interactions between wild-type and certain mutant alpha8 connexins can inhibit cell elongation of lens primary fibers. Absence of gap junction communication abolishes the exchange of GFP proteins in the lens core while the presence of either alpha3 or alpha8 connexin is sufficient for maintaining the macromolecular exchange pathway in the lens core.

Identification and characterization of gap junctional hemichannels from dissociated mouse lens epithelial cells and newly differentiating fiber cells.

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Purpose: To identify and characterize the macroscopic and single properties of connexin hemichannels in lens epithelial cells and newly differentiating fiber cells isolated from mouse lenses.
Methods: Epithelial cells and newly differentiating fiber cells were isolated from 4-8 day mouse lenses. Membrane currents were recorded from lens cells using the whole cell patch clamp technique. The following precautions were taken to minimize contamination from potassium channels and swelling-induced chloride channels: (1) cesium was used as the main cation in the pipette solution; (2) the osmolarity of the pipette and the bath solution were carefully adjusted to reduce osmotic inbalances.

Results: Many of the cells exhibited a multichannel current composed of large conductance channels in the presence of zero added external divalent cations. This current was partially closed at -60 mV, activated on depolarization, and had a reversal potential of ~0 mV. For depolarizing voltage clamp steps to potentials $\geq$ 40 mV, the current rapidly activated and then slowly inactivated. These properties resemble those of Cx50 hemichannels expressed in Xenopus oocytes. Application of 2 mM calcium caused a large reduction in current amplitude. In some cells, only a few channels were observed. These channels typically opened on depolarization and closed on hyperpolarization to -60 mV. They had a single channel conductance (γ_0 = 270-330 pS) that fell into the same range as Cx46 and Cx50 hemichannels.

Conclusion: These results support the existence of connexin hemichannels in dissociated epithelial and newly differentiating fiber cells.

Optimal lens epithelial cell proliferation is dependent on the connexin isoform providing gap junctional coupling.

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Purpose: Gap junctions between epithelial cells are essential for normal lens growth. In mice, knockout of Cx50 or targeted replacement of Cx50 with Cx46 (knockin) caused smaller lenses due to decreased epithelial cell proliferation. However, it remains unclear whether Cx50 functionally contributes to lens epithelial coupling during maximal proliferation on postnatal days 2 and 3 (P2-P3). In this study, we examined junctional coupling from epithelial cells of wild-type and knockin mice at different developmental ages.

Methods: Junctional currents were measured by dual whole cell voltage clamp. Cell proliferation was assayed by BrdU incorporation.

Results: Junctional currents exhibited a developmentally regulated sensitivity to quinine, a drug that blocks Cx50 gap junctions, but not Cx43 or Cx46. Single channel currents had a unitary conductance of 210 pS, typical of Cx50. A correlation between functional activity of Cx50 and maximal proliferation was found. In epithelial cells from P3 wild-type mice, there was a high density of BrdU labeled nuclei in both the central and equatorial epithelium and ≥ 60% of total coupling was provided by Cx50. In older cells, proliferation was greatly reduced and the contribution of Cx50 to total coupling was progressively reduced (≤45% on P12, ≤25% on P28). Functional replacement of Cx50 with Cx46 was correlated with 71% and 13% reductions in BrdU labeled cells in the P3 central and equatorial epithelium respectively and epithelial cell coupling of Cx46 knockin mice had characteristics of Cx46.

Conclusion: These results show that the contribution of Cx50 is highest during peak postnatal proliferation, but progressively declines with age thereafter.

Interactions between Gap Junctional Communication and Mitogen Activated Protein Kinase Signaling in Lens Growth

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Purpose: Knockout of Connexin50 (Cx50) reduced the number of epithelial cells undergoing mitosis during the first postnatal week and resulted in smaller lenses. The mechanisms whereby Cx50 influences mitosis are poorly understood. Growth factors stimulate lens cell proliferation and predominantly function by activating the mitogen activated protein kinase (MAPK) signaling cascade. We tested if Cx50 and the MAPK pathway could function synergistically during postnatal lens growth.
Methods: We used paired Xenopus oocytes to examine the biochemical and electrophysiological interaction of Cx50 and MAPK signaling in vitro. We cross-bred transgenic mice over expressing a constitutively active form of mitogen activated protein kinase kinase (caMEK1) in the lens with Cx50 knockout mice and analyzed lens growth.
Results: Expression of caMEK1 in oocytes casued an increase in ERK phosphorylation. Co-expression of Cx50 with caMEK1 in oocytes caused junctional conductance to increase three fold without altering Cx50 protein expression. Transgenic caMEK1 lenses were ~1.7 times larger than wild-type by 7 weeks of age and had severe nuclear and cortical cataracts, often displaying lens rupture. Cx50 knockout lenses were 40% smaller than wild-type lenses with a mild nuclear cataract. Interbreeding of caMEK1 transgenic and Cx50 knockout mice produced lenses that were 20% smaller than wild-type with severe nuclear cataract, but a transparent lens cortex.
Conclusion: These results show that the Cx50 mediated reduction in lens mitosis can rescue the macrophthalmia caused by overexpression of caMEK1. These data suggest an interaction between MAPK signaling and Cx50 may be necessary for the proper regulation of lens growth.

Lens abnormalities in Cx32 for Cx43 knockin mice

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Purpose: The lens expresses 3 connexins, Cx43, Cx46 and Cx50, each of which has unique properties of permeation and gating. Cx46 and Cx50 are only present in the lens, while Cx43 is ubiquitously expressed. Previous studies using mouse genetics have provided insight into the functions of Cx46 and 50, while little is known about Cx43. To better understand the role of Cx43, we have analyzed lenses from mice where it was replaced by Cx32 by homologous recombination (Cx43KI32).
Methods: Lenses were dissected, weighed and photographed. Differences in channel gating and permeation between Cx43 and Cx32 were analyzed by patch clamp in both isolated lens cells and transfected cell models.
Results: Cx32 and Cx43 had markedly different permeation properties. Homozygous Cx43KI32 animals exhibited a broad spectrum of defects, including lenses that were 60-70% smaller than wildtype controls and had dense cataracts, although this phenotype was not fully penetrant and ~20% of the lenses were transparent and nearly normal in size. Heterozygous Cx43KI32 animals displayed normal eyes and lenses.
Conclusion: Global replacement of Cx43 by Cx32 in mice resulted in a variety of pathologies, including micropthalmia and cataract. These defects do not result from an absence of ionic coupling, but are likely due to differences in channel permeation. We currently do not understand the lack of full penetrance of the lens phenotype.

The effect of high glucose for the transdifferentiation of lens epithelium by transforming growth factor beta.

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Purpose: It is reported that anterior capsular contraction after cataract surgery is greater in eyes of diabetic patients, especially in those with higher flare intensity. We aimed to investigate whether transdifferentiation by transforming growth factor beta (TGF-β) is affected by high glucose exposure.
Methods: Human lens epithelial cells (LECs) were cultured in the medium with 5 or 50mM glucose for 7 days, then the medium was converted to the serum free medium containing TGF-β and cultured for 7 days. The expression levels of α smooth muscle (SM)-actin, a marker for myofibroblastic cells, were examined by Western blot.
Results: Addition of TGF-β enhanced the αSM-actin expression dose-dependently indicting the transdifferentiation from LECs. There were no significant differences of αSM-actin expression induced by TGF-β between cells pretreated with 5 and 50mM glucose medium.
Conclusion: High glucose exposure did not accelerate the transdifferentiation of LECs by TGF-β. Postoperative anterior capsular contraction of diabetic patients may results from higher levels of cytokines, but not the alteration of cellular responses.

Protein Phosphatase-1 Dephosphorylates p53 and Attenuates p53-Bak Pathway to Promote Survival of Human Lens Epithelial Cells

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Purpose: Protein phosphorylation and dephosphorylation are the fundamental mechanisms to regulate various cellular activities such as gene expression, cell proliferation, differentiation, apoptosis, organgenesis, and tissue homeostasis. The serine/threonine phosphatases-1 and –2A are the major cellular phosphatases and contribute to more than 90% serine/threonine phosphatase activity in eukaryotes. In the ocular lens, PP-1 is a more abundant phosphatase than PP-2A and playing important roles in promoting survival of lens epithelial cells. Inhibition of of PP-1 activity leads to apoptosis of lens epithelial cells. Mechanistically, through dephosphorylation, PP-1 is actively modulating the functions of p53, a master regulator of apoptosis. In the present study, we demonstrate that p53 directly regulates Bak, one of the major proapoptotic members of the Bcl-2 family. PP-1 dephosphorylates p53 at multiple residues to attenuate p53 transactivity and thus negatively regulates Bak expression.
Methods: Dephosphorylation of p53 by protein phosphatase-1 was explored with in vitro dephosphorylation assay, co-immunoprecipitation and in vivo dephosphorylation assay. Gel mobility shift assay was used to demonstrate that p53 binds to Bak promoter. Luciferase reporter gene assay was used to demonstrate the differential regulation of phosphorylated and dephosphorylated p53 on Bak.
Results: p53 directly regulates Bak. PP-1 can also directly dephosphorylate p53 at Ser-20 besides Ser-15 and Ser-37. Dephosphorylation of p53 at these sites downregulates its ability to control Bak expression.
Conclusion: Bak is a p53 downstream gene. One of the major mechanisms for PP-1 to promote survival is to negatively regulate Bak and other p53 downstream genes through dephosphorylation of p53.

Activation of the unfolded protein response in the lens results in cataracts

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Purpose: Human diseases caused by mutations in extracellular matrix genes are often associated with an increased risk of cataract. Here we test the hypothesis that the presence of abnormal ECM in the lens results in cataract due to activation of the Unfolded Protein Response (UPR).
Methods: Transgenic mice were created harboring constructs consisting of the δEN/αA-crystallin promoter driving expression of either the mouse collagen α3(IV) or α4(IV) cDNA. Lens phenotypes were characterized by darkfield microscopy and conventional histology. The transgene expression and evaluation of the molecular markers of UPR was tested by immunohistochemistry, rt-PCR and Western blotting.
Results: Lenses overexpressing collagenIV chains were grossly abnormal. Immunostaining detected retention of ectopic collagen IV in transgenic lens cells and reduced collagen IV staining in the capsule. However laminin staining was seen exclusively in the capsule of transgenics indicating it is processed and secreted normally. Lenses showed a 90% reduction in total protein although the overall crystallin profile was not altered. Starting from E12.5, transgenic lenses exhibited a large upregulation of BiP, an ER resident chaperone whose expression upregulates when the protein folding capacity of the ER is challenged. Further, transgenic lenses exhibit elevated levels of processed XBP1 mRNA and protein.
Conclusion: Single Collagen IV chains can not fold and are retained in the ER. This retention attenuates global protein synthesis, activates the IRE1-XBP1 branch of the UPR resulting in elevated BiP expression and cataracts. These data support the hypothesis that activation of UPR pathways in the lens is an important cataractogenic mechanism.

The novel function of thioredoxin as a growth factor in humanlens epithelial cells

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Purpose: To investigate if TRx has a growth factor-like property in which the mitogenic action is mediated through redox signaling.
Methods: Recombinant human TRx was purified by affinity gel column and used to stimulate human lens epithelial B3 cells (HLE B3). Cell proliferation was determined by cell count or BrdU assay while intracellular reactive oxygen species (ROS) were detected by dichlorofluorescein (DCFH) via FACS analysis. NADPH oxidase activity was measured by superoxide anion production using lucigenin-amplified chemiluminescence. H_2O_2 was quantified using a colorimetric method. Cells were gradually starved from serum before use and the cells without TRx treatment were the controls.
Results: TRx (2.5-20 μM) stimulated HLE B3 cell proliferation in a concentration-dependent manner. This TRx-stimulated cell growth could be inhibited by antioxidants. Isolated HLE B3 cell membrane fraction or the whole cells in the presence of Trx produced H_2O_2 in a transient manner. Intact HLE B3 cells could be stimulated by TRx (20 μM) to produce ROS, which were suppressed in cells pretreated with catalase or DPI (NOX inhibitor). Furthermore, cells treated with TRx enhanced NOX activity twofold, which could be inhibited by a pan protein kinase C inhibitor (bisindoylmaleimide).
Conclusions: TRx could stimulate cell growth, mediated by redox cell signaling that was controlled by the ability of TRx to generate intracellular ROS via the membrane-bound NADPH oxidase system. This growth factor/cytokine like property is a novel function in mammalian cells.

The role of NADPH oxidase (NOX) in regulating platelet derived growth factor (PDGF) mitogenic signaling in Human Lens Epithelial Cells

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Purposes: NADPH oxidase (NOX) is a complex system with membrane and cytosolic components whose assembly are necessary for catalytic activity. NOX-generated reactive oxygen species (ROS) are required for growth factor-stimulated cell proliferation. This study is to investigate if p22phox, a membrane component, can regulate NOX activity and control the platelet-derived growth factor (PDGF) mitogenic signaling in human lens epithelial cells.

Methods: Human lens epithelial B3 (HLE B3) cell lines with overexpression and knockdown of p22phox (p22-OE and p22-KD, respectively) were used in comparison with non-transfected HLE B3 cells with and without PDGF stimulation. The relative NOX activity and intracellular ROS generation were detected by lucigenin-based assay and DCFH fluorescence, respectively. Cell proliferation was measured by BrdU- and PicoGreen-based assays. p22phox, P-JNK, P-ERK1/2, P-Akt, P-p38, p47phox and P-PDGF receptor in cell lysates were detected with Western blot analysis, using respective specific antibodies.

Results: Overexpression of p22phox increased NOX activity, cell proliferation, and enhanced the activation of ERK, JNK and Akt signaling cascades, whereas knockdown of p22phox showed opposite results. The overexpression of p22phox also induced more binding between p22phox and the cytosolic component p47phox, and prolonged the activation of PDGF receptor induced by PDGF, while knockdown of p22phox weakened the binding of p47phox and almost totally diminished the activation of PDGF receptor.

Conclusions: PDGF mitogenic action in human lens epithelial cells depends on NOX assembly and activation, which is controlled by the subunit p22phox.

Evaluation of Poly(ADP-Ribose)Polymerase (PARP) Inhi-bitors on Diabetic Cataract Formation

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Purpose: Activation of PARP, an enzyme that cleaves NAD+ with formation of nicotinamide and poly(ADP-ribose) polymer, leads to 1) profound metabolic abnormalities; 2) changes in transcriptional regulation and gene expression, and 3) cell death. Evidence for a key role of PARP in diabetic complications is emerging. This study evaluated two structurally unrelated PARP inhibitors on diabetic cataract formation. **Methods:** Mature (250-300g) male Wistar rats were made diabetic with streptozotocin, and injected with insulin to maintain reasonable hyperglycemia and prevent ketoacidosis and weight loss. Non-diabetic (C) and diabetic (D) rats were treated with/without PARP inhibitors, 1,5-isoquinolinediol (ISO) or 10-(4-methyl-piperazin-1-ylmethyl)-2H-7-oxa-1,2-benzo[de]anthracen-3-one (GPI-15427), at 3 $mgkg^{-1}d^{-1}$i.p. and 30 $mgkg^{-1}d^{-1}$ orally, for 10 wks after first 2 wks without treatment. Lens changes were evaluated by indirect ophthalmoscope and portable slit lamp. PARP-1 and poly(ADP-ribosyl)ated proteins were assessed by Western blot analyses. **Results:** PARP-1 is abundantly expressed in the lens. Poly(ADP-ribosyl)ated proteins were primarily detected in the 38-87 kDa range of protein spectrum, with several minor bands at 17-38 kDa. The 38-87 kDa and 17-38 kDa poly(ADP-ribosyl)ated protein expression increased by 74% and 275%, respectively, after 4 wks of diabetes. Both PARP inhibitors delayed cataract formation. At the 12 week conclusion of the study, clear lenses were detected in 50% in D+GPI and 31% in D+ISO vs 6% in D, vacuolar stage in 12% in D+GPI and 19% in D+ISO vs 25% in D, cortical opacities in 25 % of D+GPI and 38% of D+ISO vs 44% in D, and mature cataract in 12.5% of D+GPI and 12.5% of D+ISO vs 25% in D. **Conclusion:** Diabetes-induced PARP activation is present in the lens and PARP inhibitors delay cataract formation.

Sensing Oxygen in the Lens: Roles for PKC and Gap Junctions

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Purpose: Since hypoxia is a natural state in the lens there must be stress-sensing mechanisms to prevent hypoxia-induced apoptosis. The lens contains two PKC isoforms which have stress-sensing potential within their C1B domains. The purpose of this research was to identify their role in lens under hypoxic conditions.
Methods: Both PKCε and PKCγ activities were measured in N/N 1003A lens epithelial cells or whole lens in culture using a PKC assay and specific PKC immunoprecipitates. Gap junction activity was measured using dye transfer. Interactions of proteins were determined using co-immunprecipitation and confocal microscopy. Activation of mitochondrial cytochrome C oxidase was determined on purified control or knock-out mouse lens mitochondrial fractions under normoxic (21%) or hypoxic (5%) oxygen.
Results: PKCγ was activated by oxidative stress, interacted with Cx43 and Cx50, and caused inhibition of gap junction activity. In contrast, only hypoxia activated PKCε. Under hypoxia the PKCε was removed from Cx43 and Cx50, translocated to mitochondria, interacted with cytochrome C oxidase subunit IV, and activated cytochrome C oxidase activity. This effect was not observed in the PKCε knockout mouse lenses.
Conclusion: The lens has two stress-sensing PKC's: PKCγ which deals with oxidative stress through inhibition of the gap junctions, Cx43 and Cx50, and PKCε which binds to Cx43 and Cx50 under growth conditions. Once activated by hypoxia, PKCε activates cytochrome C oxidase and protects the mitochondria from initiating apoptosis. This allows the lens to protect itself from the gap junction bystander effect, through PKCγ, and mitochondrial apoptosis, through PKCε.

Supported by EY13421 to DJT.

Morphology, distribution and dynamics of mitochondria of the lens and corneal epithelium

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Purpose: Using confocal scanning laser microscopy, the morphology, distribution and dynamics of the mitochondria of the lens and corneal epithelium were compared.

Methods: Isolated bovine lenses and 9.5 mm central corneal buttons were stained using the cell-permeable mitochondria-specific fluorescent dye Rhodamine 123 (20μM). High resolution confocal images and time lapse imaging of lens epithelium and superficial cortex and corneal epithelium were acquired with a Zeiss 510 Meta 18 confocal laser scanning microscope system. Image analysis was carried out using VisArt/Physiology software packages (Zeiss Canada Ltd.).

Results: While evenly distributed mitochondria were seen in the corneal superficial epithelium, in intermediate and basal epithelium the mitochondria were abundant and ranged in length from less then 1μm to threads over 15μm. Overall the distribution pattern of the mitochondria of intermediate and basal corneal epithelium could be identified as structures forming a dense calyx around the nuclei. A similar distribution pattern was found in the lens epithelium. In the lens superficial cortex the mitochondria were much longer and were aligned along the axis of the cells. Mitochondrial dynamic movement up to 5μm/min in the lens epithelium and up to 15μm/min in the superficial lens cortex was seen. As with lens epithelium, dynamic movement was observed in the corneal epithelium.

Conclusion: This study shows similarities in the distribution of the mitochondria between the lens and corneal epithelium. The observed mitochondrial dynamics in both tissues may indicate the transmission of energy presumably allowing local transfer across the cell from regions of low to high ATP demand.

Metabolite mapping in the rat lens: new insights into amino acid uptake pathways

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Purpose: To correlate the distribution of glutathione and its precursor amino acids with the expression of their respective amino acid transporters in the rat lens.
Methods: Whole rat lenses were fixed, cryoprotected and then cryosectioned in either an equatorial or axial orientation. Sections were double labelled with GSH, cystine, glycine, glutamate, GLYT1 or GLYT2 antibodies and the membrane marker wheat germ agglutinin. Sections were imaged by confocal microscopy and intensity profiles plotted as a function of distance from the lens periphery.
Results: The distribution of cystine and glutamate levels correlated well with the expression patterns observed previously for the cystine/glutamate exchanger (Xc-) and the glutamate transporter (EAAT4/5) respectively. High levels of glycine labelling in the outer cortex correlated well with the expression of the glycine transporter GLYT1. However, the absence of GLYT1 in the core, despite an increase of glycine in this region, suggests an alternative glycine uptake system exists in the core. Equatorial sections labelled with GLYT2 antibodies, showed that GLYT2 was expressed all throughout the lens. Axial sections labelled with glycine revealed a track of high intensity glycine labelling that extended from the anterior pole through to the core that was associated with the sutures.
Conclusion: Our mapping of GSH and its precursor amino acids has shown that an alternative glycine uptake pathway exists in mature fiber cells. While GLYT1 and GLYT2 are likely to mediate glycine uptake in cortical fiber cells, GLYT2 alone appears responsible for the accumulation of glycine in the centre of the lens.

The Effects of Aging and Oxidation on Homeostasis in the Lens

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Purpose: To determine which lens transport proteins are susceptible to age dependent oxidative damage and evaluate the consequences on homeostasis.
Methods: Lenses from mice lacking the antioxidant enzyme, GPX-1, were compared to wild type (WT). Fiber cell membrane vesicle water permeability (p_m) was determined by osmotically induced swelling. In intact lenses: gap junction coupling was determined by impedance studies; $[Ca^{2+}]_i$ was measured by injecting FURA2 into fiber cells; $[Na^+]_i$ was similarly measured using SBFI.
Results: At 2 months of age, all lenses were transparent, whereas by 14 months, GPX-1 KO mice developed a cataract. At 2 months, the p_m was about 35 μm/s, but dropped to 28±4 μm/s for WT vs 8±3 μm/s for KO by 14 months. At 2 months of age, GPX KO lenses showed significant reductions in gap junction coupling, particularly for inner mature fibers (MF) where coupling was reduced to 50% of WT. KO lenses also had significantly elevated $[Na^+]_i$ and $[Ca^{2+}]_i$. Lenses from 14 month old WT mice showed larger accumulations of $[Na^+]_i$ and $[Ca^{2+}]_i$, and MF coupling was 28% of that in young lenses. In 14 month old KO lenses, MF coupling was essentially zero and $[Ca^{2+}]^i$ homeostasis was clearly lost.
Conclusion: Aging induces cumulative oxidative damage, causing significant reductions in gap junction coupling and some loss of p_m. KO of GPX-1 increased damage and caused age onset nuclear cataracts. Reductions in coupling appear to disrupt the lens circulation, leading to Ca^{2+} accumulation, which may activate Ca-dependent proteases, eventually causing a central opacity.

The use of Two-Photon Excited Flash Photolysis to study intercellular communication pathways in the mouse lens.

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Purpose: To develop methods to study intercellular communication with high spatial resolution in different regions of the mouse lens.
Methods: Lenses extracted from either wildtype (WT) or Cx46 knockout mice (Cx46KO) were cut in half, and incubated in an intracellular solution that contained low (fluorescein) or high (fluorescein-dextran, 10,000MW) molecular weight caged compounds. Cut lenses were placed upon the stage of an inverted microscope and Two-Photon Excited Flash Photolysis (TPEFP) used to create a cellular point source of fluorescent dye release. The spread of dye to adjacent cells was monitored using conventional confocal microscopy. Cage release was performed in different regions of the lens and correlated to markers of fiber cell differentiation to determine cellular position.
Results: In WT mouse lenses the extent of fluorescein spread was independent of location within the lens. In contrast, fluorescein-dextran spread following TPEFP was restricted in the outer cortex, but was extensive and similar to fluorescein spread in the inner cortex. In Cx46KO lenses fluorescein dye spread was reduced in the outer cortex relative to WT, but there was no difference in dye transfer between the low and high MW fluorophores in the inner cortex.
Conclusion: Since Cx46KO animals only express functional Cx50 gap junction channels in the outer cortex, our results show that intercellular dye transfer in the outer cortex is mediated by gap junction channels. However, in the inner cortex a macromolecule-permeable pathway appears to operate in parallel to a Cx46 mediated pathway. The implications of our findings to current models of lens homeostasis will be discussed.

Mass Spectrometry Identification of Post-Translational Modifications in Lens Fiber Connexins

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Purpose: To characterize the post-translational modifications that lens fiber connexins are subjected to *in vivo*.
Methods: Crude membranes were prepared from bovine lenses by centrifugation and the proteins present analyzed by proteolytic digestion, followed by HPLC separation of the resulting peptides and analysis by MALDI mass spectrometry and tandem mass spectrometry.
Results: We obtained a significant coverage of the cytoplasmic portions of Cx44 and Cx49, and were able to identify several phosphorylated residues in each protein. All of the phosphorylated residues in Cx44, namely Thr238, Thr300, Thr303, Ser241 and Ser245, were located in the carboxy tail. Cx49 was phosphorylated on Ser115, Ser118, and Ser134 from the cytoplasmic loop domain and on Ser258, Ser261, Ser265, Ser266, Ser297, and Ser300 in the carboxy tail. In addition, we identified other post-translational modifications including a deamidation at Asn121 in Cx49, N-terminal acetylation of both proteins, and potential *in vivo* cleavage sites in each protein, including the previously described cleavage site at Glu291 in Cx49 and novel cleavage sites at Asp125 and Leu256 in Cx44.
Conclusion: The extensive coverage of the lens fiber connexins obtained by MALDI mass spectrometry and tandem mass spectrometry in this study provide the foundation for future studies aimed at determining the identity of the kinases and proteases responsible for each specific phosphorylation and cleavage event.

Characteristics of corneal and sclerocorneal incision for micro incision cataract surgery (MICS)

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Purpose: Recently, incision sizes for cataract surgery decreased and it is more important than before to understand the characteristics of incision. In this study, characteristics of corneal and sclerocorneal incisions were compared experimentally.

Methods: First, pig eyes were prepared and micro incision cataract surgery (MICS) were performed by corneal or scleralcorneal incision (2.4mm). The widths of each incision were measured using an inner gauge before and after IOL implantation and the percentages of extension ratios were calculated. Second, 2 kg of albino rabbits were prepared and 2.8mm of MICS were performed. After that shapes of cornea were evaluated using topography on postoperative 1, 2, and 4 weeks. At the same time, the eyes of rabbits were extracted and these different incisions were analyzed histologically.

Results: Before the implantation, the widths of corneal and sclerocorneal were 2.41±0.04 mm and 2.49±0.07mm respectively. After the implantation, the widths of corneal and sclerocorneal were 2.56±0.09 mm and 2.64±0.07 mm and the percentages of extension ratios were 6.5±3.7% in the corneal incisions and 10.1±2.9% in the sclerocorneal incisions. The topography revealed the corneal incision had the tendency to form more flatly around the incision. The histological results showed the corneal incision was resturcutured earlier and the inflammatory cells were disappeared faster than the sclerocorneal incision.

Conclusion: It is different between the size of slit knife and the actual size of incision after IOL implantation. Understanding the characteristics of incisions will be important for development the advantages of MICS in future.

Experimental *in vivo* anti-PCO treatments: Status

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Purpose: To determine best method to prevent lens epithelium proliferation following endocapsular cataract surgery.
Methods: Mechanical, cryogenic, hyperthemia and pharmacological approaches were investigated in NZW rabbits following extraction of cortex and nucleus via a 4-5mm capsulorhexis with and without implantation of an IOL or via a peripheral 1mm capsulorhexis with injection of a gel designed to restore accommodation. PCO was evaluated weekly at the slit-lamp and after enucleation, by Miyake macroscopy and by LM histology; success was determined as no LECs and no regenerated cortex after 3 months.
Results: In control animals, PCO was detectable as early as 1 week. Capsule polishing, cryogenic and thermal treatment schemes failed to prevent PCO. When MTX, 5FU, MMC, et al lavage concentrations were sufficient to stop LEC proliferation, corneal decompensation, uveitis and retinal damage occurred; at safe concentration, these drugs were found clinically useless except for 5FU when released by a biodegradable annular implant. Three minutes treatment with de-ionized distilled water only delayed proliferation by ~1 month. Implantation of IOL tended to prevent PCO but did not prevent lens regeneration at the equator; lens refilling didn’t prevent LEC proliferation. Endocapsular thermal treatment combined with slow release of paclitaxel within the capsular bag seems most promising.
Conclusion: Combining treatment modalities might prevent lens epithelium proliferation but their application to the clinical setting is difficult because of time constraint.

First Demonstration of Laser-Assisted, Aspiration-Only Crystalline Lens Extraction and Laser Created Capsulorhexis

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Purpose: The combination of emerging IOL technologies and rapid growth projected in the cataract surgery patient population over the next 10-20 years creates a compelling need to develop advanced surgical techniques. Such techniques should offer safer and easier lens extraction. A novel photodisruption laser beam delivery system has been developed specifically for crystalline lens surgical procedures, including presbyopia treatment. This study investigates the advantages of using an advanced photodissruption laser system to slice and dice the lens nucleus and epinucleus for aspiration only lens extraction. In addition, a laser created capsulorhexis is evaluated.
Methods: Five matched pairs of enucleated porcine eyes were used to compare traditional phacoemulsification lens extraction and manual capsulorhexis to laser assisted lens extraction and laser created capsulorhexis. In addition, the two eye comparison was performed in a living rhesus monkey with bilateral IOL placement and postop followup. Key measurement parameters are total lens removal time, total time of ultrasound utilization, including if aspiration-only removal was achieved, and image analysis of the RMS deviation of the capsulorhexis from a circle.
Results: Preliminary results in porcine eyes show the laser offers: a significant reduction in total lens removal time, the ability to achieve aspiration-only lens extraction, and the ability to create a precise circular capsulorhexis. Further results pending.
Conclusion: A photodisruption laser beam delivery system designed for crystalline lens surgery has potential advantages over current lens extraction surgery including aspiration only lens extraction and highly precise capsulorhexis. Future work remains to be completed in human studies.

Different surface modification and CCC (continuous curvilinear capsulorhexis) contraction after cataract surgery

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Purpose: Clarification of mechanisms of anterior CCC (continuous curvilinear capsulorhexis) contraction.
Methods: Acrylic IOLs (YA-60BB, HOYA) were prepared and 2 different surface modifications were performed to modify the surface characteristics of IOLs. MPC (2-methacryloyloxyethyl phosphocholine-co-n-butyl-methacrylate) polymer coating was performed for decreasing adhesion or UV/Ozone modification was performed for increasing adhesion. First, to evaluate the effect of modifications (adhesion with capsule), type 4 collagen gels were prepared and cultured with lens epithelial cells on surface of different characteristics of acrylic IOLs and shrinkages of gels were observed. Second, eight-week old albino rabbits were anesthetized and phacoemulcification were performed after that the different modified IOLs were implanted. The rabbits were anesthetized and anterior segment were photographed using EAS-1000 analyzing system (NIDEK) after two weeks. The percentages of contraction of CCC were calculated and analyzed.
Results: The experimental collagen gels shrinkages were 56.6% (MPC coated IOL), 27.4% (UV/Ozone modified IOL) and 41.3% (control IOL). The percentages of contraction of CCC after cataract surgeries were 28.4% (MPC coated IOL), 4.0% (UV/Ozone modified IOL), 3.9% (control IOL) and there are statistically significance ($p<0.05$, multiple comparisons using Fisher's PLSD). Contraction of CCC was increased using the MPC polymer treated acrylic IOLs by decreasing adhesion with anterior capsule.
Conclusion: The formation of adhesion structure between surface of IOL and anterior lens capsule is one of the important factors of CCC contraction.

Engineering the Crystalline Lens

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Purpose. Following endocapsular lens/cataract extraction, the residual lens epithelial cells may be engineered to form a natural replacement lens by implantation of a biodegradable or non-degradable scaffold.

Methods. Lens regeneration has been demonstrated in other mammals, including mice, cats, dogs, and monkeys. We present data from the rabbit endocapsular lens extraction model. Following removal of the natural clear or cataractous lens, a hyaluronic acid based scaffold or a synthetic polymer was injected intra-lenticularly, and the capsulotomy was closed.

Results. The regenerative process began as early as two weeks, and new lens growth filled the capsule bag as early as two months postoperatively. Initially, new lens growth was noted in the equator and along the posterior capsule surrounding the scaffold. Lens growth gradually progressed from the periphery toward the center in a fairly uniform manner. The regenerated lenses have had good clarity and structure anterior and peripheral to the scaffold and are more opacified posterior to the scaffold.

Conclusion. The lens regenerative process may be enhanced by providing an intact external capsular support and an internal bio-degradable or non-degradable scaffold.

In-situ observation of dynamic accommodation

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Purpose: Analysis of *ex-vivo* primate lenses suggests that the underlying mechanism for accommodation is the interfacing of fibers ends at suture branches. We report results of ongoing *in-situ* slit-lamp analysis of suture appearance in the accommodated vs. the unaccommodated state that is consistent with the *ex-vivo* studies.
Methods: Young (20 - 30 years, n=8) and older adult (45 - 65 years, n=8) lenses were photographed using a Haag-Streit slit-lamp, with particular emphasis on individual suture branches, while the subjects were either not accommodating or attempting to accommodate to a 10D stimulus.
Results: Suture branches were characterized as a pair of apposed, slightly translucent, diffuse linear regions. In young unaccommodated lenses, the diffuse regions bracketed a sharp transparent region. However, in young accommodating lenses, the diffuse regions were directly apposed. By comparison, in the accommodated state, older lenses, the two broader diffuse regions appeared to be at least partially coalesced. Whereas in the older unaccommodated state, the bands were less distinct and did not bracket a central translucent region.
Conclusion: If the transparent region represents the end to end arrangement of fibers at a suture branch when there is no accommodation, and the elimination of the transparent region is due to the fibers interfacing at the suture during accommodation, then the *in-situ* slit-lamp analysis is completely consistent with the *ex-vivo* microscopic analysis. However, more lenses will be analyzed for statistical significance. If this is accomplished in the future, it will be possible to determine the accommodative abilities of a lens *in-situ*.

Proteomic characterization of lens epithelial cells in mice null for the antioxidant protein, Peroxiredoxin 6

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Purpose: The antioxidant protein Peroxiredoxin 6 (Prdx6) protects cells by removing H_2O_2 and mediating survival signaling. We have reported that PRDX6-depleted lens epithelial cells (LECs) displayed phenotypic alterations and elevated α-smooth muscle actin (α-SMA) and βig-h3 expression, indistinguishable from transforming growth factor β (TGF-β)-induced changes. The present study quantified the protein changes in LECs that were deficient in PRDX6. **Methods:** *Prdx6*$^{-/-}$ and *Prdx6*$^{+/+}$ LECs were derived from eye lenses of Prdx6 knockout and wild type mice and maintained in complete DMEM. DIGE (Fluorescence 2-D Difference Gel Electrophoresis) with M_R's of 10-100kDa and *pI*'s of 4.0-7.0 and mass spectrometry were utilized to resolve LEC protein expression profiles. Results were validated with Western analysis using specific antibodies and real-time quantitative PCR.
Results: MS and database searching revealed a total of 919 differentially expressed protein spots, with 22 spots differentially up-regulated and 20 spots differentially down-regulated. The differentially expressed proteins are involved in cytoskeleton ($^{e.g.}$ tropomyosin 2β, vimentin associated with cell differentiation), oxidoreductase, and other processes. Real-time PCR analysis and/or western analysis revealed that altered expression of proteins involved in TGF-β, tropomyosin 1α and 2β, α-SMA and antioxidant enzymes. These differences may reflect mechanisms aimed at compensating for increased oxidative stress. Alteration of protein levels may contribute to the morphological abnormalities in *Prdx6*-/- LECs previously observed.
Conclusion: These findings provide insight into oxidative stress-dependent alterations in LEC protein expression. The study may help to elucidate underlying molecular mechanisms involved in the etiology and progression of catractogenesis.

Clarification of Mechanisms of Gas Induced Cataract after Vitrectomy and Gas Exchange

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Purpose: To determine the mechanisms of the gas induced cataract after fluid-gas exchange in vitrectomy.
Methods: Around 2 kg of albino rabbits were prepared and anesthetized by intramuscular injection of ketamine. After two ports vitrectomy (PREMIERE, STOLZ), fluid-gas exchange was performed using different types of gas (room air and pure nitrogen gas). Control group was performed only vitrectomy. The changes of the lens were observed with stereoscopic microscope (OMS.75 TOPCON) from 0 to 90 minutes. Each tissue was fixed with Carnoy's fluid at selected time period and stained for hematoxylin-eosin solution and for 4HNE 4-hydroxynon-2-enal:a marker for oxidation . Histological changes were observed with an optical microscope (DX-51, OLYMPUS).
Results: In macroscopic findings, no significant changes were observed in control group. Opacifications along posterior Y suture were developed on 10 minutes and it was deceased gradually and disappeared at 90minutes after the room air exchange. However, no opacifications were observed using nitrogen gas. Histological results showed no significant changes in control group. In room air group, small particles were detected at posterior pole of lens and 4HNE-positive stain was observed at this small particles. In nitrogen gas group, few small particles were detected, however, 4HNE-positive stain does not observed.
Conclusion: Oxidative stress induced small particles along with posterior pole of lens is one of the important causes for gas induced cataract after vitrectomy and gas exchange.

Thioredoxin reductase, but not GSH or catalase, defends human lens epithelial cells against UVA light.

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Purpose: To investigate the roles of GSH and thioredoxin reductase (TrxR) in protecting cultured human lens epithelial cells (LECs) against UVA-induced damage.
Methods: SRA 01/04 cells were depleted of GSH by 58% or 66% by treating with 1-chloro-2, 4-dinitrobenzene (CDNB, 0.02mM, 10 min) or buthionine sulfoximine (BSO, 0.5mM, overnight), respectively. The cells were exposed to UVA light, 7mW/cm^2 (338-400nm wavelength, peak at 365nm), for 1h at 36°C, in PBS, at either 3% or 20% O_2. Cells were counted after 24 h, and changes in cell morphology were evaluated by light microscopy. Real Time RT-PCR was used to analyze upregulation of mRNA.
Results: Treatment of normal cells with UVA light, at either 3% or 20% O_2, showed no loss of GSH, change in morphology or decrease in growth after 24 h, even though catalase activity had decreased by 85%. Minimal effects on cell growth, and no effect on morphology, were seen after UVA-exposure of BS0-treated cells. Treatment with CDNB, however, either alone or in combination with UVA, at 3% or 20% O_2, produced significantly greater effects, compared to BSO. For example, CDNB+UVA at 20% O_2 caused significant cell damage and death after 24 h, whereas BSO+UVA at 20% O_2 had no effect on cell morphology. CDNB+UVA at 20% O_2 inhibited the activity of TrxR by 75%, whereas BSO had much less effect on the enzyme. CDNB+UVA produced a 5-fold increase in TrxR1 message (the cytoplasmic form of the enzyme) at 3% O_2 and a 17-fold increase at 20% O_2.
Conclusion: TrxR plays a greater role than GSH or catalase in defending HLE cells against UVA-induced damage. TrxR is known to use NADPH and thioredoxin to reduce reactive oxygen species, and produce deoxyribonucleotides needed for DNA synthesis.

Dynamics of rat lens epithelial cells in sugar cataractogenesis. ~Possible association of basic fibroblast growth factor~

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Purpose: To investigate the profile of expression of basic fibroblast growth factor (bFGF) and its association between the dynamics of lens epithelial cells (LECs) in the progression of rat sugar cataract.
Methods: Sprague–Dawley rats (6W) were fed diet containing 50% galactose in the presence or absence of aldose reductase inhibitor (ARI, fidarestat). The temporal profiles of bFGF-expression in the protein and mRNA levels were examined by ELISA and real-time RT PCR assays, respectively. To know the localization of bFGF expression, immunohistochemical staining was performed. To evaluate the LECs movement, thymidine was injected into anterior chamber, then the distance from lens edge to the labeled cells was measured.
Results: At 2 weeks after galactose feeding, cortical opacity and the formation of multilayered LECs accompanied by focal cell growth were observed. At this time period and thereafter, the level of bFGF protein and mRNA expression were dramatically elevated. Multilayered LECs exhibited the strong bFGF expression, but not beta-crystallin the marker of lens fibers. These increases of bFGF expression were normalized by ARI. There were no significant differences in the differentiation indicated by the movement of LECs into the equatorial region.
Conclusion: Our results suggest that enhanced bFGF expression may be involved in the formation of multilayered LECs, but not affect the differentiation from LECs to lens fibers during the progression of sugar cataract.

The features of HOYA IOL and IOLs trend in Japan

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Similar as worldwide market, IOLs in Japan have been developed as the innovation of operation tools and requirement of patients and doctors. As a result, doctors can choose a best IOL out of the variety of IOL selections now. Then, in this section, I will talk about the current state of Japanese IOLs and its trend. And after that, I will explain about features of the IOL we are developing from this background

Difference of cataract development by different frequency in millimeter wave

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Purpose: The validity of the safety standard (10-300 GHz 10 W/m2 in public, ICNIRP) of a wide-ranging millimeter wave was examined from lens epithelial injury after exposure.
Methods: Pigmented rabbits were exposed unilaterally to 40 or 60 GHz millimeter wave with a lens antenna for 6 minutes. 1,500 or 3,000 mW/cm2 focused beam was exposed to the center of the rabbit cornea. The corneal surface temperature was measured with a thermography camera. Ocular changes were evaluated by slit lamp and ocular inflammation was measured by laser flare meter immediately after and 3 day after exposure. Enucleated lens was observed with lens epithelial cell flat mounting.
Results: 40 GHz 1,500 and 3,000 mW/cm2 for 6 min exposure developed cornea and lens opacity. Meanwhile, 60 GHz, 3,000 mW/cm2 exposure showed only transitory corneal opacity and find mitotic lens epithelial cells in the pupillary aria, but not developed cataract. 60 GHz 1,500 mW/cm2 exposure did not show any ocular changes.
Conclusion: It was suggested that biological reactions differed from 40 and 60 GHz, even when the maximum incident power density was the same.

Myopia Induced by Retrodots and Nuclear Cataract

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Purpose: Ophthalmologists know that nuclear cataract (N) causes myopic shift in refraction. We report retrodots (RD) do too. We compared RD and N for ocular refraction.
Methods: Some 246 cases (444 eyes), participants of the Monzen Eye Study, 2005 and cataract surgery patients from Kanazawa Medical University Hospital from December 2005 to March 2007were enrolled. Posterior subcapsular cataractous, and cortical opacified eyes within 3mm diameter of the pupil were excluded. RD eyes were categorized (0 to 4) according to area of opacity. N were graded using the WHO classification system. A-mode ultrasound (AL2000, TOMEY) was used to measure axial length. Refraction was measured with an auto kerato-refractometer (ARK. NIDEK). Lens refractive power was calculated using SRK.
Results: There were no significant differences in axial length and corneal refraction among the 4 groups. The average RD refraction for respective steps was 0.67D, 0.80D, 0.59D, -0.85D and -0.43D. And average refractive power of lens was 17.50D, 17.45D, 16,98D, 19,69D and 18.86D. We found a tendency of myopia and increase of lens refractive power ($p<0.05$). The mean refraction of N grade eyes was 0.67D, 0.47D, -1.95D, and -3.53D, with lens refractive power 17.50D, 17.86D, 21,62D and 23,97D respectively. There was also a tendency for myopia and increase in refractive power ($p<0.01$). Lens refractive power and refraction of concomitant step 3 RD/N>grade 2 were 24.00D and -3.59D.
Conclusion: RD affects lens refraction. Nuclear cataract affects refraction more than RD does. Concomitant nuclear cataract and RD worsens myopia.

"NASCA" – The NASA Study of Cataract in Astronauts. Baseline Analyses

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Purpose: NASCA is a five-year study of risk factors associated with cortical (C), nuclear (N), and posterior subcapsular opacities (PSC) in US astronauts exposed to space radiation, and controls with no such exposure. Risk factors studied include space radiation exposure, solar UV exposure, age, gender, smoking history, and nutrition.
Methods: Standardized areal measures of C and PSC and pixel density of N were obtained by analysis of standardized Nidek EAS 1000 digital images. Nuclear color (NC) was assessed with LOCS III. Space radiation exposure was estimated from flight data and expressed as mean lens dose (mSv). Solar UV exposure and nutrition data were estimated from standardized validated questionnaires. Distributions of lens endpoint variables all skewed. Confounding variables: history of asthma, hypertension, gout, obesity, tobacco use, and meds. Propensity scores used in view of heterogeneity of cohorts and differences in confounding variables. Nutritional variables studied were related to risk of age-related cataract and included: carotenoids, vitamins A, C, E, lycopene, lutein, zeaxanthin, omega-3 fatty acids, and multivitamin supplements.
Results and Conclusions: Actively recruited (declined participation/excluded at baseline exam): Astronauts: 288 (65/4), Payload Specialists: 5 (0/0), Military Aircrew: 103 (5/3), Ground-based Controls: 100 (1/0), TOTAL: 496 (71/7). 418 subjects given baseline examination. Age is most important predictor of C. Astronauts who have flown in space had more C and more pleomorphic opacities than non-exposure controls. Space radiation exposure was not related to measures of N and PSC. Solar radiation exposure and nutritional variables had no discernable effect on any measure of opacity.

Quantitative Evaluation of Selective Diffusion within the Anterior Lens Capsule

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Purpose: The lens capsule is a thick basement membrane completely surrounding the lens. It is considered selectively permeable to molecules important for normal lens function but limiting passage of others. Recently adenoviruses and plasmids have been reported to pass through intact lens capsules bringing this selective permeability into question. Anionic sites within basement membranes have been proposed to contribute to this apparent selective permeability. We test the hypotheses that the lens capsule is selectively permeable to molecules based on their size, charge, and shape and that anionic domains are a major contributor to selective permeability.
Methods: We employed Fluorescence Recovery After Photobleaching (FRAP) on the capsules of intact mouse lenses and a novel mathematical algorithm to determine diffusion coefficients and binding constants of FITC labeled neutral dextrans and several proteins (IgG, albumin, EGF, and transferrin). Diffusion rates were compared between normal capsules and those treated with a cationic masking agent, polybrene.
Results: Proteins have significantly slower diffusion rates and higher binding constants within the lens capsule than neutral dextrans of similar Stokes radii. Diffusion rates in polybrene treated capsules significantly increased for dextrans and proteins with Stokes radii less than 5 nm and remained unchanged for larger molecules up to 8 nm.
Conclusion: We demonstrate that the anionic sites within the lens capsule influence the diffusion rates of molecules in the capsule based on Stokes radius and ECM binding potential.

The Visual Environment Affects the Refractive Development of the Eye but Not the Lens –The Example of the Fish Eye

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Purpose: – Research with young mammals and chicks has shown that the visual environment can affect the refractive development of the eye by enhancing or slowing axial eye growth, but the lens is only minimally affected. This research investigates the role of the lens in induced refractive error development in fish species.
Methods: Translucent goggles were directly sutured over the right eye of tilapia (*Oreochromis niloticus*) for 4 weeks to induce form deprivation myopia while the left eye served as an untreated contralateral control. Refractive state was measured by retinoscopy, ocular dimensions were determined from frozen sections and with ultrasound biomicroscopy, and a scanning laser system was used to determine the optical quality of excised lenses.
Results: All the deprived fish eyes developed significant amounts of myopia ranging from -3.75 to -26.25 diopters (D), with the average amounting to -10.27±1.14 D. Tthe vitreous and anterior chambers of the treated eye are significantly longer axially than those of the contralateral eyes. No significant change in optical quality was found between lenses of the myopic and non-myopic eyes and the fish recovered completely from the myopia five days after the goggle was removed.
Conclusion: Although fish are capable of lifelong growth, this research shows that the visual environment is an important factor controlling ocular development in lower vertebrates, as well as in higher ones, and eye development is not strictly genetically determined. However, this study also indicates that lens growth and optical development is independent from the refractive development of the whole eye.

αA^{G98R}-Crystallin: Altered structure, stability and chaperone activity contribute to cataract

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Purpose: To understand the molecular mechanism of cataract formation following G98R mutation in human αA-crystallin
Methods: Site-directed mutagenesis was employed to introduce the desired G98R mutation in human αA-crystallin cDNA cloned into pET vector. The mutant and wild-type proteins were expressed in E.coli and purified. The purified proteins were characterized by spectroscopic methods and the chaperone activity was determined using alcohol dehydrogenase (ADH), restriction enzyme Sma1, lactalbumin and insulin. The aggregation status of the protein at different temperatures was investigated by DLS method.
Results: The homo-aggregates of G98R are larger than the wild-type and have increased hydrophobicity and greater intrinsic fluorescence. The mutant proteins also show altered near and far-UV CD spectra and decreased stability. The mutant protein showed complete loss of chaperone activity during DTT-induced insulin reducing assay. On the other hand assays with ADH and Sma1 performed at 37°C showed that the G98R mutation did not result in lowering of αA-crystallin chaperone activity. However, chaperone assays with ADH performed at 43°C or higher temperature (where G98R is less stable) showed that the G98R has diminished chaperone activity.
Conclusion: The G98R mutation alters stability of αA-crystallin. The apparent difference in the chaperone activity of G98R toward different substrates can be attributed to the differences in the stability of the chaperone and substrate protein complexes.

Alpha-Crystallins and their role in cataract and other diseases

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Purpose: In spite of intensive work trying to elucidate the 3 dimensional structure of alpha-crystallin, we still do not have a high-resolution structure. This is because alpha-crystallin is ploydispersed and cannot be crystallized. We can infer its structure and function from other small heat-shock proteins belonging to the alpha-crystallins family that were crystallized.
Methods: To study its function in the lens, we are at present using a genetic approach. Xiaohua Gong and his collaborators have recently identified 2 mouse cataractous mutant lines. One such mutation is αA-R54C, and the second is αA-Y118D. The two mutations cause a recessive and a dominant cataract respectively.
Results: The first mutation (αA-R54C) which is in N-terminal region of alpha-crystallin seem to affect lens epithelial and fiber cell during development, the αA-Y118D mutation which is in the "α-crystallin domain" of the molecule affect the interaction of alpha-crystallin with the other major lens crystallins namely beta and gamma-crystallins.
Conclusion: This approach and these kinds of animal models will help us understand the multiple roles of the alpha-crystallin in the lens and in the other tissues. The involvement of alpha-crystallin in age-related macular degeneration and various types of cancer will also be discussed.

Identification of Microtubule Interactive Domains in αB crystallin

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Purpose: αB crystallin is a small heat-shock protein (sHSP) found in high concentration in lens cells, that protects against aggregation of unfolding/misfolding proteins. Recent studies identified a regulatory effect on microtubule assembly during cell proliferation and in response to stress. Understanding the interactions the microtubule - αB crystallin interaction will provide insight into the mechanism of αB crystallin functions in cells.
Methods: The function of five interactive sequences in αB crystallin were investigated on the assembly of microtubules and aggregation of tubulin using synthetic peptides and mutants of αB crystallin. Assembly of microtubules was measured using a fluorescence DAPI assay (Ghosh et al. (2007) PloS one 6:3498). Aggregation of tubulin was measured by light scattering.
Results: αB crystallin inhibited microtubule assembly at concentrations >2:1 αB crystallin:tubulin and promoted microtubule assembly at low concentrations of 1:4 – 2:1. The synthetic peptide corresponding to the αB crystallin interactive sequence, ${}_{113}$FISREFHR${}_{120}$, partially inhibited microtubule assembly. In contrast, the peptides ${}_{131}$LTITSSLSSDGV${}_{142}$ and ${}_{156}$ERTIPITRE${}_{164}$ promoted microtubule assembly. These three peptides also partially prevented the aggregation of tubulin. Mutagenesis to these regions in full length αB crystallin affected the nature of the interactions with microtubules.
Conclusion: Three interactive sequences on the surface of human αB crystallin collectively modulate microtubule assembly through a dynamic subunit exchange mechanism that depends on the ratio of αB crystallin to tubulin. These are the first experimental results in support of the functional importance of the dynamic subunit model of small heat shock proteins.

Kinetics of Amyloid Fibril formation in vitro by Human gamma Crystallins

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Purpose: Human gamma D crystallin (HγD-Crys) and human gamma C crystallin (HγC-Crys) are two of the most abundant gamma crystallins found in the nuclear region of human eye lens. Both proteins have been found in the insoluble fraction of cataract removed by surgery. This report presents our recent study on the aggregation kinetics of wild type and mutant forms of HγD-Crys and HγC-Crys as a function of protein concentration, pH and temperature.
Methods: Proteins were expressed in E. coli and purified using affinity or size-exclusion chromatography. Mutant protein constructs were generated by site-directed mutagenesis. Protein aggregation was measured by monitoring absorbance at 350nm using a UV-Vis spectrophotometer. Protein aggregates were characterized by thioflavin T (ThT) binding, transmission electron microscopy (TEM) and infrared (IR) spectrometry.
Results: Incubation of purified proteins in pH3 buffer at 37^{0}C resulted in the formation of insoluble aggregates. Characterization of HγD-Crys and HγC-Crys aggregates by ThT binding, TEM and IR revealed that the aggregates share many similarities with amyloid fibrils. The fibril formation rate increased with increased protein concentration, decreased pH or increased temperature. Mutants which decreased protein stability were more aggregation prone. In contrast, the proteins remained soluble in neutral buffers at 37^{0}C.
Conclusion: Comparison of the aggregation properties between mutant and wild type gamma crystallins suggests that factors which decrease protein stability or promote partially unfolded conformations will promote aggregation. The amyloid fibrils formed at low pH by gamma crystallins could serve as a nucleus for further aggregation and could be significant to cataract formation.

Indoleamine 2,3-dioxygenase Over-expression in the Lens

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Purpose: To determine if enhanced production of kynurenines results in lens protein pigmentation and crosslinking that lead to cataract formation.
Methods: Indoleamine 2,3-dioxygenase (IDO) is the first enzyme in tryptophan oxidation, which leads to the formation of a number of kynurenines. Kynurenines can react to form brown-pigmented products that can crosslink lens proteins. Human IDO was over-expressed in mice using chick delta1-crystallin lens enhancer/αA crystallin promoter. IDO was measured by an HPLC method. Kynurenines were measured by C18-RPHPLC. Kynurenine modifications were measured by ELISA and Western blotting using two monoclonal antibodies.
Results: IDO activity was negligible in wild type (WT) lenses and was 0.83 nmoles kynurenine/mg protein/min in transgenic (TR) animals. Immunostaining for IDO showed it to be present mostly in outer cortical fiber cells and epithelial cells. Kynurenine was not detected in WT lenses, but in TR the levels were 0.51 ± 0.12 nmoles/mg lens. Incubation of TR lenses with tryptophan showed considerable 3OHkynurenine and kynurenine-derived modifications in the lens.
Conclusion: Over-expression of IDO results in enhanced production of kynurenines in the lens. Supplementation of tryptophan is likely to accelerate lens protein browning and crosslinking in mouse lenses, which may serve as a useful model to study biochemical changes in cataractogenesis.

Supported by R01EY-09912 (RHN), P30EY-11373, RPB and OLERF (CWRU)

Metal Analysis in Human Alzheimer Disease Brain & Lens

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Purpose: Alzheimer's disease (AD) is characterized by cerebral accumulation of β-amyloid (Aβ). We previously reported specific AD-linked Aβ amyloid pathology and co-localizing subequatorial supranuclear cataracts in the lenses of patients with AD (Goldstein et al, 2003). Previous research has shown high-affinity binding of specific biometals (Zn, Cu, Fe) to human Aβ and biometal co-localization with amyloid lesions in AD brain. We hypothesize that pathogenic AD-linked Aβ-mediated protein aggregation is mediated *via* metalloprotein interactions in both brain and lens. Here we sought to identify and localize specific biometals and quantitate absolute regional tissue metal concentrations in lenses and brain specimens obtained from human AD and control donors.

Methods: *Human tissue:* Massachusetts Alzheimer's Disease Research Center, NDRI. *Techniques:* X-ray fluorescence microscopy (beamline 10.3.2, Advanced Light Source, LBNL), ICP-mass spectrometry, metal histochemistry, EM autometallography, quasi-elastic light scattering, turbidometry.

Results: XRFM, metal histochemistry, EM autometallography revealed Zn accumulation that regionally co-localized with AD-linked Aβ amyloid pathology in both lens and brain. Mechanistic studies demonstrated that specific chelation potently blocks Aβ autoaggregation and Aβ-mediated lens protein aggregation in vitro.

Conclusion: These results yield important data regarding the role of trace biometals in AD-linked Aβ-mediated protein aggregation in both the lens and brain. Furthermore, these data suggest new therapeutic strategies for interdicting pathogenic Aβ-mediated protein aggregation *in vivo*.

Cataract Development in Estrogen Receptor Knock-Out and Transgenic Mouse Models

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Purpose: The increased risk of age-related cataracts in postmenopausal women and studies in animal models suggest that estrogen may have a protective role in the lens. However, very little is known regarding the role of estrogen and its receptors in the lens. For this reason, we examined whether estrogen receptors (ER) are required for maintenance of lens transparency using ER knock-out (ERKO) and ERΔ3 mouse models. ERΔ3 mice express a dominant negative ERα repressor that results in the formation of spontaneous cortical cataracts only in estrogen-producing females.
Methods: To examine whether the loss of ER will induce cataracts, slit-lamp examinations were performed on aging mice lacking expression of ERα and/or ERβ and the observed lenticular phenotypes were confirmed by histopathology. We also examined cataract development in ERΔ3 and ERKO crossbred mice.
Results: Regardless of their genotype, cataracts were not detectable in αERKO, βERKO, αβERKO, or wild-type (WT) male or female mice up to 16 months of age. In contrast, ERΔ3- induced cataracts were evident in females from αERKO/ERΔ3, βERKO/ERΔ3, and αβERKO/ERΔ3 mice, but not in males or WT mice.
Conclusions: Our data demonstrate that ERα and ERβ are not required to maintain lens transparency; however, these receptors (and their estrogenic ligands) may still have a protective role in the lens against other factors that stimulate cataractogenesis. Indeed, the most severe cataracts were detected in ERΔ3 mice lacking ERα. Continued investigations into the role of ER in the lens will be crucial for assessing the cataract preventative capabilities of estrogen.

βA3/A1-crystallin is expressed in astrocytes and the Nuc1 mutation produces abnormalities in the retinal vasculature

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Purpose: To determine the cause of retinal abnormalities in the Nuc1 mutant rat.
Methods: To localize the Nuc1 mutation, a genome-wide scan was performed on backcross progeny from Lewis/Sprague-Dawley hybrids using 207 microsatellite markers. Candidate genes were sequenced to identify the specific mutation. The retina phenotype in the Nuc1 rats was characterized immunohistochemically on retinal sections and flat mounts analyzed by confocal microscopy, by Evans Blue leakage, and by ADPase staining. Laser capture microdissection and Real-Time quantitative PCR were used to localize expression within the retina.
Results: The Nuc1 locus was mapped to rat chromosome 10p22-26. The specific mutation was determined to be an insertion into exon 6 of the βA3/A1-crystallin gene in which a conserved glycine residue is replaced by 10 new amino acids. In the neural retina, βA3/A1-crystallin was found to be expressed only in astrocytes. Astrocytes are macroglial cells that direct the formation and maturation of the retinal vasculature, however the specific mechanisms involved are poorly understood. In the Nuc1 homozygote, astrocytes lack the normal compact stellate structure and do not form the highly ordered, honeycomb-like template on which the retinal vasculature normally forms. Vessels in the Nuc1 retina are shown to be leaky and the vascular pattern sparse.
Conclusion: Mutation of βA3/A1-crystallin affects astrocytes and the retinal vasculature in a recessive manner, whereas cataract is a dominant effect of the same mutation. We believe that the retinal phenotype results from the loss of the “non-crystallin” function of A3/A1 and hope that Nuc1 will enable us to identify that function.

Linking risk for nuclear and cortical lens opacities, dietary carbohydrate intake and glycemic index, and protein quality control

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Purpose: We sought to monitor effects of glycation on the ubiquitin proteolytic pathway and to determine if recent dietary carbohydrate intake or glycemic index (GI), a measure of carbohydrate intake quality, is associated with the presence of cortical or nuclear opacities in non-diabetics.
Methods:FFQ used to obtain dietary information. Participants 60 – 80 y, AREDS cohort. Only single type opacities used.
Results: For persons in the highest quartile, dietary GI was associated with a higher prevalence of all pure nuclear opacities (grade > 2) (odds ratio (OR) = 1.29, 95% CI, 1.04-1.59; *Ptrend* = 0.02) and moderate nuclear opacities (grade ≥ 4) (OR = 1.43, 95% CI, 0.96-2.14; *Ptrend* = 0.052). The OR comparing the highest with the lowest quartile of total carbohydrate intake was 1.27 (95% CI, 0.99-1.63; *Ptrend* = 0.09) for cortical opacities of any severity (>0% area opaque). The OR increased somewhat for moderate cortical opacities (> 5% area opaque) (OR = 1.71, 95% CI, 1.00-2.95; *Ptrend* = 0.056). Results not modified by gender, education level, vitamin C intake, smoking status, or body mass index. Carbohydrate can induce compromises to the ubiquitin proteolytic pathway of protein quality control.
Conclusions: Carbohydrate can induce cataract-related compromises to the protein quality control machinery. This is corroborated by the cross-sectional analysis of AREDS baseline data which suggest that carbohydrate nutrition may be associated with prevalent nuclear and cortical opacities, albeit play different roles in the development of cortical and nuclear lens opacities.

Funding U.S.D. A. 58-1950-4-401, 1950-5100-060-01A, NIH R01-13250 and R03-EY014183-01A2, Johnson and Johnson Focused Giving Program, American Health Assistance Foundation.

Multifuctional Antioxidants Delay Cataract Formation

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Purpose: Oxidative stress is a major component of cataract development. Diabetic cataracts are initiated by sorbitol accumulation; however, reactive oxygen species (ROS) are generated during this process through endoplasmic reticulum (ER) stress initiated by osmotic imbalance induced by sorbitol accumulation. While aldose reductase inhibitors (ARIs) prevent diabetic cataracts, the purpose of this study was to determine whether multifunctional antioxidants can reduce ROS sufficiently to delay cataract formation.
Methods: Young streptozotocin diabetic rats were divided into 4 groups while a 5th group of was composed of untreated non-diabetic controls. In the diabetic groups, one was not treated while a second group received the ARI AL1576 administered at a concentration of 0.0125% in rat chow. The two remaining diabetic groups received one of two novel multifunctional antioxidants at chow concentrations of 0.025%. Lens changes were monitored by slit lamp at 3-4 day intervals following dilation with 1% tropicamide.
Results: All rats were equally diabetic with HbA1C levels > 11.9%. No lens changes were observed in either the non-diabetic control or ARI treated diabetic rats. In the untreated diabetic rats, vacuole formation began by 14 days after initiation of diabetes with cortical opacities observed by 29 days and mature cataracts by 44 days. In the two groups of antioxidant treated rats, vacuole formation was delayed by 3 and 14 days respectively. This delay was proportional to the lens levels of drug achieved. Moreover, antioxidant administration did not reduce lens sorbitol accumulation.
Conclusion: While ARIs prevent diabetic cataract formation, oral administration of multifunctional antioxidants can delay diabetic cataract formation presumably by reducing ROS generated by ER stress.

Slit lamp and histological detection of huntingtin polyglutamine repeats in mouse lenses

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Introduction: Aggregates in lenses of mice overexpressing huntingtin 72 polyglutamine repeats were observed using slit lamp and fluoresence microscopy methods.
Methods: Mice expressing 25 and 72 length EGFP-tagged huntingtin polyglutamine repeats were generously provided by Dr. Paul Muchowski (Gladstone Institute, UC San Francisco). Mice aged 9 days to 2 years expressing the huntingtin repeats, EGFP only, and wild-type were examined in a photo slit lamp at 40X using excitation(465-498nm), emission(515-560nm), and barrier(475-560nm) filters optimal for EGFP. Lenses were fixed in 2% paraformaldehyde in 0.136M phosphate buffer, pH 7.35 for 5 hours at RT, and embedded in paraffin. Lens sections were observed directly in a fluoresence microscope at 10X.
Results: In the slit lamp, EGFP fluorescence was observed in mouse lenses expressing the 72 polyglutamine repeats as early as 2 weeks in a diffuse ring extending from the inner cortex through the outer nucleus. EGFP-tagged aggregates were observed in the same regions of the lens by 3 weeks in paraffin sections. EGFP was observed by slit lamp in lenses expressing 25 polyglutamine repeat peptides at lower levels than the 72 repeats, but no aggregates were observed in paraffin sections. Mouse lenses expressing EGFP alone exhibited a diffuse and widespread cortical and nuclear fluorescence distribution at all ages observed (4-10 months). Modest background light scattering was observed in most lenses, including the wild type.
Conclusions: Protein aggregates were detected in lenses expressing huntingtin 72 repeats but not in lenses with 25 glutamine repeats.

Contrast sensitivity of high value-added intraocular lenses.

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Purpose: Recently, intraocular lenses (IOLs) started to have additional function such as aspheric, yellow tinted, and both; however, the clinical effects of these functions are still unclear. In this study, we evaluated visual functions, in particular contrast sensitivity after different functionalized (spherical, aspheric, yellow tinted, and both of aspheric and yellow tinted) IOLs implantation.
Methods: 54 patients were implanted 3 different types of IOLs randomly: Spherical IOL (SP; ALCON SA60AT, 21 eyes), Aspheric IOL (AP; AMO ZA9003, 34 eyes), and Yellow tinted IOL (YT; HOYA YA60-BBR 32 eyes). Ophthalmologic examination including best-spectacle corrected visual acuity (BCVA), intraocular pressure (IOP), flare in anterior chamber, corneal endothelial cell density (CECD), glare disability, and contrast sensitivity were measured preoperatively, 1 day, 1 week, 1 month, and 3 months postoperatively. Contrast sensitivity was measured under different light conditions (under half-dark condition; 18-20 lux, under dark condition; 1-2 lux).
Results: There are no significant difference about BCVA, IOP, flare and CECD in each IOL. Glare disabilities of AP IOLs (5.0±3.5 %) were smaller than ones of SP IOLs (10.0±5.9 %) and YT IOLs (7.7±4.6%). Furthermore, the results of contrast sensitivities showed that AP IOLs were greater than SP and YT IOLs significantly under half-dark and dark conditions.
Conclusion: The clinical results of functionalized IOLs were preferable. The aspheric IOL demonstrated greater visual performance under dark conditions.

Identification of Estrogen-Regulated Genes in the Lens Using the ERΔ3 Transgenic Mouse Model with Inducible Cataracts

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Purpose: Numerous reports suggest that estrogen reduces the risk of age-related cataracts. Unfortunately, information on how estrogen may exert this protective effect is limited. Classical estrogen actions are mediated by estrogen receptors (ER) which are ligand-dependent transcription factors. In our ERΔ3 transgenic mouse model, spontaneous cortical cataracts occur in postpubertal females from inhibition of estrogen action by ERΔ3 (a dominant negative ERα variant). Estrogen (which activates the ERΔ3 repressor) also induces cataracts in prepubertal ERΔ3 males and females within 48 hours. Using this inducible model, we sought to identify estrogen-regulated genes that may be involved in cataract development.
Methods: To identify differentially expressed genes, microarray analysis was performed using lens RNA obtained from prepubertal ERΔ3 females 6 hours after treatment with 17β-estradiol (E_2) or vehicle.
Results: Microarray analysis revealed differential expression patterns after estrogen treatment for a wide variety of biological pathways which have been linked to cataract development, such as morphogenesis, organogenesis, cell differentiation, cell-cell signaling, and cell cycle. To validate the microarray results, lenticular expression of select genes are being analyzed by real-time RT-PCR in both ERΔ3 and wild-type (WT) females treated with and without E_2. For example, expression of Pax6 was modified in both ERΔ3 and WT females after estrogen exposure.
Conclusions: These results will permit identification of individual genes with potential or known roles in lens physiology and cataractogenesis that may be directly- or indirectly-regulated by estrogen. Identifying such targets should advance our understanding of estrogen action in the lens and its potential use for cataract prevention.

Crosslinks of pentosidine and pyridinoline in human diabetic cataract lenses

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Purpose: At the last US-Japan CCRG meeting, we reported two types of crosslinks, pentosidine and pyridinoline, as indicators to investigate the effects of aging in human senile cataractous lenses. Pentosidine is known as a kind of advanced glycation end products (AGEs) associated with diabetic mellitus (DM). This time, we report the result of analysis of the relation between these two types of crosslinks in DM and non-DM cataractous lenses.
Methods: The subjects were classified, according to the presence or absence of DM, into the DM group (n=12, 60.0±10.9 yrs.) and non-DM group (n=17, 66.6±7.71 yrs.). The lenses were obtained during cataract surgery with same stage in Emery's classification under informed consent. Crosslink levels were determined using high-performance liquid chromatography and spectrofluorometer (Ex/Em: 307/390 nm) after acid hydrolysis at 110oC for 20 hours.
Results: In non-DM group lenses, levels of pentosidine were significantly and positively related to that of pyridinoline (r=0.649, p=0.0038) and age (r=0.590, p=0.0126). Whereas in the DM group, pentosidine was significantly related to neither pyridinoline (r=0.572, p=0.0511) nor age (r=-0.088, p=0.792). Pyridinoline levels and age were not significantly related in both groups.
Conclusion: This results show that no relation was observed between pentosidine and pyridinoline nor between pentosidine and age in the DM lenses, though both relations were maintained in the non-DM lenses. It suggests that the increase of crosslinks due to glycation changed these relations in the DM lenses, which is considered to contribute to the course of cataract development in DM, regardless of aging.

Change of Light Scattering Intensity in Eyes with Posterior Vitreous Detachment: Monzen Eye Study

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Purpose: We report that prevalence of nuclear cataract is higher in eyes with posterior vitreous detachment (PVD) than in those without. In this study we measured the light scattering intensities (LSI) in lens layers to investigate the correlation between PVD and LSI.
Methods: Participants comprised 501 Japanese subjects aged 55 yrs and over (mean: 67.7±7.3yrs) who participated in the Monzen Eye Study in 2005. Scheimpflug slit and retroillumination images were taken of eyes under maximum pupil dilatation using the anterior segment analysis system (EAS1000, NIDEK). The LSI of the anterior capsule (A), anterior cortex (B), adult nucleus (C), anterior, and posterior fetal nucleus (D), (F) and central clear zone (E) were measured. Grading of cortical, nuclear, and posterior subcapsular cataract (PSC) was performed according to the WHO grading system. PVD was judged as presence of Weis ring.
Results: PVD was observed in 414 eyes (52%) and noted to increase with aging. Odds ratio of nuclear cataract between PVD(+) and PVD(-) was 1.9 p 0.009 χ examination , however, there was no significant difference for cortical and PSC. LSI increased with aging in six layers. After adjusting for age and axial length, the LSI in eyes with PVD(+) was higher in layers D (p=0.036) and E (p=0.004) compared with those with PVD (-) in females, however, there was no significant difference in males.
Conclusion: PVD might play an important role for developing nuclear cataract. Our result showed that progression of vitreous liquefaction might be a risk factor for nuclear cataract formation

A Common Mechanism for Age-related Cataract in 4 Species

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Purpose: To compare the formative mechanisms of age-related cataract development in 4 species.
Methods: Age-related changes **in** intact, live lenses from mice, rats, dogs, and humans were studied by confocal microscopy using fluorescent dyes specific for DNA and ROS. In fixed sections, specific antibodies against the DNA oxidation adduct 8-oxo-G and the DNA repair protein XRCC1 were used. Dispersed lens cells (LEC) were also studied by comet assay for the repair rate following x-irradiation DNA strand damage.
Results: All 4 species lenses showed the same changes with age and the presence of age-related cataract (ARC). These consisted of abnormal retention of nuclear fragments and free DNA deep into the lens interior, with detachment of the LEC from the surface at inappropriate sites far from the bow region, and thence LEC migration to the interior of the lens, accompanied by complete opacities. These sites of denuded lens surface and LEC migration pathways contained large amounts of ROS. The development of ARC was mimicked in soft x-ray induced ARC by confocal study of living lenses and, also in lens sections, by both the DNA migration and the oxidative findings, and with additional evidence for DNA strand damage and attempted repair.
Conclusion: The mechanistic processes of ARC are quite similar for the 4 species studied. Additionally, x-ray induced cataractogenesis, an oxidatively driven process, mimics ARC, providing an easy valuable way to provide subject matter to study this process.

Quantitative Analysis of TGF-β_2 in Human Diabetic Cataractous Lenses

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Purpose: Quantitative analysis of transforming growth factor (TGF) -β_2 in different part of diabetic cataractous lenses and comparison with senile cataractous lenses without diabetes mellitus.
Methods: Nine diabetic cataractous lenses (mean age; 66.8 yo) and 8 senile cataractous lenses (mean age; 71.9 yo) were obtained from patients using intracapsular cataract extraction after informed consent. Each cataractous lens was separated into capsule + lens epithelial cells (C+LEC), cortex and nucleus. Active form and total amount of TGF-β_2 in each component was measured by enzyme-linked immunosorbent assay (ELISA). Inactive form of TGF-β_2 was determined by calculating the difference of active form and total amount of TGF-β_2.
Results: In C+LEC, 1.1 % of total TGF-β_2 was active form in diabetic cataractous lens, while 61.7 % of total TGF-β_2 was active form in senile cataractous lens. In cortex, 2.6 % of total TGF-β_2 was active form in diabetic cataractous lens, whereas 35.8 % of total TGF-β_2 was active form in senile cataractous lens. In diabetic cataract, active form of TGF-β_2 was significantly lower, and inactive form of TGF-β_2 was significantly higher than in senile cataract in both of C+LEC and cortex ($p < 0.01$, respectively).
Conclusion: Low rate of TGF-β_2 activation in capsule + lens epithelial cells and cortex might be one of causes in diabetic cataract formation.

The Biology of Transparency: Non-crystallin Function of αB-crystallin Suggests a Physiological Basis for the Distribution of Crystallins in the Ocular Lens

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Purpose: Transparency is associated with the presence of high concentrations of proteins (crystallins) in the lens fiber cells. The non-crystallin function of crystallins is generally believed to be inconsequential to this process. As a part of an effort to understand the biological basis of the emergence of the phenotype of transparency we have investigated the status of small heat shock protein αB-crystallin by studying the non-crystallin functions of this protein in the ocular lens.
Methods: Differential cell fractionation, sucrose density gradients, immunoblotting with immunolocalization and confocal microscopy were used to investigate the distribution of αB-crystallin in the rat ocular lens.
Results: In the fetal and the early post-natal lens αB-crystallin is associated exclusively with the Golgi complex. By separating the Golgi membranes and the Golgi derived vesicles and employing αB-crystallin as the marker, we find that as postnatal lens grows the Golgi fragments into vesicles. The proportion of αB-crystallin in the Golgi membranes in comparison to the vesicular fraction shows gradual decrease with age. The analyses of the patterns of distribution obtained with lenses of various ages reveal that αB-crystallin starts life as a Golgi membrane protein. Its status changes with the maturation of the fiber cells resulting in the appearance of αB-crystallin as "Soluble" protein via its association with the vesicular fraction.
Conclusion: These data suggest that non-crystallin function of αB-crystallin (its association with the Golgi) has a fundamental role in dictating the distribution of proteins in the fiber cells and therefore, the emergence of the phenotype of transparency.

Superoxide-dependent NADH photo-oxidation enhanced by lambda-crystallin

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Purpose: In the rabbit lens, high levels of NADH can function as near-UV filter, and appear to be achieved by specific nucleotide binding to lambda-crystallin. The present study has been made to reveal that lambda-crystallin enhances NADH photo-oxidation by superoxide radicals generated via NADH photosensitization.
Methods: lambda-Crystallin was partially purified from rabbit (Japanese white) lens soluble fraction by a two-step isolation procedure comprised of gel filtration and Affi-Gel Blue affinity-column chromatography. NADH (10-50 µM) with or without partially purified lambda-crystallin was subjected to near-UV irradiation (300-430 nm, about 600 $\mu W/cm^2$) or exposed to superoxide generated enzymatically in oxidation of xanthine (20 µM) by xanthine oxidase (5.5 µg/ml). The rates of NADH oxidation were determined by measuring absorbance decreases at 340 nm.
Results: Under near-UV irradiation, free NADH was oxidized very little in the absence of lambda-crystallin. In contrast, partially purified lambda-crystallin largely increased NADH oxidation by superoxide generated by either NADH photosensitization or xanthine/xanthine oxidase. The lambda-crystallin-enhanced NADH oxidation exceeded the superoxide levels generated without the enzyme-crystallin. It was suggested from the above results that NADH bound to lambda-crystallin rapidly reacted with superoxide generated through a radical chain reaction mechanism. Reactivity of bound NADH with superoxide was almost equivalent to that of ascorbic acid.
Conclusion: We presume that lambda-crystallin with bound NADH contributes to efficient scavenging of superoxide radicals in the rabbit lens, where oxygen tension is very low.

Transparency in Zebrafish Lens and Cornea

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Purpose: Although the zebrafish is a promising model for studying development and structure of the eye, many biophysical and cellular properties of the normal zebrafish lens and cornea relating to transparency need to be characterized.

Methods: Slit lamp microscopy was performed on mature zebrafish anesthetized in 0.02% Tricaine (3-amino benzoic acid ethylester) and unanesthetized mice and recorded by digital video. Quantitative light scatter (QLS) measurements were taken from the cornea and lens of anesthetized zebrafish and mice. Total protein content was measured in zebrafish lenses.

Results: Slit lamp and QLS results indicate that light scattering properties of the zebrafish cornea and lens are more similar than the mouse cornea and lens. It can be anticipated that the differences in transparency are the result of differences in protein composition that occur during development.

Conclusion: Although zebrafish lens and cornea have many similarities with mammalian eyes, important differences in the molecular basis for transparency need to be "clarified".

Clinical Evaluation of Micro Incision Cataract Surgery (MICS)

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Purpose: Development of phacoemulsification techniques, IOLs and injectors decease the sizes of incisions for cataract surgery. In this study, the early postoperative results by using the different sizes of small incisions (2.4mm, 3.0mm, and 4.0mm) were evaluated.
Methods: The study was conducted on 208 eyes of 148 patients that had undergone cataract surgery. The cataract surgeries were performed using different sizes of sclerocorneal incisions (2.4mm, 89eyes; 3.0mm, 81eyes; and 4.0mm, 38eyes). Ophthalmologic examination including best-spectacle corrected visual acuity (BCVA), intraocular pressure (IOP), flare in anterior chamber, corneal endothelial cell density (CECD) were measured preoperatively, 1 week, 1 month, and 3 months postoperatively. Statistical comparisons were made according to Fisher's PSLD ($p<0.05$). And surgically-induced astigmatisms (SIA) were analyzed by the methods of Jaffe and Holladay at the same time periods.
Results: There are no significant difference about BCVA, IOP, flare and CECD in each incision. 3 months postoperatively, SIA by Jaffe in 2.4mm incision (0.58±0.71D) and 3.0mm incision (0.72±0.53D) were significant smaller than that in 4.0mm incision (1.37±0.96D). There is no significant difference about SIA by Holladay.
Conclusion: Micro incision cataract surgery (MICS) is effective for postoperative visual function by reducing the surgically-induced astigmatism.

Na-Cl Cotransport (NCC) in Human Lens Epithelial Cells (LECs).

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Purpose: Na-K-2Cl cotransport (NKCC) moves RbCl/KCl+NaCl/LiCl. To compare in FHL124 LECs Li fluxes through NKCC in hyposmotic high K media (to block IK channel-mediated regulatory volume decrease (RVD)), with Rb fluxes in high Na with hypotonicity-induced RVD.
Methods: Li and Rb uptake by atomic absorption spectrophotometry to determine LiKCC or NRbCC through bumetanide-sensitive (BS) and Cl-dependent fluxes in Na/K or Na-free/K-free (N-methyl-D-glucamine) and Cl or Cl-free (sulfamate or nitrate) media ± ouabain (O) or [O+B], ± thiazides, at varying Rb, Li or Cl mol fractions (MF); RT-PCR, Western blots/immunochemistry with NCC-specific antibodies.
Results: Li influx in isosmotic (300 mOsM) K was 1/3 of Rb influx in Na, mainly mediated by a Cl-dependent Li flux (LiKCC) and a [O+B+Cl]-insensitive (IS) Li “leak” that was 5x the Rb leak. In 200 mOsM high K, LiKCC was abolished, wheras in Na, NRbCC remained active. LiKCC and BS-Li influx (BS-LiKCC) showed bell-shaped curves for 0.1-1 Li MF, maxing at ~0.6 MF. BS-LiKCC was ¼ of LiKCC. The difference, i.e. the BIS/Cl-dependent-Li influx, saturated with Li and Cl MFs. K_{ms} for Li were 11 with and 7-8 mM without external K, respectively; and ~40 ± 12 (SE) mM for Cl. At 0.6 Rb MF, Li had no effect on bumetanide-inhibition. Neither furosemide (<100 μM) nor thiazide derivatives inhibited LiCC. RTPCR, Western blots and immunochemistry revealed NCC RNA and protein, besides the expected NKCC1.
Conclusion: By preventing IK-channel-mediated RVD in FHL124 LECs, a K-independent/Cl-dependent Li flux (LiCC) revealed the presence of NCC, as verified molecularly.

Cell-autonomous involvement of *Mab21l1* is essential for lens placode development

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Purpose: – The *mab-21* gene was originally identified as a call fate determinant in *C. elegans*. It is now known to constitute a family of genes that are highly conserved from vertebrates to invertebrates, and two orthologues *Mab21l1* and *Mab21l2* have been identified in many species. Although some functions of vertebrate MAB21 family proteins have already been investigated using RNA interference and antisense oligonucleotide strategies, a genetic approach is needed to achieve a deeper and more precise understanding of these proteins.
Methods: We generated *Mab21l1* deficient and chimera mice, and revealed essential roles in mouse development.
Results: The mutant mouse eye has a rudimentary lens resulting from insufficient invagination of the lens placode caused by deficient proliferation. Chimera analyses suggest that the lens placode is affected in a cell-autonomous manner, although *Mab21l1* is expressed in both the lens placode and the optic vesicle. The defects in lens placode development correlate with delayed and insufficient expression of *Foxe3*, which is also required for lens development, while *Maf, Sox2, Six3* and PAX6 levels are not significantly affected.
Conclusion: We conclude that *Mab21l1* expression dependent on PAX6 is essential for lens placode growth and for formation of the lens vesicle; lack of Mab21l1 expression caused reduced expression of Foxe3 in a cell-autonomous manner.

Roles for Heat Shock Proteins and Chaperones in Modeling Dietary Strategies for Astronaut Cataract Risk Reduction.

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Purpose: –To explore the role of heat shock proteins and related chaperone and protective proteins in porcine lenses exposed to elevated temperature and gamma radiation stress.
Methods: Pig lenses obtained from a local abattoir were dissected aseptically and incubated in medium M199 without serum for 4 days to stabilize, and those with protein leakage less than 10 mg/L were taken for heat shock. Lenses were exposed to radiation stress by exposure to ^{60}Co γ-radiation. Heat stress was performed by incubation for 1 hr in M199 wthout serum at a variety of temperatures from 37C to 55C. After post-stress incubation for 24 hr., the lenses were weighed and homogenized. The homogenates were analysed for Hsps 90, 70, 47, 27, HSF1, Akt, SOD1, MnSOD, Cu.ZnSOD, and αB-crystallin by western blotting. Scantox^{TM} and Scion Image analysis of lens photographs were used to estimate cataract image blurring.
Results: The degree of cataract blurring of the images increased with both temperature and radiation dose, while the percent differences in lens weights decreased with increasing temperature. In response to the stress, the following bands changed in intensity with increasing temperature: Hsps 70, 27 increased. With radiation dose , ubiquitin decreased, and Hsps 70, 27, and Cu/Zn SOD increased.
Conclusion: The lens stress responses appear to be tailored to the sort of stress the lens encounters.

Support: Canadian Space Agency

Influence of the tilting of aspheric intraocular lens on the ocular higher order aberration and the contrast sensitivity function

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Purpose: The influence of the tilting of aspheric intraocular lens (IOL) on the visual function was evaluated by ocular higher Order Aberration And Contrast Sensitivity Function.
Subjects And Methods: Twenty-nine pseudophakic eyes (Group-Z, mean age: 70.8 yrs) with acrylic aspheric IOL (TECNIS® ZA9003, AMO) and 48 pseudophakic eyes (Group-K, mean age: 77.3 yrs) with silicone aspheric IOL (KS-3Ai, Canon-Staar) were selected as subjects. Twenty-four pseudophakic eyes (Group-S, mean age: 78.7 yrs) with acrylic spherical IOL (AcrySof® SA60AT, Alcon) were selected as a control. The ocular higher order aberrations were measured by Wave-Front Analyzer (KR-9000PW; Topcon) at about one month after surgery in all cases. Tilting of IOL was evaluated using the EAS-1000 (NIDEK). Contrast sensitivity function was measured by CAT-2000 (NEITZ, Japan).
Results: There were significant differences among 3 groups in mean ocular spherical aberration (Group-Z: 0.016μm K: 0.135μm S:0.541μm of 6mm pupil diameter). There was no significant difference among 3 groups in mean IOL tilting (Group-Z: 3.22°, K: 3.38°, S: 3.11°). Coma aberration of the Group-Z significantly increased with IOL tilting ($p<0.05$), but there were no significant correlations between the coma aberration and IOL tilting in other 2 groups (K- & S-groups). In the aspheric IOL groups (Z & K), there were no significant correlations between the IOL tilting and contrast sensitivity function.
Conclusion: Aspheric IOL was developed for reduction of the ocular spherical aberration. Although contrast sensitivity of the pseudophakic eye with aspheric IOL is higher than the one of the case with spherical IOL, the appropriate positioning in the capsular bag is the key to exhibit the advantage of aspheric IOL, without which might result in the increase of coma aberration.

In Vivo Detection of Alzheimer's Disease-Linked Aβ Peptide Accumulation in the Lens

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Purpose: Alzheimer's disease (AD) is characterized by cerebral accumulation of β-amyloid (Aβ) that begins years before cognitive decline. We previously discovered AD-linked Aβ amyloid pathology and distinctive co-localizing supranuclear cataracts in AD lenses (Goldstein et al, **Lancet,** 2003). Here, we developed and tested purpose-built low-energy quasi-elastic light scattering (QLS) instrumentation for early quantitative detection and monitoring of AD-linked amyloid lens pathology *in vivo.*
Methods: *AD Transgenic Mice:* Tg2576 (Tg) and age-matched wild-type (Wt) controls. *Techniques:* Non-invasive infrared QLS, slitlamp stereophotomicroscopy, amyloid histochemistry, immunohistochemistry, western blot, ELISA, immunogold EM, peptide sequencing.
Results: We sequenced human Aβ from Tg mouse lens and detected human Aβ-immunoreactive microaggregates in the cytoplasm of Tg lens fiber cells. Human Aβ accumulated in conditioned medium from Tg lens explant cultures, indicating lenticular origin of detected Aβ. *In vivo* QLS discriminated non-anesthetized Tg mice from age-matched Wt controls by 10 months of age *before* β-amyloid pathology was detectable in lens or brain. Human Aβ promoted aggregation of mouse lens protein that replicated QLS signal changes detected *in vivo*. Second-generation instruments incorporate confocal optics, autocorrelation spectroscopy, and high-affinity fluorescent amyloid-binding ligands. **Conclusions:** Our data support AD-linked Aβ accumulation in the lens as an optically accessible early AD biomarker and provide "proof of concept" preclinical testing of novel non-invasive technology for AD screening, diagnosis, and monitoring.

Effect of 5-S-GAD (Eye drops) on UV-B-Induced Cataract in Rats

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Purpose: 5-S-GAD is a novel antibacterial substance purified from Sarcophaga peregrina (Flesh Fly) that has both a radical scavenging activity and an anti-oxidative activity. We investigated the effect of 5-S-GAD (Eye drops) on UV-B-induced cataract in rats.
Methods: Brown Norway male rats (N=8, 6-weeks years old) were treated with either 5-S-GAD (0.1% or 1%) eye drops or astaxanthin (AST) 0.1% suspension or the vehicle alone (the solution without 5-S-GAD) three times a day (3 times with 5 min intervals). The treatment was scheduled as 2 days before UV-B exposure and 2 days after UV-B exposure. 100-200 mJ/cm2 UV-B exposure was performed once a week between drug treatments for 9 weeks continuously with a total dose of 1,200 mJ/cm^2 UV-B. Ocular penetration of 5-S-GAD was analyzed using high-pressure liquid chromatography (HPLC).
Results: Cataract formations were documented by an anterior eye segment analysis system (EAS-1000, NIDEK) once a week in mydriasis (Atropine). The light scattering intensity of the anterior superficial cortex regions was measured.The light scattering intensity of lenses from 5-S-GAD (0.1% and 1%) treated animals was significantly lower than that of the control group. This difference was found 8 to 9 weeks after the start of UV-B exposure. On the other hand, the AST treated group showed no significant difference in the light scattering intensity. 5-S-GAD in the aqueous humor was detected.
Conclusion: 5-S-GAD eye drop application may have the potential to delay the progression of UV-B-induced cataract in rats.

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